Quantity One®

User Guide for Version 4.6.3
Windows and Macintosh

P/N 10002940 Rev C

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Preface

1. About This Document

This user guide is designed to be used as a reference in your everyday use of Quantity One® Software. It provides detailed information about the tools and commands of Quantity One for the Windows and Macintosh platforms. Any platform differences in procedures and commands are noted in the text.

This guide assumes that you have a working knowledge of your computer operating system and its conventions, including how to use a mouse and standard menus and commands, and how to open, save, and close files. For help with any of these techniques, see the documentation that came with your computer.

This guide uses certain text conventions to describe specific commands and functions.

Example	Indicates
File > Open	Choosing the Open command under the File menu.
Dragging	Positioning the cursor on an object and holding down the left mouse button while you move the mouse.
Ctrl+s	Holding down the Control key while typing the letter *s*.
Right-click/ Left-click/ Double-click	Clicking the right mouse button/ Clicking the left mouse button/ Clicking the left mouse button twice.

Some of the illustrations of menus and dialog boxes found in this manual are taken from the Windows version of the software, and some are taken from the Macintosh version. Both versions of a menu or dialog box will be shown only when there is a significant difference between the two.

2. Bio-Rad Listens

The staff at Bio-Rad are receptive to your suggestions. Many of the new features and enhancements in this version of Quantity One are a direct result of conversations with our customers. Please let us know what you would like to see in the next version of Quantity One by faxing, calling, or e-mailing our Technical Services staff. You can also use Solobug (installed with Quantity One) to make software feature requests.

1. Introduction

1.1 Overview of Quantity One

Quantity One is a powerful, flexible software package for imaging and analyzing 1-D electrophoresis gels, dot blots, arrays, and colonies.

The software is supported on Windows and Macintosh operating systems and has a graphical interface with standard pull-down menus, toolbars, and keyboard commands.

Quantity One can image and analyze a wide variety of biological data, including radioactive, chemiluminescent, fluorescent, and color-stained samples acquired from densitometers, phosphor imagers, fluorescent imagers, and gel documentation systems.

An image of a sample is captured using the controls in the imaging device window and displayed on your computer monitor. Image processing and analysis operations are performed using commands from the menus and toolbars.

Images can be magnified, annotated, rotated, and resized. They can be printed using standard and video printers.

All data in the image can be quickly and accurately quantitated using the Volume tools.

The lane-based functions can be used to determine molecular weights, isoelectric points, VNTRs, presence/absence and up/down regulation of bands, and other values. The software can measure total and average quantities, determine relative and actual amounts of protein, and count colonies in a Petri dish.

The software can cope with distortions in the shape of lanes and bands. Lanes can be adjusted along their lengths to compensate for any curvature or smiling of gels.

Image files can be shared among all The Discovery Series™ software. Images can also be easily converted into TIFF format for compatibility with other Macintosh and Windows applications.

1.2 Digital Data and Signal Intensity

The Bio-Rad imaging devices supported by Quantity One are light and/or radiation detectors that convert signals from biological samples into digital data. Quantity One then displays the digital data on your computer screen, in the form of gray scale or color images.

A data object as displayed on the computer is composed of tiny individual screen pixels. Each pixel has an X and Y coordinate, and a value Z. The X and Y coordinates are the pixel's horizontal and vertical positions on the image, and the Z value is the signal intensity of the pixel.

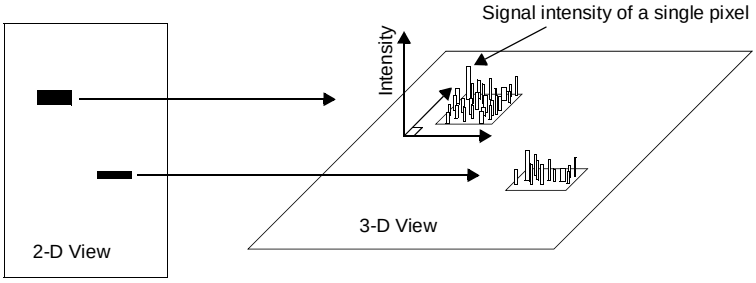


Fig. 1-1. Representation of the pixels in two digitally imaged bands in a gel.

For a data object to be visible and quantifiable, the intensity of its clustered pixels must be higher than the intensity of the pixels that make up the background of the image. The total intensity of a data object is the sum of the intensities of all the pixels that make up the object. The mean intensity of a data object is the total intensity divided by the number of pixels in the object.

The units of signal intensity are Optical Density (O.D.) in the case of the GS-700™ imaging densitometer, GS-710™ calibrated imaging densitometer, and GS-800™ calibrated densitometer, the Gel Doc EQ™ system, ChemiDoc EQ™ system, ChemiDoc XRS™ system with a white light source, or the Fluor-S™ MultiImager system, Fluor-S™ MAX MultiImager system, Fluor-S™ MAX2 MultiImager system and VersaDoc™ imaging systems with white light illumination. Signal intensity is expressed in counts when using the Personal Molecular Imager™ system or the Molecular Imager FX™ system, Molecular Imager FX Pro™ fluorescent imager,

Molecular Imager FX Pro Plus™ multiimager system, or in the case of the Gel Doc EQ, ChemiDoc EQ, ChemiDoc XRS, Fluor-S, Fluor-S MAX, or VersaDoc when using the UV light source.

1.3 Gel Quality

Quantity One is very tolerant of an assortment of electrophoretic artifacts. Lanes do not have to be perfectly straight or parallel. Bands do not have to be perfectly resolved.

However, for accurate lane-based quantitation, bands should be reasonably flat and horizontal. Lane-based quantitation involves calculating the average intensity of pixels across the band width and integrating over the band height. For the automatic band finder to function optimally, bands should be well-resolved.

Dots that appear as halos, rings, or craters, or that are of unequal diameter, may be incorrectly quantified using the automatic functions.

1.4 Quantity One Workflow

The following steps are involved in using Quantity One.

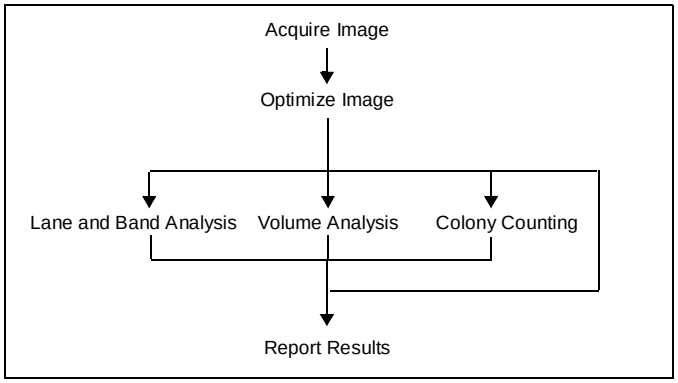


Fig. 1-2. Quantity One workflow.

1.4.a Acquire Image

Before you can use Quantity One to analyze a biological image, you need to capture the image and save it as an image file. This may be done with one of the several Bio-Rad imaging instruments supported by this software: the Molecular Imager FX and Personal Molecular Imager systems; the GS-800 Imaging Densitometer; the Gel Doc EQ, ChemiDoc EQ, and ChemiDoc XRS Gel documentation systems; and the VersaDoc.

The resulting images can be stored in files on a computer hard disk, network file server, or removable disks.

1.4.b Optimize Image

Once you have acquired an image of your sample, you may need to reduce noise or background density in the image. Quantity One has a variety of functions to minimize image background while maintaining data integrity.

1.4.c Analyze Image

Once a “clean” image is available, you can use Quantity One to gather and analyze your biological data. In the case of 1-D gels, the software has tools for identifying lanes and defining, quantifying, and calculating the values of bands. Volume tools allow you to easily measure and compare the quantities of bands, spots, or arrays. The colony counting controls allow you to count the number of colonies in a Petri dish, as well as perform batch analysis.

Qualitative and quantitative data can be displayed in tabular and graphical formats.

1.4.d Report Results

When your analysis is complete, you can print your results in the form of simple images, images with overlays, reports, tables, and graphs. You can export your images and data to other applications for further analysis.

1.5 Computer Requirements

This software is supported on Windows XP and Windows 2000, or on a Macintosh PowerPC running Mac OS 9.2.2 or Mac OS 10.2.6.

The computer memory requirements are mainly determined by the file size of the images you will scan and analyze. High-resolution image files can be very large. For this reason, we recommend that you archive images on a network file server or high-capacity removable disk.

PC

The following is the **recommended** system configuration for installing and running on a PC:

Operating system:	Windows 2000 Windows XP
Processor:	Pentium 333 MHz or faster
RAM:	≥ 256 MB for all Bio-Rad imaging systems.

Hard disk space:	≥ 3 GB
Monitor:	17" monitor, 1024 x 768 resolution (required), True color.
USB port:	Required for the Hardware Security Key (HSK).
Printer:	Optional.

Macintosh

The following is the **recommended** system configuration for installing and running on a Macintosh:

Operating system:	MacOS 9.2, MacOS 10.2 and 10.3.
Processor/Model:	PowerPC G3 processor or faster.
RAM:	≥ 256 MB for all Bio-Rad imaging systems.
Hard disk space:	≥ 3 GB
Monitor:	17" monitor, 1024 x 768 resolution (required), Millions of colors.
USB port:	Required for the Hardware Security Key (HSK).
Printer:	Optional.

Note: The default amount of memory assigned to this program on the Macintosh is 128 MB. If the total RAM in your Macintosh is 128 MB or less, you should reduce the amount of memory assigned to the program to 10 MB less than your total RAM. With the application icon selected, go to File > Get Info in your Finder to reduce the memory requirements for the application. See your Macintosh computer documentation for details.

1.6 Installation

Refer to the Installation Guide for detailed information regarding installation of The Discovery Series.

1.6.a Hardware Security Key (HSK)

The Discovery Series software is password-protected using a Hardware Security Key (HSK), which is included in your software package. You must attach the Hardware Security Key to your computer before you can run the software.

Fig. 1-3. Hardware Security Key

Before proceeding with installation, plug the HSK into any available USB port on your computer.

The code for the HSK is EYYCY, which is printed on the key itself. Use this code to identify the HSK that belongs to The Discovery Series software.

Note: Initial installation of a network server requires the Hardware Security Key included in the software package. Installation of an additional Network Client User to a Network License Server System does not require an HSK. Please refer to the Network License Installation Guide that ships with Network License.

1.6.b Installing The Discovery Series for Windows

Note: You must be a member of the Administrators group to install Discovery Series software.

Insert The Discovery Series CD-ROM. The installation wizard will start automatically. (If the CD does not auto-start, click Start in your taskbar then click Run. In the Open field type d:\setup.exe where d is the letter of your CD-ROM drive.) Select the software application you want to install. On each panel, click Next when you are ready to proceed. You must accept the license agreement to continue with installation.

The installer program guides you through the installation. The installer creates a default directory under Program Files on your computer called Bio-Rad\The Discovery Series (to select a different directory, click Browse). The application program will be placed in the Bin folder inside The Discovery Series folder. An additional folder for storing sample images is also located in The Discovery Series folder. User profiles will be created and stored in the Documents and Settings folder for each user.

The installer places a shortcut to the application and user guide on your desktop and creates a The Discovery Series folder in Programs on your Windows Start menu.

After installation, you must reboot your computer before using an imaging device.

Note: If you are installing in a Windows 2000 environment, you must start the application before allowing any other user access to the application.

Uninstalling The Discovery Series from Windows

If you need to uninstall The Discovery Series for any reason, go to Add/Remove Programs in the Control Panel. Highlight the application you want to remove and click Remove.

Note: Uninstalling The Discovery Series software does not remove any system files in the Documents and Settings folder located in Application Data. If you want to remove these files as well, you must do so manually.

1.6.c Installing the Discovery Series on a Macintosh

The Mac install process involves installation of the application, dongle drivers, and Roper support. Insert the Discovery Series CD-ROM into your Macintosh. The TDS-Mac folder opens on your desktop, displaying the installers for The Discovery Series applications. Double-click on the installer for your application.

Fig. 1-4. Installation program icon (Macintosh).

Insert the Discovery Series CD-ROM. The TDS-Mac folder opens on your desktop, displaying the installers for The Discovery Series applications. Double-click the installer for your application. You must accept the license agreement to proceed with installation. The ReadMe contains important information about the HSK. Click Continue.

The installation wizard lists the types of installations in the pull down list. If you want to install the application and documentation only, select Easy Install from the list.

If you want to install drivers as well, select Custom from the pull down list. (Remember to also select the application in custom install.)

Note: If the computer is going to be used to acquire images from Gel Doc EQ, ChemiDoc EQ, ChemiDoc XRS, or the VersaDoc system, then select the driver for your particular imaging device now.

Install Location identifies where the application will be installed. If you want to specify a different location, click the pull-down button.

Click Install to proceed. Once installation is complete, click Quit. To return to the installer, click Continue.

Uninstalling The Discovery Series from a Macintosh

Insert the Discovery Series CD-ROM. The TDS-Mac folder opens on your desktop, displaying the installers for The Discovery Series applications. Double-click the installer for your application. In the installer screen, select Uninstall from the pull down list.

1.7 Software License

When the software opens for the first time, you will see a *Software License* screen that shows the current status of your software license.

With a new HSK or network license, you receive a 30-day temporary license ("Your license will expire on _____"). The temporary license is designed to give you time to purchase the software, if you have not already done so.

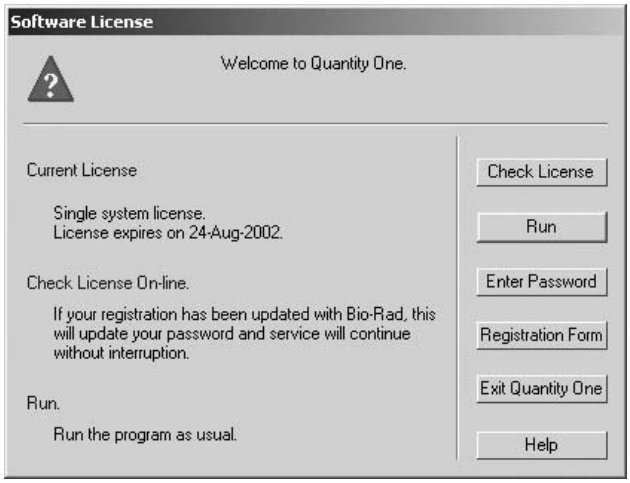


Fig. 1-5. Temporary license screen.

During the 30-day period, the *Software License* screen will appear every time you open the software. To use the software during this period, click on the **Run** button.

Network license holders can click on the **Check License** button at any time during the 30-day period to activate their full network license. (If your network license is not activated when you click on **Check License**, notify your network administrator.)

HSK users have 30 days to purchase the software and obtain a purchase order number and software serial number from Bio-Rad. When you have this information, click on the **Check License** or **Registration Form** button in the *Software License* screen to register your software.

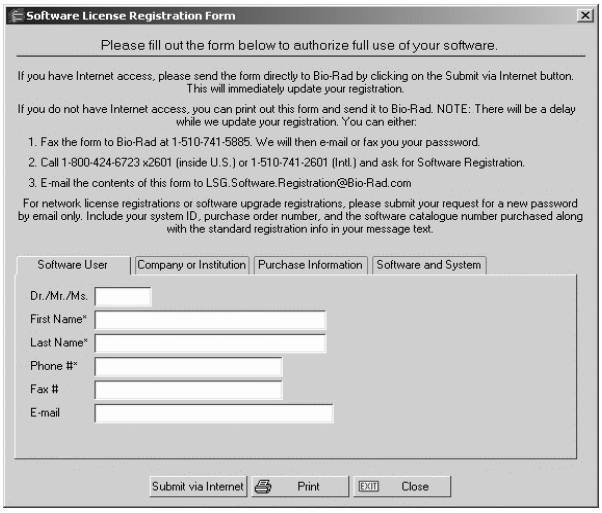


Fig. 1-6. Software License Registration Form.

Fill out the information in the *Software License Registration Form*. Be sure to enter your purchase order number and software serial number under the *Purchase Information* tab when registering.

1.7.a Registering by Internet

If you have Internet access from your computer, click on the Submit via Internet button to send the *Software Registration Form* directly to Bio-Rad.

Your information will be submitted, and a temporary password will be generated automatically and sent back to your computer. Simply continue to run the application as before.

Bio-Rad will confirm your purchase information and generate a permanent license. After 2–3 days, click on **Check License** in the *Software License* screen again to update to a permanent password. (The *Software License* screen will not appear automatically after the temporary password has been generated; the software will

simply open normally. Go to the *Help* menu and select **Register** to open the *Software License* screen.)

1.7.b Registering by Fax or E-mail

If you do not have Internet access, click on the **Print** button in the *Software License Registration Form* and fax the form to Bio-Rad at the number listed on the form. Alternatively, you can enter the contents of the form into an e-mail and send it to Bio-Rad at the address listed in the *Registration Form*.

Bio-Rad will contact you by fax or e-mail in 2–3 days with a full license.

1.7.c Entering a Password

If you fax or e-mail your registration information, you will receive a password from Bio-Rad. You must enter this password manually.

To enter your password, click on **Enter Password** in the *Software License* screen. If you are not currently in the *Software License* screen, select **Register** from the *Help* menu.

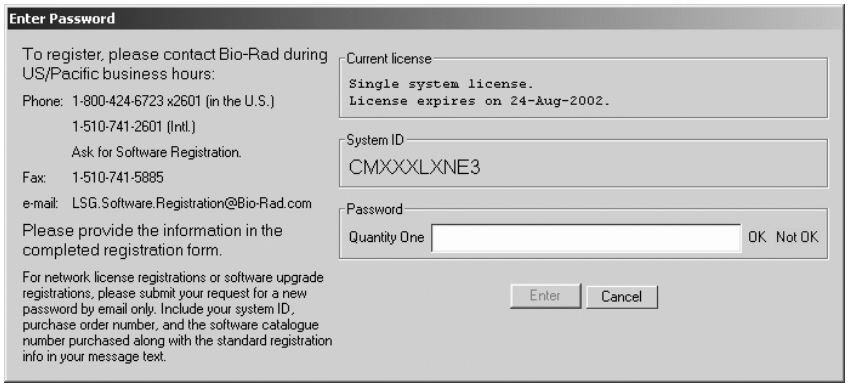


Fig. 1-7. Enter Password screen.

In the *Enter Password* screen, type in your password in the field.

Once you have typed in the correct password, the **OK** light next to the password field will change to green and the **Enter** button will activate. Click on **Enter** to run the program.

1.8 Downloading from the Internet

You can download a trial version of the software from Bio-Rad's Web site. Go to The Discovery Series download page at **www.bio-rad.com/softwaredownloads** and select from the list of applications. Follow the instructions to download the installer onto your computer, then run the installer.

After installation, double-click on the application icon to run the program. The software will open and the *Software License* screen will be displayed.

Note: If you attempt to start the downloaded program and receive an "Unable to obtain authorization" message, you will need a Hardware Security Key to run the program. Contact Bio-Rad to obtain a key.

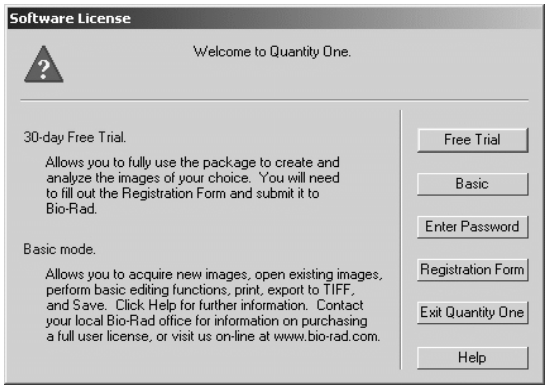


Fig. 1-8. Free Trial screen.

In the *Software License* screen, click on the **Free Trial** button. This will open the *Software License Registration Form*. Enter the required information (you will not

have a purchase order number or software serial number, and can leave these fields blank) and click on **Submit Via Internet**.

A free trial password will be automatically downloaded to your computer. This password will allow you to use the software for 30 days.

If you decide to purchase the software during that period, contact Bio-Rad to receive a software package and a Hardware Security Key. You can then complete the registration process as described in the previous sections.

1.9 Quantity One Basic

Quantity One can be run in Basic mode. Quantity One Basic does not require a software license. The program can be installed and used simultaneously on unlimited numbers of computers. Quantity One Basic is a limited version of the flexible and powerful Quantity One.

The following functionality is active in Basic Mode: Image acquisition with Bio-Rad imaging devices, Transform, Crop, Flip, Rotate, Text Tool, Volume Rectangle Tool, Volume Circle Tool, Density Tools, ReadyAgarose 96 PLUS, Manual Excision, Print, Export to TIFF, and Save.

1.10 Contacting Bio-Rad

Bio-Rad technical service hours are from 8:00 a.m. to 4:00 p.m., Pacific Standard Time in the U.S.

Phone: 800-424-6723
510-741-2612

Fax: 510-741-5802

E-mail: LSG.TechServ.US@Bio-Rad.com

For software registration:

Phone: 800-424-6723 (in the U.S.)

+1-510-741-6996 (outside the U.S.)

2. General Operation

This chapter describes the graphical interface of Quantity One, how to access the various commands, how to open and save images, how to set preferences, and how to perform other basic file commands.

2.1 Menus and Toolbars

2.1.a Menu Bar

Quantity One has a standard menu bar with pull-down menus that contain all the major features and functions available in the software.

- *File*—Opening and saving files, imaging device controls, printing, exporting.
- *Edit*—Preferences, other settings.
- *View*—Image magnification and viewing tools, tools for viewing image data.
- *Image*—Image transform, advanced crop, image processing and modification.
- *Lane*—Lane-finding tools.
- *Band*—Band-finding and band-modeling tools.
- *Match*—Tools for calculating molecular weights and other values from standards, tools for comparing lanes and bands in lanes.
- *Volume*—Band quantity and array data tools.
- *Analysis*—Colony counting, Differential Display, VNTR analysis.
- *Excision*—Cut lanes or bands, or individual spots using EXQuest Spot Cutter.
- *Reports*—Band and lane analysis reports, Phylogenetic Tree, Similarity Matrix.
- *Window*—Commands for arranging multiple image windows.
- *Help*—Quick Guides, on-line Help, software registration.

Below the menu bar is the main toolbar, containing some of the most commonly used commands. Next to the main toolbar are the status boxes, which provide information about cursor selection and toolbar buttons.

2.1.b Main Toolbar

The main toolbar appears below the menu. It includes buttons for the main file commands (**Open, Save, Print**) and essential viewing tools (**Zoom Box, Grab**, etc.), as well as buttons that open the secondary toolbars and the most useful Quick Guides (*Printing, Volumes, Molecular Weight,* and *Colony Counting*).

Fig. 2-1. Main toolbar.

Tool Help

If you hold the cursor over a toolbar icon, the name of the command will pop up below the icon. This utility is called Tool Help. Tool Help appears on a time delay basis that can be specified in the *Preferences* dialog box (see section 2.5, Preferences). You can also specify how long the Tool Help will remain displayed.

2.1.c Status Boxes

There are two status boxes, which appear to the right of the main toolbar.

Fig. 2-2. Status boxes.

The first box displays any function that is assigned to the mouse. If you select a command such as **Zoom Box**, the name and icon of that command will appear in this status box and remain there until another mouse function is selected or the mouse is deassigned.

The second status box is designed to supplement Tool Help (see above). It provides additional information about the toolbar buttons. If you hold your cursor over a

button, a short explanation about that command will be displayed in this second status box.

2.1.d Secondary Toolbars

Secondary toolbars contain groups of related functions. You can open these toolbars from the main toolbar or from the *View > Toolbars* submenu.

The secondary toolbars can be toggled between vertical, horizontal, and expanded formats by clicking on the resize button on the toolbar itself.

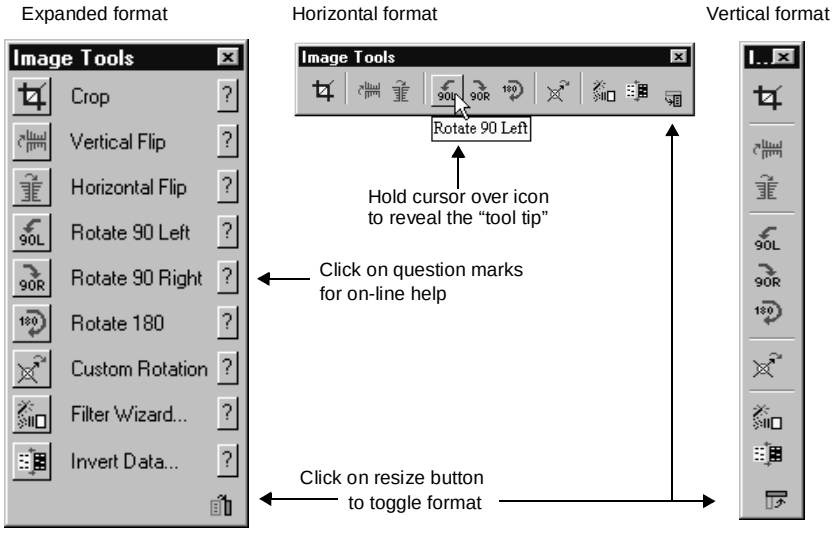


Fig. 2-3. Secondary toolbar formats and features.

The expanded toolbar format shows the name of each of the commands. Click on the **?** icon next to the name to display on-line Help for that command.

2.1.e Quick Guides

The Quick Guides are designed to guide you through the major applications of the software. They are listed under the *Help* menu; four of these are also available on the main toolbar.

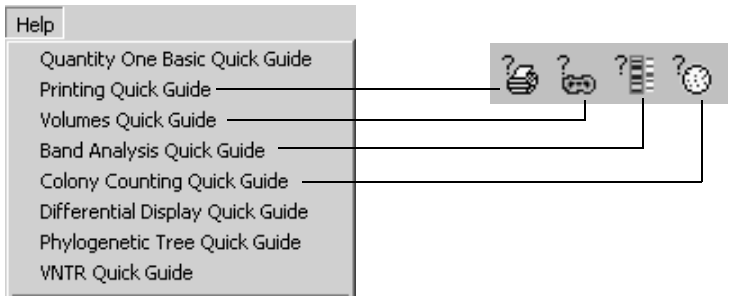


Fig. 2-4. Quick Guides listed on Help menu and main toolbar.

The Quick Guides are similar in design to the secondary toolbars, but are application-specific. Each Quick Guide contains all of the functions for a particular application, from opening the image to outputting data.

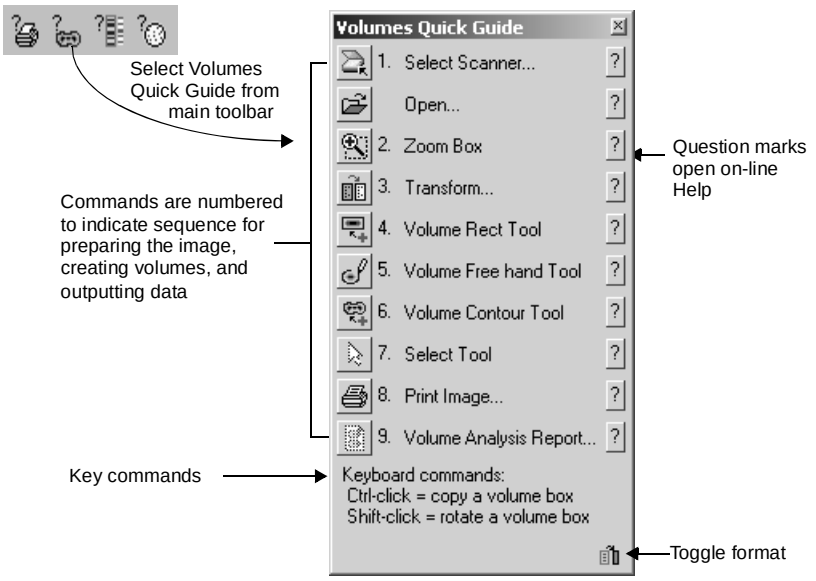


Fig. 2-5. Example of a Quick Guide: Volumes

In their expanded format, the Quick Guide commands are numbered as well as named. The numbers provide a suggested order of operation; however, not every command is required for every application.

As with the secondary toolbars, you can click on the **?** next to the name of a function to display the Help text.

2.1.f Right-Click Context Menu

With an image open, right-click anywhere on the image to display a context menu of common commands.

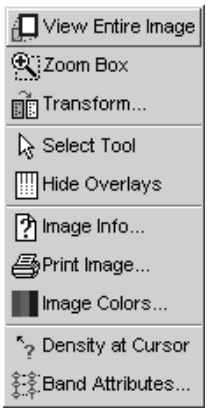


Fig. 2-6. Selecting Zoom Box from the right-click context menu.

You can select commands from this menu as you would from a standard menu.

2.1.g Keyboard Commands

Many commands and functions can be performed using keyboard keys (e.g., press the F1 key for **View Entire Image**; press Ctrl+S for **Save**). Select **Keyboard Layout** from the *Help* menu to display a list of keys and key combinations and their associated commands.

The pull-down menus also list the shortcut keys for the menu commands.

2.2 File Commands

The basic file commands and functions are located on the *File* menu.

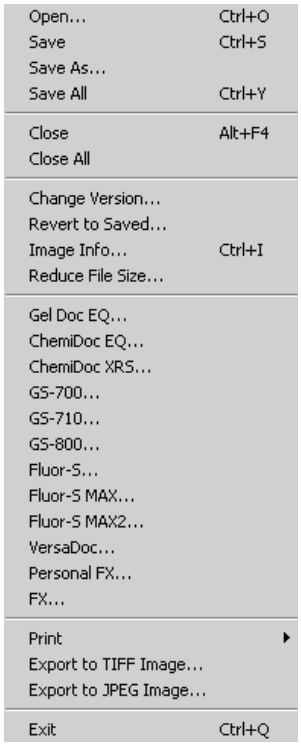
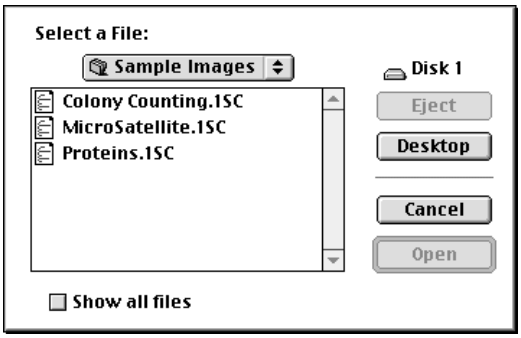


Fig. 2-7. File menu.

2.2.a Opening Images

To open a saved image, select **Open** from the *File* menu or click on **Open** button on the main toolbar. This opens the standard *Open* dialog box for your operating system.

Macintosh version:



Windows version:

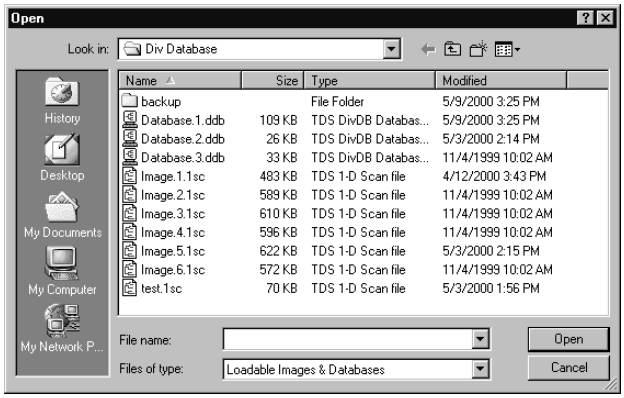


Fig. 2-8. Open dialog box.

In the dialog box, open a file by double-clicking on the file name. To open multiple files, first select them using Ctrl-click or Shift-click key combinations, and then click on the **Open** button.

Note: Due to operating system limitations, it is possible to have image files open in two or more locations over a network simultaneously. To safeguard your image files keep them in a protected folder or on your local machine.

You can also open images from other The Discovery Series software (PDQuest, Diversity Database, DNACode).

Note: It is possible to move image data between The Discovery Series software applications on different platforms (Windows and Macintosh).

The application comes with a selection of sample images. In Windows, these are located in The Discovery Series/Sample Images/1D directory. On the Macintosh, they are stored in the Sample Images folder in the Quantity One folder.

Opening TIFF Images

The **Open** command can also be used to import TIFF images created using other software applications.

There are many types of TIFF formats that exist on the market. Not all are supported by The Discovery Series. There are two broad categories of TIFF files that are supported:

1. 8-bit Grayscale. Most scanners have an option between line art, full color, and grayscale formats. Select grayscale for use with The Discovery Series software. In a grayscale format, each pixel is assigned a value from 0 to 255, with each value corresponding to a particular shade of gray.

2. 16-bit Grayscale. Bio-Rad's Molecular Imager FX and Personal Molecular Imager and Fluor-S, and VersaDoc imaging systems use 16-bit pixel values to describe intensity of scale. Molecular Dynamics™ and Fuji™ imagers also use 16-bit pixel values. The Discovery Series understands these formats and can interpret images from both Bio-Rad and Molecular Dynamics storage phosphor systems.

Note: The program can import 8- and 16-bit TIFF images from both Macintosh and PC platforms.

TIFF files that are *not* supported include:

1. 1-bit Line Art. This format is generally used for scanning text for optical character recognition or line drawings. Each pixel in an image is read as either black or white. Because the software needs to read continuous gradations to perform gel analysis, this on-off pixel format is not used.

2. 24-bit Full Color or 256 Indexed Color. These formats are frequently used for retouching photographs and are currently unsupported in The Discovery Series,

although most scanners that are capable of producing 24-bit and indexed color images will be able to produce grayscale scans as well.

3. Compressed Files. The software does not read compressed TIFF images. Since most programs offer compression as a selectable option, files intended for compatibility with The Discovery Series should be formatted with the compression option turned off.

2.2.b Saving Images

To save a new image or an old image with changes, select **Save** from the *File* menu. In Windows, new images will be given a **.1sc** extension when they are first saved.

Save As can be used to save a new image, rename an old image, or save a copy of an image to a different directory. The standard *Save As* dialog box for your operating system will open.

To save all open images, select **Save All** from the *File* menu or click on the button on the main toolbar.

2.2.c Closing Images

To close an image, select **Close** from the *File* menu. To close all open images, select **Close All**. You will be prompted to save any changes before closing.

2.2.d Revert to Saved

To reload the last saved version of an image, select **Revert to Saved** from the *File* menu. Because any changes you made since last saving the file will be lost, you will be prompted to confirm the command.

2.2.e Image Info

Image Info on the *File* menu opens a dialog box containing general information about the selected image, including scan date, scan area, number of pixels in the image, data range, and the size of the file. Type any description or comments about the image in the *Description* field.

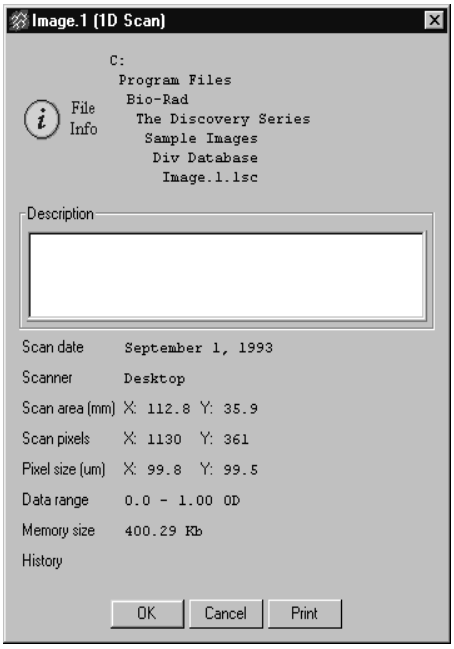


Fig. 2-9. Image Info box.

History lists the changes made to the image including the date. If you have Security Mode active, the name of the user who made the change is also listed (See Section 2.5, Preferences for information on Security Mode).

To print the file info, click on the **Print** button in the dialog box.

Changing the Image Dimensions

You can change the dimensions of certain images using the *Image Info* dialog box. This feature is only available for images captured by a camera or imported TIFF images in which the dimensions are not already specified.

For these types of images, the *Image Info* dialog box will include fields for changing the image dimensions.

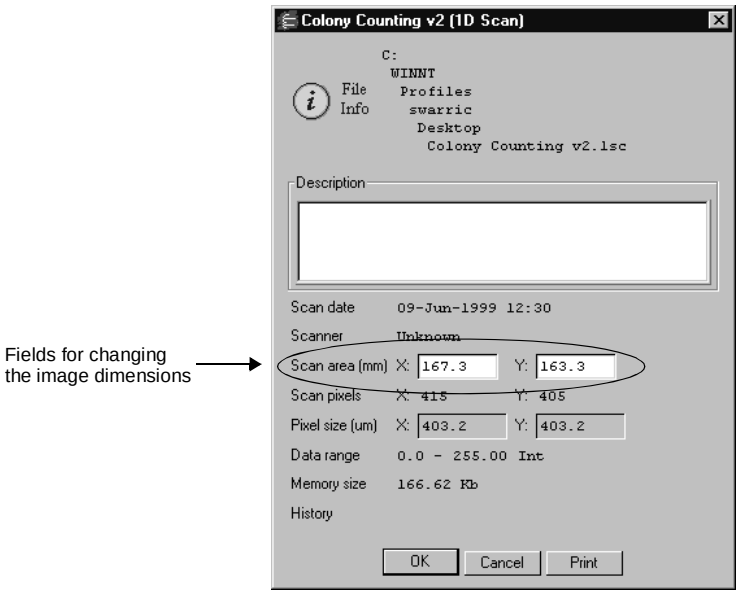


Fig. 2-10. Image Info dialog box with fields for changing the image dimensions.

Enter the new image dimensions (in millimeters) in the appropriate fields. Note that the pixel size in the image (in micrometers) will change to retain the same number of pixels in the image.

2.2.f Reduce File Size

High-resolution image files can be very large, which can lead to problems with opening and saving. To reduce the file size of an image, you can reduce the image resolution by reducing the number of pixels in the image. (You can also trim unneeded parts of an image to reduce its memory size. See section 3.9.a, Cropping Images.)

This function is comparable to scanning at a lower resolution, in that you are increasing the size of the pixels in the image, thereby reducing the total number of pixels and thus the file size.

Note: In most cases, reducing the resolution of an image will not affect quantitation. In general, as long as the pixel size remains less than 10 percent of the size of the objects in your image, changing the pixel size will not affect quantitation.

Select **Reduce File Size** from the *File* menu to open the *Reduce File Size* dialog box. The dialog box lists the size of the pixels in the image (**Pixel Size: X by Y microns**), the number of pixels in the image (**Pixel Count: X by Y pixels**), and the memory size of the image.

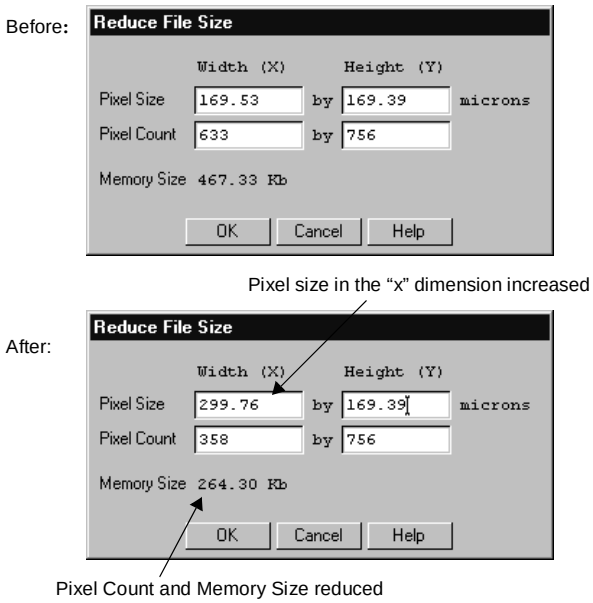


Fig. 2-11. Reduce File Size dialog box, before and after pixel size increase.

Lower the resolution by entering *lower* values in the *Pixel Count* fields or *higher* values in the *Pixel Size* fields (see the figure for an example).

Note: Since with most 1-D gels you are more concerned with resolving bands in the vertical direction than the horizontal direction, you may want to reduce the file size by making rectangular pixels. That is, keep the pixel size in the "y" dimension the same, while increasing the size in the "x" dimension.

When you are finished, click on the **OK** button.

A pop-up box will give you the option of reducing the file size of the displayed image or making a copy of the image and then reducing the copy's size.

Reducing the file size is an irreversible process. For that reason, we suggest that you first experiment with a copy of the image. Then, when you are satisfied with the reduced image, delete the original.

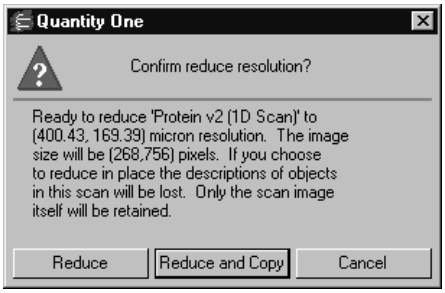


Fig. 2-12. Confirm Reduce File Size pop-up box.

2.3 Imaging Device Acquisition Windows

The *File* menu contains a list of Bio-Rad imaging devices supported by Quantity One. These are:

1. Gel Doc EQ
2. ChemiDoc EQ
3. ChemiDoc XRS
4. GS-800 Calibrated Densitometer

5. VersaDoc
6. Personal Molecular Imager FX
7. Molecular Imager FX
8. PharosFX

To open the acquisition window for an imaging device, select the name of that device from the *File* menu.

See the individual chapters on the imaging devices for more details.

2.4 Exit

To close Quantity One, select **Exit** from the *File* menu. You will be prompted to save your changes to any open files.

2.5 Preferences

You can customize basic features of Quantity One—such as menu options, display settings, and toolbars—using the *Preferences* dialog box. Select **Preferences** from the *Edit* menu to open this dialog.

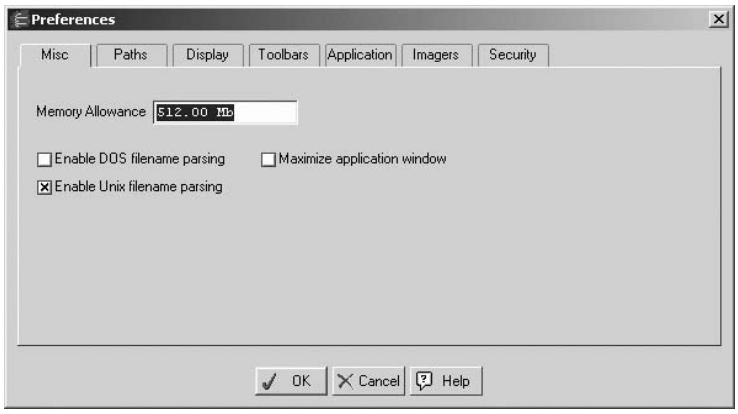


Fig. 2-13. Preferences dialog box.

Click on the appropriate tab to access groups of related preferences. After you have selected your preferences, click on **OK** to implement them.

2.5.a Misc.

Click on the *Misc* tab to access the following preferences.

Memory Allowance

To specify the amount of virtual memory allocated for the application at start-up, enter a value (in megabytes) in the **Memory Allowance** field. The default value of 512 megabytes is recommended. If you receive a warning message that the amount of virtual memory is set too high, you can enter a smaller value in this field. However, this should be considered a temporary fix, and you should consider expanding your hard drive.

Enable DOS Filename Parsing

If this checkbox is selected, for 8-character file names ending in two digits, the final two digits are interpreted as version and exposure numbers. For example, the file name IMAGE-11.1sc would be parsed as IMAGE ver 1 xpo 1.1sc. This is designed to

enable backwards compatibility for users with DOS image files. You should only check this box if you are using these image files.

Enable UNIX Filename Parsing

This is similar to DOS file name parsing. Windows and Macintosh users are unlikely to run into difficulties with UNIX parsing; therefore, this setting is checked by default.

Maximize Application Window

In the Windows version, select the **Maximize Application Window** checkbox to automatically maximize the application window when Quantity One first opens. If this is unchecked, the menu and status bars will appear across the top of the screen and any toolbars will appear “floating” on the screen.

2.5.b Paths

Click on the *Paths* tab to access the following preference.

Temporary File Location

Temporary image files are normally stored in the TMP directory of your The Discovery Series folder. The full path is listed in the field. To change the location of your temporary files, type in a new path or click **Browse** and select a new directory. To return to the default TMP directory, click **Default**.

2.5.c Display

Click on the *Display* tab to access the following preferences.

Zoom %

Zoom % determines the percentage by which an image zooms in or out when you use the **Zoom In** and **Zoom Out** functions. This percentage is based on the size of the image.

Pan %

Pan % determines the percentage by which the image moves side to side or up and down when you use the arrow keys. This percentage is based on the size of the image.

Band Style

Bands in your gel image can be marked with brackets that define the top and bottom boundaries of the band, or they can be marked with a dash at the center of the band. Indicate your preference by clicking on the **Brackets** or **Lines** button. (This setting can be temporarily changed in the *Band Attributes* dialog box. However, all newly opened images will use the preferences setting.)

Auto “Imitate Zoom”

When this checkbox is selected, the magnifying and image positioning commands used in one window will be applied to all open windows. This is useful, for example, if you want to compare the same band of group of bands in different gels; magnify the band(s) in one gel, and the same area will be magnified in all the other gel images.

Note that the images must be approximately the same size.

AutoScale Annotations

Select AutoScale Annotations if you want to display annotations in the image as a percentage of the image window not the image. This allows you to zoom in on an area of the image but have displayed annotations remain the same size in the image window.

Disable hardware Acceleration for 3D Viewer

If you are having problems using the 3D Viewer, install the latest drivers for your graphics card. If, after updating your drivers, you are still having problems, select Disable hardware acceleration for 3D viewer. This allows Quantity One to emulate a graphics driver to render a 3D image.

Note: Disabling hardware acceleration reduces performance for the 3D Viewer.

2.5.d Toolbar

Click on the Toolbar tab to access the following preferences, which determine the behavior and positioning of the secondary toolbars and Quick Guides.

Show Default Quick Guide at Startup

If this checkbox is selected, the *Volumes Quick Guide* will open automatically when you open the program. If you are in Basic mode, the Basic Quick Guide opens instead.

Align Quick Guide with Document

If this checkbox is selected, the Quick Guides will open flush with the edge of your documents. Otherwise, they will appear flush with the edge of the screen.

Guides Always on Top

If this checkbox is selected, Quick Guides will always appear on top of images and never be obscured by them.

Quick Guide Placement and Toolbar Placement

These checkboxes determine on which side of the screen the Quick Guides and toolbars will first open.

Placement Behavior

This setting determines whether a Quick Guide or toolbar will always pop up in the same place and format (**Always Auto**), or whether they will pop up in the last location they were moved to and the last format selected (**Save Prior**).

Toolbar Orientation

These option buttons specify whether toolbars will first appear in a vertical, horizontal, or expanded format when you open the program.

Tool Help Delay and Persistence

Specify the amount of time the cursor must remain over a toolbar icon before the Tool Help appears by entering a value (in seconds) in the **Tool Help Delay** field.

Specify the amount of time that the Tool Help will remain on the screen after you move the cursor off a button by entering a value (in seconds) in the **Tool Help Persistence** field.

2.5.e Application

Click on the Application tab to access the following preferences.

Relative Quantity Calculation

The **Relative Quantity Calculation** option allows you to define how the relative quantities of defined bands in lanes will be determined for all reports, histograms, and band information functions: either as a percentage of the signal intensity of an entire lane or as a percentage of the signal intensity of the defined bands in a lane.

Selecting **% of Lane** means that the total intensity in the lane (including bands and the intensity between bands) will equal 100 percent and the intensity of a band in that lane will be reported as a fraction thereof.

Selecting **% of Bands in Lane** means that the sum of the intensity of the defined bands in a lane will equal 100 percent, and the intensity of an individual band will be reported as a fraction of that sum.

If you create, adjust, or remove bands in a lane with **Relative Quantity** defined as **% of Bands in Lane**, the relative quantities of the remaining bands will be updated.

Relative Front Calculation

The **Relative Front Calculation** option lets you select the method for calculating the relative positions of bands in lanes . This affects the calculation of both Relative Front and Normalized Rf values.

Relative front is calculated by either:

1. Dividing the distance a band has traveled down a lane by the length of the lane (**Follow Lane**). This is useful if your gel image is curved or slanted.

2. Dividing the vertical distance a band has traveled from the top of a lane by the vertical distance from the top of the lane to the bottom (**Vertical**).

Note: "Lane" and "band" refer here to lanes and bands as defined by overlays on the gel image. For example, the top of a lane refers to the beginning of the lane line created in Quantity One, not necessarily the actual gel lane.

Note that if a lane is straight and vertical, both calculation methods will give the same result.

2.5.f Devices

This tab contains preferences for imaging devices and the EXQuest Spot Cutter.

Specify the imaging devices that you want to appear on the File menu. By default, all supported imaging devices are selected; clear the checkboxes of the imagers that you do not want to include on the File menu.

If you have connected the EXQuest Spot Cutter, select it under Spot Cutter. Click Setup spot cutter to run the spot cutter calibration wizard.

Note: If you are changing the spot cutter settings, you need to restart the application before you can perform calibration.

2.5.g Security

The Security tab of the Preferences dialog box contains the security options for CFR mode and the option for enabling Secure mode.

CFR Mode

Setting the Security preferences for CFR mode requires the user have administrator privileges. To locate the Security Preferences, select Preferences from the Edit menu and click the Security tab.

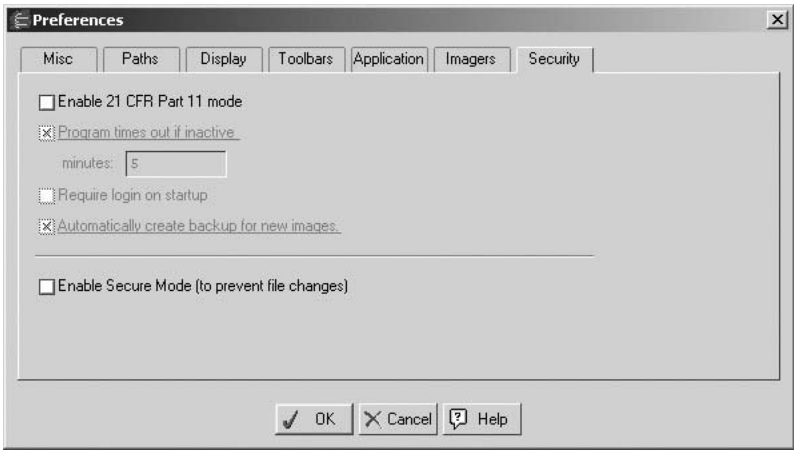


Fig. 2-14. Security Preferences

The following options are available on the Security tab of the Preferences dialog box:

Enable 21 CFR Part 11 mode - If this box is unchecked, all other options are inactive. To activate the other options and CFR mode check this box.

Program times out if inactive - When this box is checked, the user must enter their password to resume using Quantity One. The minutes field indicates the amount of time that must elapse before the application times out.

Require login on startup - Check this box to require the user to enter the user's password when the application opens. This password is the same password as the one used for logging on to Windows. This is an added security measure to prevent unauthorized users from starting Quantity One on a machine where they are not logged in.

Automatically create backup for new images - This option, when selected, creates a backup file when you save a newly acquired or imported image. Locate the backup file in the same folder as the original.

See Appendix K, 21 CFR Part 11, for further information.

Secure Mode

Secure mode is not associated with CFR. However, you can enable Secure mode when CFR is enabled. Secure mode prevents any user from making changes to raw image data on the local machine. If a user opens any image on a machine where Secure mode is enabled, any commands that change the data, such as Subtract background, Filter Wizard, etc. as well as all analysis commands will be inactive (grayed out).

To activate Secure mode, select Secure mode. Once checked, you will be prompted for a new Secure mode password which will be required for disabling Secure Mode.

Note: Secure mode only affects images opened on the local machine. Images stored on a network can still be opened and modified on other machines where Secure mode is disabled.

2.6 User Settings

If Quantity One is on a workstation with multiple users, each user can have his or her own set of preferences and settings.

In multiple-user situations, the preferences and settings are associated with individual user names. Under Windows, your user name is the name you use to log onto the computer. On a Macintosh, your user name is the **Owner Name** on the *File Sharing* control panel.

If you do not log onto your Windows PC or do not have a Owner Name on your Macintosh, then you do not have a user name and your preferences and settings will be saved in a generic file.

3. Viewing and Editing Images

This chapter describes the viewing tools for magnifying and optimizing images. This chapter also describes the tools for cropping, flipping, and rotating images, reducing background intensity and filtering noise, and adding text overlays to images.

These tools are located on the *View*, *Image*, *Window*, and *Edit* menus.

Note: The following chapters contain instructions for analyzing X-ray films, wet and dry gels, blots, and photographs. For the sake of simplicity, these are all referred to as “gels.”

3.1 Magnifying and Positioning Tools

The magnifying and positioning tools are located on the *View* menu and *Window* menu; some of these functions are also found on the main toolbar.

These commands will only change how the image is displayed on the computer screen. *They will not change the underlying data.*

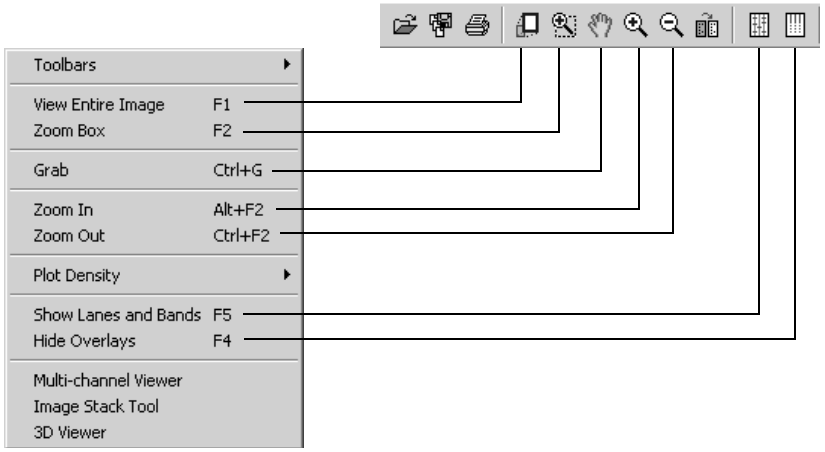


Fig. 3-1. Viewing functions on View menu and main toolbar.

Zoom Box

Use **Zoom Box** to select a small area of the image to magnify so that it fills the entire image window.

Click on the **Zoom Box** button on the main toolbar or select the command from the *View* menu. Then drag the cursor on the image to enclose the area you want to magnify, and release the mouse button. The area of the image you selected will be magnified to fill the entire window.

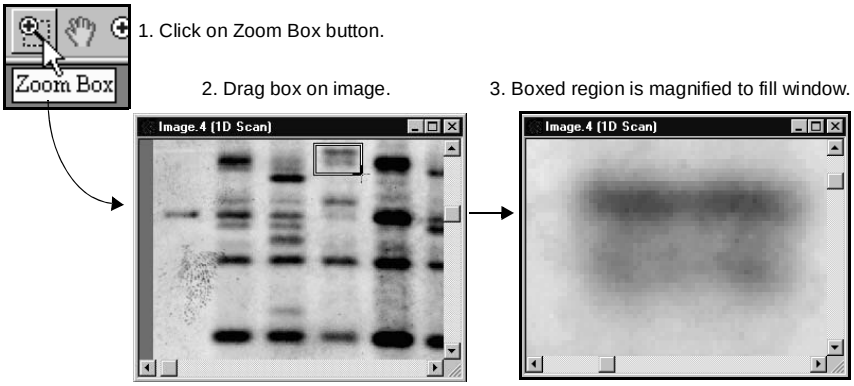


Fig. 3-2. Zoom Box tool.

Zoom In/Zoom Out

These tools work like standard magnifying tools in other applications.

Click on the **Zoom In** or **Zoom Out** button on the main toolbar (or select from the *View* menu). The cursor will change to a magnifying glass. Click on an area of the image to zoom in or out a defined amount, determined by the setting in the *Preferences* dialog (see section 2.5, Preferences).

Grab

This tool allows you to change the position of the image in the image window. Select **Grab** from the main toolbar or *View* menu. The cursor will change to a "hand" symbol. Drag the cursor on the image to move the image in any direction.

Arrow Keys

You can also move the image inside the image window by using the **Arrow** keys on the keyboard. Click on an arrow button to shift the image incrementally within the window. The amount the image shifts is determined by the **Pan %** setting in the *Preferences* dialog (see section 2.5, Preferences).

View Entire Image

If you have magnified part of an image or moved part of an image out of view, select **View Entire Image** from the main toolbar or *View* menu to return to the original, full view of the image.

Centering an Image

You can center the image window on any point in an image quickly and easily using the **F3** key command. This is useful if you are comparing the same region on two gel images and want to center both image windows on the same point.

Position the cursor on the point on the image that you want at the center of the image window, then press the **F3** key. The image will shift so that point is at the center of the image window.

Imitate Zoom

To magnify the same area on multiple images at the same time, use the **Imitate Zoom** command on the *Window* menu.

First, adjust the magnification in one of the images. Then, with that image window still selected, select **Imitate Zoom**. The zoom factor and region of the selected image will be applied to all the images.

Note: **Imitate Zoom** only works on images with similar dimensions.

Tiling Windows

If you have more than one image open, the **Tile** commands on the *Window* menu allow you to arrange the images neatly on the screen.

Select **Tile** to resize all the windows and arrange them on the screen left to right and top to bottom.

Select **Tile Vertical** to resize all windows and arrange them side-by-side on the screen.

Select **Tile Horizontal** to resize all windows and stack them top-to-bottom on the screen

3.2 Density Tools

The density tools on the *View* > *Plot Density* submenu and the *Density Tools* toolbar are designed to provide a quick measure of the data in a gel image.

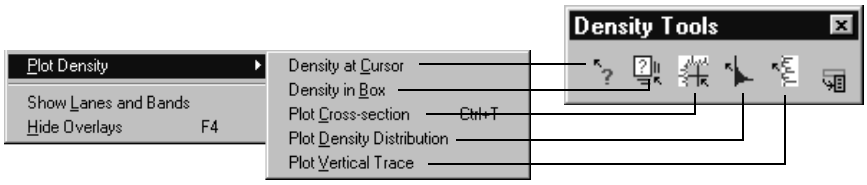


Fig. 3-3. Density tools on the menu and toolbar.

Note: The density traces will be slightly different than the traces for functions such as **Plot Lane** or **Plot Band**, because the sampling width is only one image pixel.

Density at Cursor

Select **Density at Cursor** and click on a band or spot to display the intensity of that point on the image. It also shows the average intensity for a 3 x 3 pixel box centered on that point.

Density in Box

Select **Density in Box** and drag a box on the image to display the average and total intensity within the boxed region.

Plot Density Distribution

Select **Plot Density Distribution** to display a histogram of the signal intensity distribution for the part of the image displayed in the image window. The average intensity is marked in yellow on the histogram.

The histogram will appear along the right side of the image. Magnify the image to display the data for a smaller region.

Plot Cross-section

Select **Plot Cross-section** and click or drag on the image to display an intensity trace of a cross-section of the gel at that point. The horizontal trace is displayed along the top of the image, and the vertical trace is displayed along the side of the image.

The intensity at the point you clicked on is displayed, as is the maximum intensity along the lines of the cross-section.

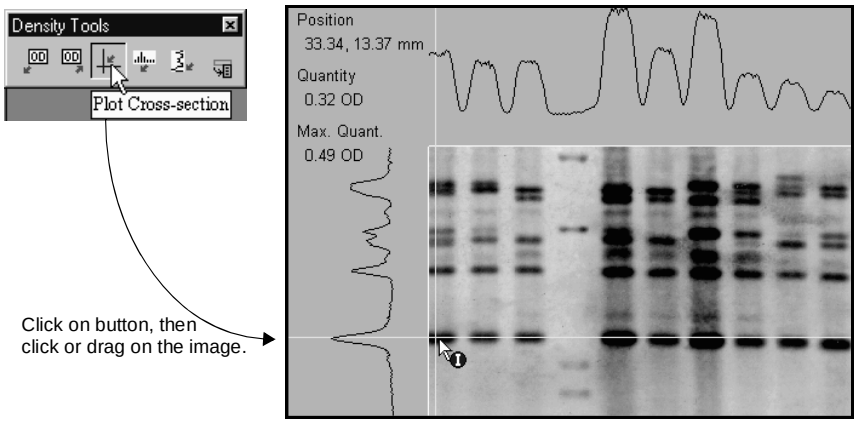


Fig. 3-4. Plot Cross-section tool.

Plot Vertical Trace

Select **Plot Vertical Trace** and click or drag on the image to plot an intensity trace of a vertical cross-section of the image centered on that point.

3.3 Showing and Hiding Overlays

To conceal all plots, traces, info boxes, and overlays on an image, select **Hide Overlays** from the main toolbar or *View* menu.

Note: Click once on **Hide Overlays** to conceal the overlays. Click twice to deassign any function that has been assigned to the mouse.

To redisplay the lane and band overlays, select **Show Lanes and Bands** from the *View* menu or main toolbar.

3.4 Multi-Channel Viewer

The *Multi-Channel Viewer* can display different types and levels of fluorescence in a gel that has been imaged at different wavelengths. You can merge the data from up to three different images of the same gel.

Note: The gel images being compared must be exactly the same size. When changing image filters, be careful not to move the gel. If the images are not exactly the same size, you can use the **Crop** tool (see Section 3.9.a, Cropping Images) to resize them.

With at least one image open, select **Multi-Channel Viewer** from the *View* menu. The first open image will be displayed in the viewer window using the **Red channel**, and the image name will be displayed in the field at the top of the viewer.

Fig. 3-5. Multi-Channel Viewer.

Note: The color channel used to display the image in the viewer has no relation to the filter used to capture the image. The red, green, and blue channels are only used to distinguish different images.

To add another image to the viewer, make sure the image is open and click on the pull-down button next to the **Green** or **Blue** name field. Select the image name from the pull-down list. Add a third image using the same procedure.

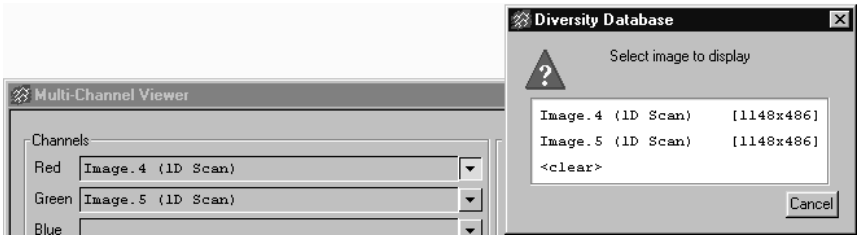


Fig. 3-6. Selecting images to display in the viewer.

To reassign the different images to different channels, use the pull-down buttons to the right of the name fields. Select **<clear>** from the pull-down list to remove an image from that channel of the viewer.

Viewing Options

To remove a particular color channel from the display, click in the checkbox associated with that channel to deselect it.

Select the **Auto-Scale Image When Assigned** checkbox to automatically adjust the brightness and contrast of each loaded image based on the data range in the image. This invokes the **Auto-scale** command from the *Transform* dialog (see section 3.8, Transform) when an image is first opened in the viewer. Note that this setting affects only how the image is displayed in the viewer, not the actual data.

Note: If you deselect this checkbox, any images currently displayed will remain auto-scaled. Click on the **Transform** button in the viewer and click on the **Reset** button in the *Transform* dialog to undo auto-scaling.

Buttons for various viewing tools are included in the *Multi-Channel Viewer*. Tools such as **Zoom Box** and **Grab** will change the display of all the images in the viewer at once.

Click on the **Transform** button to open the *Transform* dialog. In the dialog, you can adjust the display of each channel independently by selecting the appropriate channel option button. Similarly, the **Plot Cross-section** command will report the intensity of each channel separately.

Exporting and Printing

Click on the **Export** button to export a 24-bit TIFF image of the merged view. This will open a version of the *Export to TIFF* dialog (see section 13.6, Export to TIFF Image). Note that you cannot export data from the *Multi-Channel Viewer*—only the current view of the image (designated as **Publishing Mode** in the *Export* dialog). The colors in the viewer will be preserved in the exported TIFF image.

To print a copy of the merged view to a color or grayscale printer, click on the **Print** button.

3.5 3D Viewer

The *3D Viewer* allows you to see a three-dimensional rendering of a portion of your image. This is important for such instances as determining whether a selected band is actually two or more separate bands.

To see a 3D rendering of a portion of your image, select 3D Viewer from the View menu. Your curser turns into a crosshair. Click and drag your curser over the image area you would like to view creating a box.

Note: viewing a large area of your image may reduce performance.

- To reposition the box, position your cursor at the center of the box. The cursor appearance will change to a multidirectional arrow symbol. You can then drag the box to a new position.
- To resize the box, position your cursor on a box corner. The cursor appearance will change to a bi-directional arrow. You can then drag that corner in or out, resizing the box.
- To redraw the box, position your cursor outside the box and click once. The box disappears, and you can then draw a new box.

To view the selected area, position your cursor inside the box slightly off-center. The cursor appearance will change to an arrow. Click once to open the 3D Viewer.

Note: If you are having problems using the 3D Viewer, install the latest drivers for your graphics card. If, after updating your drivers, you are still having problems, go to the Display tab of the Preferences dialog box and select Disable hardware

acceleration for 3D viewer. This allows Quantity One to emulate a graphics driver to render a 3D image. See Section 2.5.c, Display, for further information.

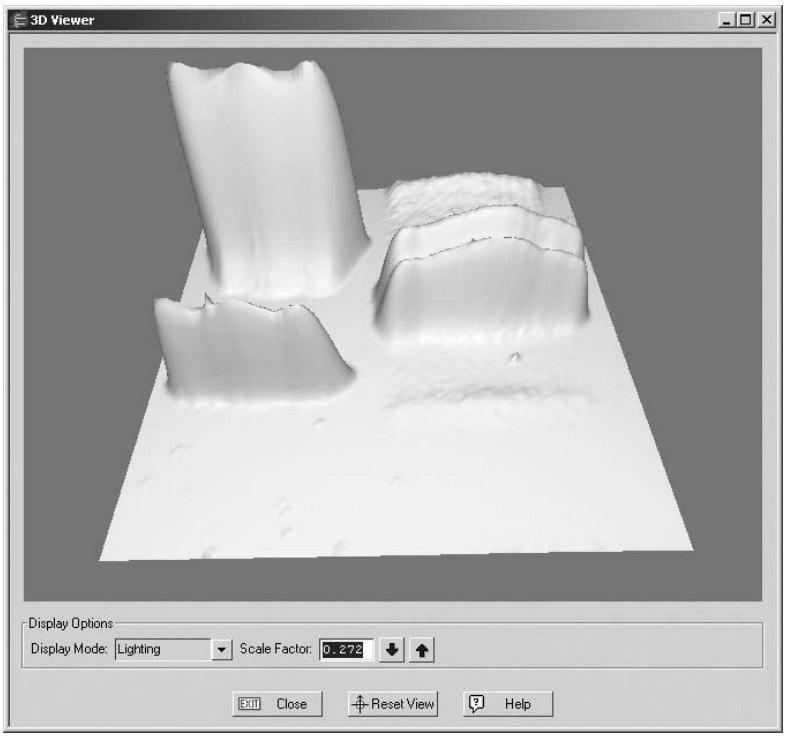


Fig. 3-7. 3D Viewer

3.5.a Positioning the Image

Windows

For Windows operating systems, use your mouse to reposition and rotate the image.

- **Rotate the image** - Left click and drag to rotate the image.
- **Reposition the image** - Right click and drag to reposition the image.
- **Zoom in/out** - To zoom in or out, Click the center mouse button or roll the wheel. If you do not have a three button mouse or a mouse with a wheel, hold down the shift key and left click and drag to zoom in or out.

Macintosh

For Macintosh operating systems, use mouse and keyboard combinations to reposition and rotate the image.

- **Rotate the image** - Click and drag to rotate the image.
- **Reposition the image** - Ctrl>click and drag to reposition the image.
- **Zoom in/out** - Shift>click and drag to zoom in or out.

3.5.b Display Mode

The 3D Viewer window allows you to view the image in three different modes; wire frame, lighting, and textured.

- Wire-frame shows the image in a transparent frame view.
- Lighting shows the image with different areas of light and shadow depending on the angle of view. Use the slider bar to adjust the intensity of the lighting.
- Texture gives the image texture.

Use the Scale function to scale the image. This is useful for viewing shallow spots in the 3D Viewer.

If you lose the image because you moved it too far past the window border, or rotated it and disoriented the view, click Reset View to return the image to the original view.

Note: Reset view does not change the scale factor. To reset the scale factor, close the 3D Viewer and click the box again to re-open the 3D Viewer with the original scale factor.

3.6 Image Stack Tool

Use the *Image Stack Tool* to scroll through a series of related gel images. You can easily compare bands that appear, disappear, or change size in different gels run under different conditions.

Note: The images should be close to the same size, with bands in the same relative positions. You can use the **Crop** tool to resize images.

With all the images open, select **Image Stack Tool** from the *View* menu. The *Image Stack Tool* window will open.

Fig. 3-8. Image Stack Tool.

In the *Image Stack Tool* window, all open gels are listed in the field to the right of the display window. To select an image to display, click on a gel name. The name will appear highlighted with an arrow and the image will appear in the window.

Click on another gel name to display that image.

Buttons for various viewing tools are aligned next to the *Image Stack Tool* window. These commands will change the display of all the images in the stacker at once (e.g., magnifying one image will magnify the same relative area in all the images).

Using the controls below the list of names, you can reorder the images and/or scroll through them in the stacker.

Reordering Images

To reorder the images in the stacker, first select an image name in the list, and then click on the **Move displayed image** arrow buttons to move it up or down in the list.

Image Playback

Using the controls under **Playback**, you can scroll through the images in the stacker.

First, highlight some or all of the gel names using **Shift-click** or **Ctrl-click** key commands. With multiple images selected, the **Step** arrow buttons become active. Click on the arrow buttons to scroll through the list of selected gels.

Alternatively, click on the **Auto** checkbox next to the arrow buttons to begin automatically scrolling through the list. You can adjust the auto-scroll speed using the **Slow/Fast** slider.

3.7 Colors

Select **Colors** from the *Edit* menu to open a dialog in which you can adjust the colors of the image, as well as windows, buttons, overlays, and other features of the display.

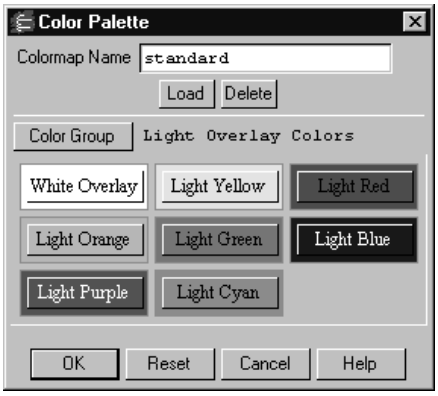


Fig. 3-9. Colors dialog.

Selecting a Color Group

In the *Colors* dialog, click on the **Color Group** button to select the colors of a particular group of objects (e.g., pop-up boxes, image colors, etc.).

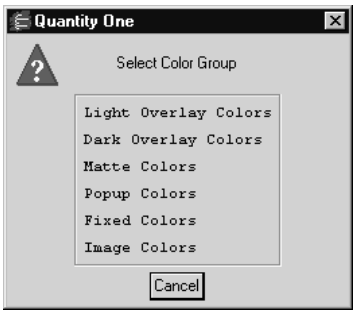


Fig. 3-10. List of Color Groups.

Click on a color group in the list to select it.

Changing a Color

After you have selected the color group to change, click on the specific color button. In the *Color Edit* dialog, adjust the RGB values of the color you selected.

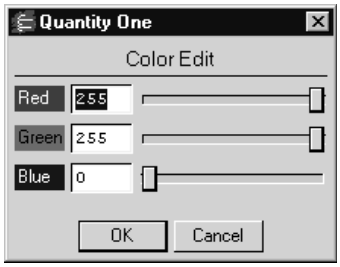


Fig. 3-11. Color Edit dialog.

Saving/Selecting a Defined Set of Colors

After you have changed the colors within color groups, you can save these settings for future use on other images. The **Colormap Name** field displays the name of a defined set of colors and color groups. There are several predefined colormaps, or you can create your own.

To select a predefined colormap, click on the **Load** button.

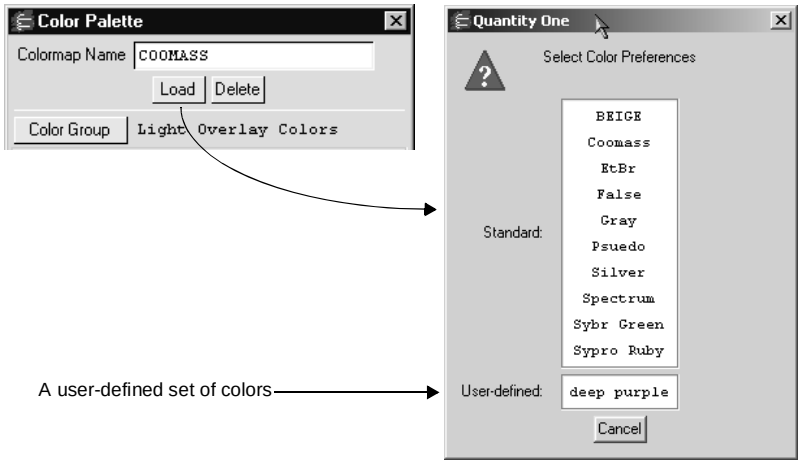


Fig. 3-12. Selecting a Colormap.

From the list displayed, click on the set of colors you want to apply.

To create your own colormap, adjust the colors within the color groups as described above and type in a new colormap name. Click on **OK** to apply the changes.

To remove a colormap, click on the **Delete** button. Select the colormap to be deleted from the displayed list. A pop-up box will ask you to confirm the deletion.

To return to the **Standard** colormap, click on the **Reset** button. All colors will reset to their default values.

3.8 Transform

Use the *Transform* dialog to adjust the image brightness and contrast and optimize the image display. These controls affect the image display only, and *will not change* the underlying data.

With an image open, select **Transform** from the *Image* menu or main toolbar.

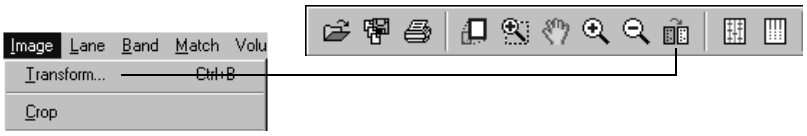


Fig. 3-13. Transform command.

The *Transform* dialog contains a Preview Window, a Frequency Distribution histogram, a Transform Plot, and three main methods of optimizing the image: **Auto-scale**, **High** and **Low** sliders, and a **Gamma** slider. You can use these controls to adjust the way the software transforms raw image data into the visual display.

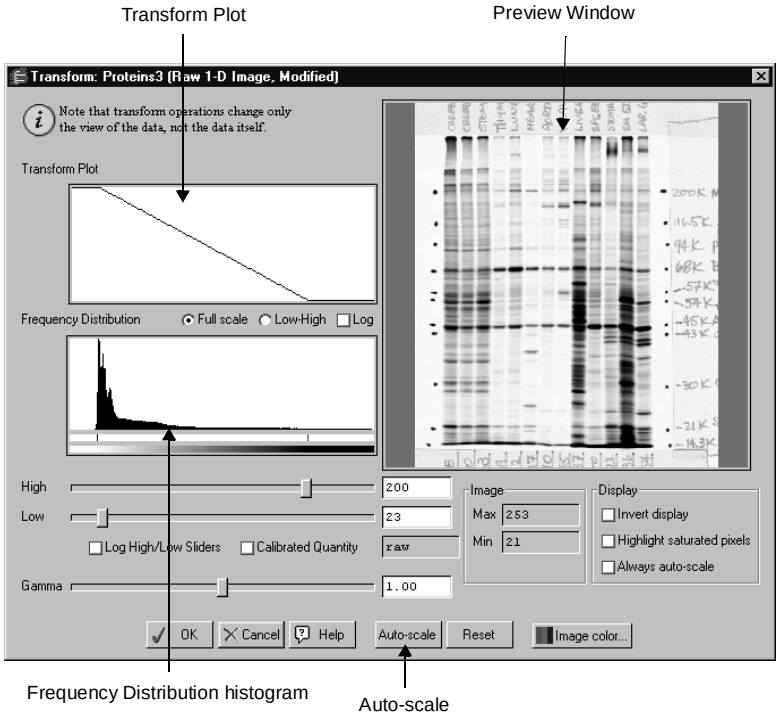


Fig. 3-14. Transform dialog.

3.8.a Transform Subwindows

Preview Window

The Preview Window shows a smaller view of the same image that is displayed in the main image window. Changes in the controls are automatically reflected in the Preview Window. They are only applied to the main image when you click on **OK**.

You can use viewing tools such as **Zoom Box, Grab** and **View Entire Image** in the Preview Window just as you can in the main image window, to focus on particular regions of interest.

Frequency Distribution Histogram

The Frequency Distribution histogram shows the total data range in the image and the amount of data at each point in the range. In a typical scan, there is a signal spike at the left ("gray") end of the histogram due to background noise.

Transform Plot

The Transform Plot is a logarithmic representation of how the raw pixel data are mapped to the pixels of the computer screen.

3.8.b Transform Controls

Auto-scale

Click on the **Auto-scale** button to optimize the image automatically. The lightest part of the image will be set to the minimum intensity (e.g., white), and the darkest will be set to the maximum intensity (e.g., black). This enhances minor variations in the image, making fine details easier to see. You can then "fine-tune" the display using the **High, Low**, and **Gamma** sliders described below.

High/Low Sliders

If **Auto-scale** doesn't give you the appearance you want, use the **High** and **Low** sliders to redraw the image manually. Drag the **High** slider handle to the left to make weak signals appear darker. Drag the **Low** slider handle to the right to reduce background noise.

As you drag the sliders, the slider markers on the Frequency Distribution histogram will move. Everything to the left of the **Low** marker will be remapped to minimum intensity, while everything to the right of the **High** marker will be remapped to maximum intensity. Using the histogram, you can position the markers at either end of the data range in the image, and use the low slider to cut off the "spike" of background noise.

You can also type specific **High** and **Low** values in the text boxes next to the sliders. Click anywhere on the slider bars to move the sliders incrementally.

Log High/Low Sliders changes the feedback from the slider handles, so that when you drag them, the slider markers move a shorter distance in the histogram. This allows for finer adjustments when the data is in a narrow range.

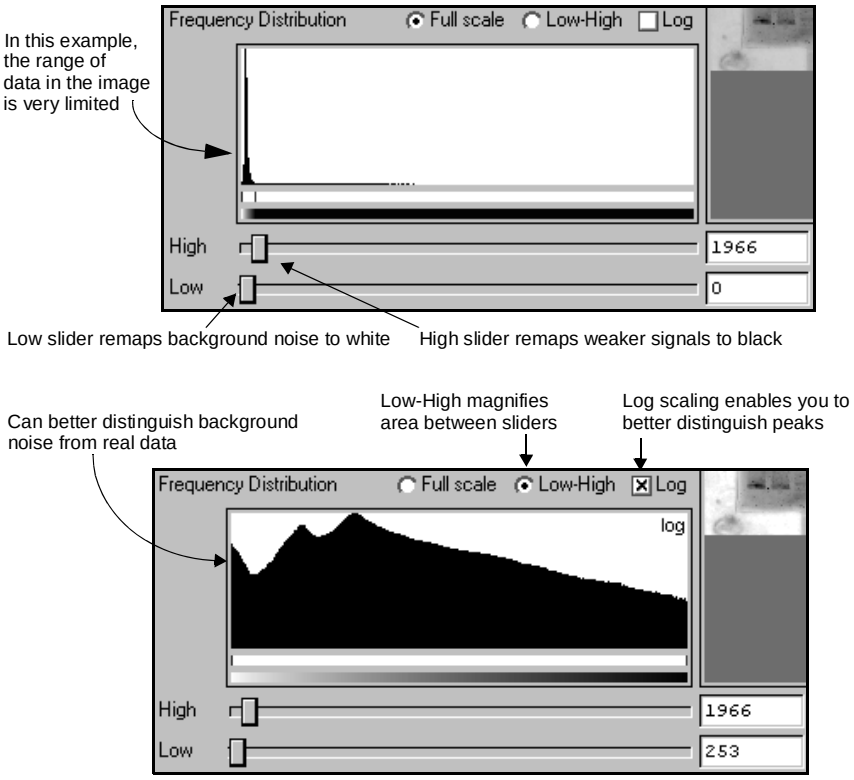


Fig. 3-15. Two views of the Frequency Distribution histogram.

Gamma Slider

Some images may be more effectively visualized if their data are mapped to the computer screen in a nonlinear fashion. Adjust the **Gamma** slider handle to expand or compress the contrast range at the dark or light end of the range. This is reflected in the Transform Plot and Preview Window.

3.8.c Other Features

Full Scale and Low-High

The **Full Scale** and **Low-High** option buttons adjust how the range of data in the image is displayed in the Frequency Distribution histogram and Transform Plot. They do not change how the data is displayed in the image window.

Select **Full Scale** to adjust the displays so that they show the full intensity range of the image.

Select **Low-High** to magnify the range between the **Low** and **High** sliders. This makes it easier to view the data if it does not occupy the full intensity range of the image.

Log

The **Log** checkbox changes the way the data is displayed in the histogram so you can better discern subtle changes in signal intensity.

Image Max/Min and Units

Image **Max** and **Min** display the range of intensity in the gel image.

The image units are determined by the type of scanner used to create the image. For images measured in O.D.s, you can display the maximum and minimum O.D values in the image by selecting the **Calibrated Quantity** checkbox. If this box is not selected, the maximum and minimum numeric pixel values are displayed.

Image Color

Click on this button to open a list of color maps, which you can define using the **Colors** command on the *Edit* menu (see section 3.7, Colors). Select a color map from the list to change the image in both the *Transform* window and the image window.

Invert Display

Select the **Invert Display** checkbox to change light bands on a dark background to dark bands on a light background, and visa versa. The image data will not change—only the display.

Highlight Saturated Pixels

Select the **Highlight Saturated Pixels** checkbox to highlight areas of saturation in the image in red.

Always Auto-Scale

Select this checkbox to automatically **Auto-Scale** every new image that you open. The software will examine the data range in every image and optimize it accordingly. This setting disables the other image-optimization controls in the *Transform* dialog.

Reset

To return to an unmodified view of the image, click on **Reset**.

3.9 Resizing and Reorienting Images

The *Image* menu and toolbar contain commands for changing the size and orientation of images.

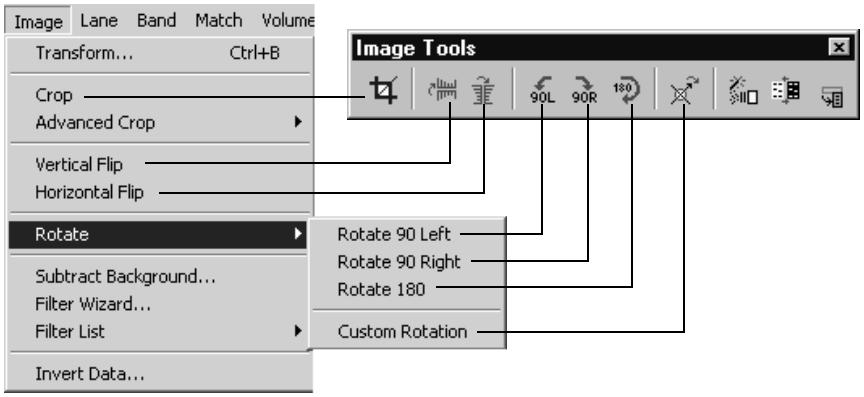


Fig. 3-16. Resizing and reorienting tools.

Note: Many of these commands will permanently change the image. You will be prompted to confirm any permanent changes.

3.9.a Cropping Images

Use the **Crop** tool to eliminate unwanted parts of an image, such as border space around the gel. You can also use this command to reduce the file size of an image.

Select **Crop** from the *Image* menu or toolbar. The cursor will change to a Crop symbol.

Define the region to be cropped by dragging the cursor across the image, creating a box. Everything outside the box will be deleted.

The dimensions of the crop area (in millimeters and number of pixels) and the file size of the image inside the crop area are listed at the bottom of the crop box.

1. To *reposition* the crop box, position the cursor at the center of the box. The cursor will change to a multidirectional arrow. Then drag the box to a new position.

2. To *resize* the box, position the cursor on a box border line or corner. The cursor will change to a bidirectional arrow. You can then drag the border or corner in or out, resizing the box.

3. To *redraw* the box, position the cursor outside the box. The cursor will change back to the Crop tool, and you can redraw the box.

After you are satisfied with the size of crop box, position the cursor inside the box slightly off-center. The cursor will change to a scissors symbol. Then click to perform the crop.

A pop-up box will prompt you to: (1) crop the original image, (2) crop a copy of the original image, or (3) cancel the operation.

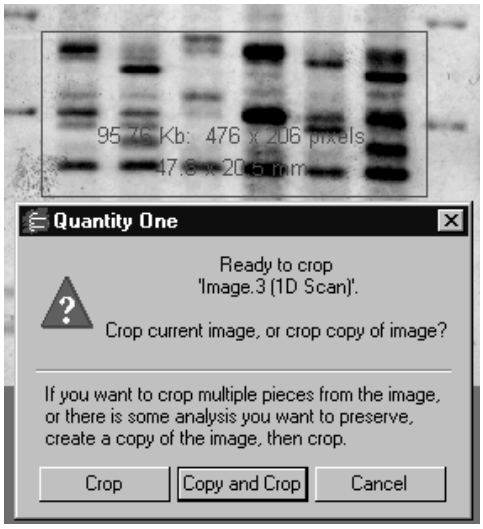


Fig. 3-17. Crop box and pop-up Crop dialog.

If you click on the **Copy and Crop** button, you will be prompted to enter the name and version number of the image copy before cropping.

Advanced Crop Commands

To ensure that your scans are exactly the same size and shape, you can use the tools on the Image > Advanced Crop submenu to save the crop box from one scan and apply it to others. These tools also allow you to crop a gel of the same scan.

To define and save a crop box and apply it to another scan:

1. Select Define Crop Area from the submenu and drag a crop box on an image. Position the box as described in Cropping Images.
2. Select Place Crosshair from the submenu and click a landmark inside the box that is present in all the gels you want to crop. This could be a spot or some other image detail. The crosshair will make it easier to position the box in the other images so that it encloses the same area.
3. Select Save Crop Settings from the submenu, enter a name for the current crop settings in the pop-up box, and click Apply.
4. Complete the crop action in the current image by positioning your cursor inside the box slightly off-center and clicking to perform the crop, as described in Cropping Images.
5. Open or select the next image you want to crop, select Load Crop Settings from the submenu, and select the name of the settings you saved. The crop box and crosshair will appear on the image.
6. Reposition the crop box so that the crosshair is correctly aligned with the appropriate image object, then complete the crop as described in step 4 above.

To delete any crop settings you have saved, select Delete Crop Settings from the submenu and choose the settings to be deleted from the list.

3.9.b Flipping and Rotating Images

Use the image flipping and rotating commands to reorient lanes and bands for proper analysis.

Note: These actions will erase any analysis you have performed on the image. You will be prompted to confirm the changes.

Flipping

To flip the image right-to-left, select **Horizontal Flip** from the *Image* menu or toolbar. To flip the image top-to-bottom, select **Vertical Flip**.

90° Rotations

Select **Rotate 90 Left**, **Rotate 90 Right**, or **Rotate 180** from the *Image* > *Rotate* menu or *Image* toolbar to perform the specified rotation.

Custom Rotation

Use the **Custom Rotation** command to rotate the image in increments other than 90°. Select **Custom Rotation** from the *Image* > *Rotate* submenu or *Image* toolbar. A green “plus” sign will appear next to the cursor. Click on the image and a circular overlay with an orange arrow will appear. A pop-up box will indicate the angle of rotation in degrees and radians.

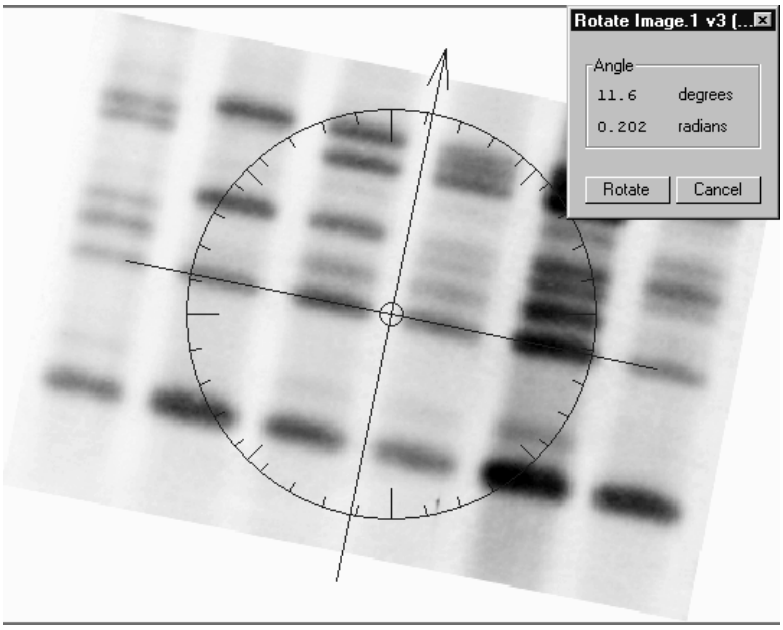


Fig. 3-18. Custom rotation; the arrow points in the direction of the new top of the image.

To perform the rotation, position the cursor on the arrowhead and drag. As you drag, the arrow will rotate and the angle in the box will change. Position the arrow so that it points in the direction of the new top of the image.

Note: To center the arrow on a particular point on the image (e.g., to align along a particular lane), position the cursor on the point and press the F3 key. The center of the arrow will shift to the new position.

To complete the rotation, click on the **Rotate** button in the pop-up box. Another window will open containing the rotated image, and you will be prompted to save this image under a new name or version number.

If you are not satisfied with the rotated image, close the window without saving and start over.

Note: Because an image is composed of square or rectangular pixels, **Custom Rotation** performs some minor smoothing on the image for rotations other than 90^{o}. Also, any analysis performed on the image will be lost.

3.10 Whole-Image Background Subtraction

Image background due to gel opacity, random signal noise, or other factors can interfere with quantitation and data analysis. Quantity One has several tools for subtracting background intensity from gel images. This section describes whole image background subtraction. You can also subtract background from individual lanes (see section 4.2, Lane-Based Background Subtraction) and bands (see section 7.6, Volume Background Subtraction).

Whole-image background subtraction is useful for reducing background resulting from the opacity of the carrier medium (film, gel matrix, or blot matrix) or film fogging.

Note: Whole-image background subtraction permanently changes the image. You will be prompted to confirm the change.

Select **Subtract Background** from the *Image* menu.

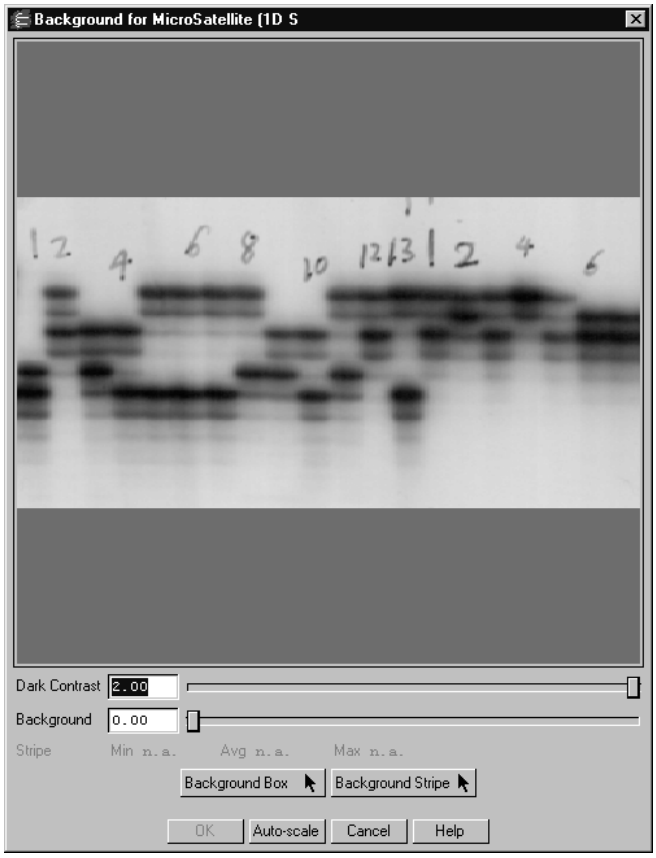


Fig. 3-19. Subtract Background dialog.

The *Subtract Background* dialog has a preview window, which contains a smaller view of the image. Changes in the subtract background controls are reflected in the preview window and are only applied to the main image when you click on **OK**.

Auto-scale

Click on the **Auto-scale** button to automatically adjust the **Dark Contrast** and **Background** settings to optimal levels. You can then manually adjust these settings using the other controls.

Dark Contrast Slider

Use the **Dark Contrast** slider to reveal the level of background in the image before subtraction. This slider is similar to the **High** slider in the *Transform* dialog. Drag the slider handle to the left to make faint signals appear stronger. Click on the slider bar to move the slider incrementally, or type a value into the field next to the slider.

Note: The **Dark Contrast** slider itself does not eliminate background intensity; therefore, the **OK** button will not activate if you only adjust this slider. If you want to adjust the display contrast *without* subtracting background, use the **Transform** command (see section 3.8, Transform).

Before adjustment: the image seems clear of background

After adjustment: background levels are more apparent

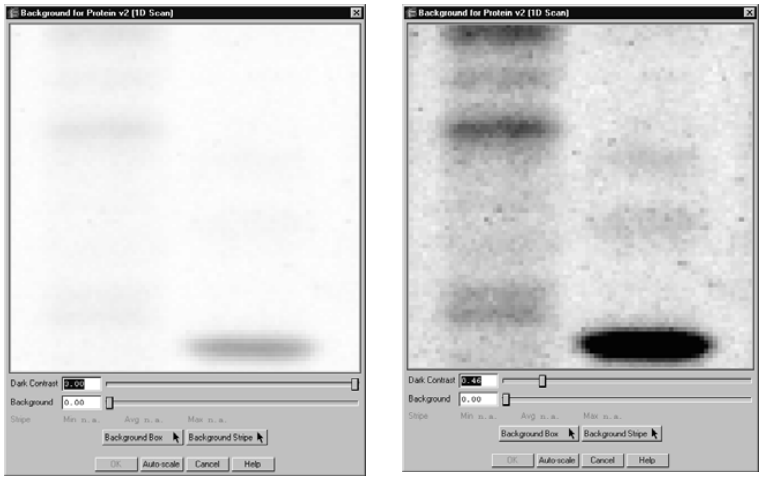


Fig. 3-20. Dark Contrast adjustment reveals true levels of background in the image.

Background Slider

To manually adjust the background subtraction levels, drag the **Background** slider to the right. You can also move the slider incrementally by clicking on the slider bar, or type a specific value into the field next to the bar.

Objects with signal intensities lower than the subtraction level will be eliminated from the image when you click on **OK**.

Background Box

Use the **Background Box** function to define a background area in the gel that is representative of the background in the entire image. This method of subtraction is useful for images with uniform backgrounds.

Click on the **Background Box** button, then drag on a background area of the image. The average intensity of the pixels in the box will be used as the background level to be subtracted from the entire image.

Background Stripe

The **Background Stripe** function is useful for gels in which the background changes from top to bottom (e.g., gradient gels).

Click on the **Background Stripe** button, then drag on a background region to create a rectangular box down the length of the image. The average intensity of each horizontal row of pixels in the stripe will be subtracted from each pixel in that row across the entire gel. This way, if the image has more background at the bottom than at the top, more background will be removed from the lower regions of the image.

Note: Make sure that the background stripe runs the entire length of the lanes down the gel. The average of the topmost row in the stripe will be subtracted from all rows above the stripe, and the average of the bottommost row will be subtracted from all rows below.

The minimum and maximum intensities in the stripe are displayed next to the **Min** and **Max** labels in the box. Also, the average intensity value for the entire stripe is displayed next to **Avg**.

Completing the Subtraction

When you are satisfied with the background subtraction shown in the preview image, click on **OK**. Because whole-image background subtraction is irreversible, you will be prompted to subtract from the original image, subtract from a copy, or cancel the operation.

If you choose **Copy and Subtract**, enter the name and/or version number for the new copy in the pop-up box and click on **OK** to complete the command.

3.11 Filtering Images

Filtering is a process that removes small noise features on an image while leaving larger features (e.g., bands) relatively unaffected. A wide range of filters are available for removing different types of noise from images. Depending on the nature of your data, you will probably need to use only one or two of the available filters. However, you should experiment with several different filters before selecting the ones that work best for your images.

The filtering commands are located on the *Image* menu and toolbar.

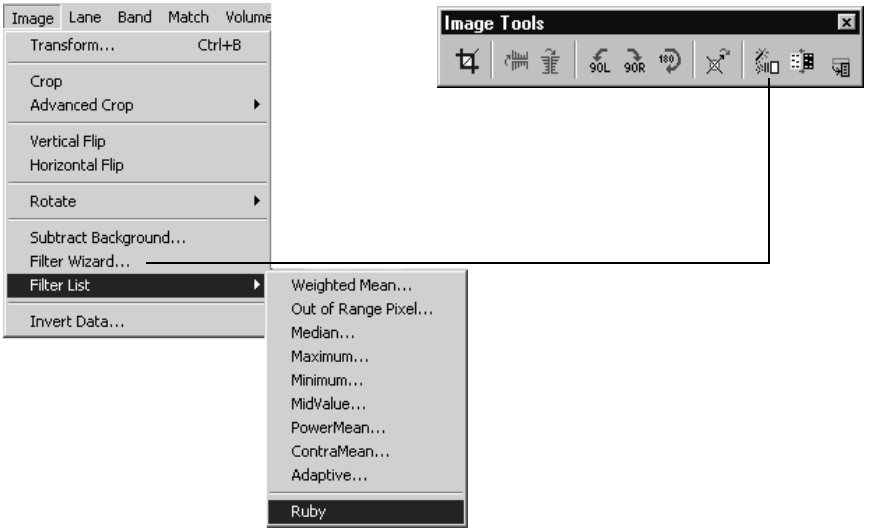


Fig. 3-21. Filtering commands.

Note: Since filtering is an irreversible process, you will be asked if you want to create a copy of the original image before you filter. If you are experimenting with various filters, you should create copies of the image and compare them side-by-side. If you filter the original image and save it, *you cannot return to the original, unfiltered state*.

3.11.a Filter Wizard

The *Filter Wizard* is designed to guide you through the filter selection process. First, you identify the type of noise in the image. Next, select the size of the filter to use on that noise. Finally, filter the image.

Select **Filter Wizard** from the *Image* menu or toolbar to open the dialog.

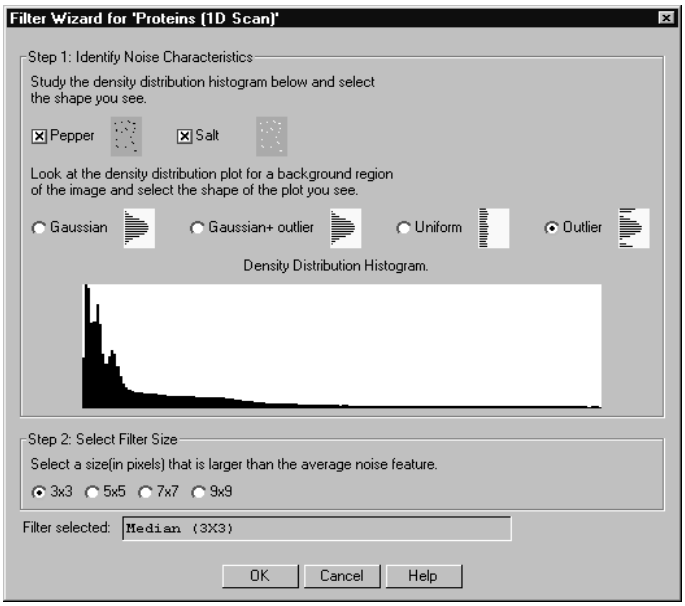


Fig. 3-22. Filter Wizard dialog.

The dialog contains settings for identifying the different types of noise in the image. It also includes a density distribution histogram of the noise in the image to aid in filter selection.

Step I: Identify Noise Characteristics

The first step is to identify the type of noise in the image. Examine both the image and the density distribution histogram, then select one, both, or neither of the following checkboxes:

- **Salt.** This type of noise appears as specks that are lighter than the surrounding background. The density distribution histogram of this type of noise displays noise peaks at the high end of the range (right end of the plot). This type of noise is common in electronic cameras with malfunctioning pixels. It can also be

caused by dust or lint in the imaging optics or scratches on photographic film. Salt is a type of outlier noise (see below).

- **Pepper.** This type of noise appears as specks that are darker than the surrounding background. The distribution histogram of this type of noise displays noise peaks at the low end of the range (left end of the plot). Its causes are similar to those of salt noise. Pepper is a type of outlier noise (see below).

Next, select one of the following option buttons to describe additional features of the noise.

- **Gaussian.** The distribution histogram of this type of noise has a Gaussian profile, usually at the bottom of the data range. This type of noise is usually an electronic artifact created by cameras and sensors, or by a combination of independent unknown noise sources.
- **Uniform noise.** This type of noise appears in the histogram as a uniform layer of noise across the data range of the image.
- **Outlier noise.** This category of noise includes salt and pepper noise (see above). The distribution histogram of this type of noise displays noise peaks at the high and low ends of the range.

After you have identified the type of noise, go to the next step.

Step 2: Select Filter Size

Image noise is filtered by means of a filtering window (or kernel), which is measured in pixels. This filtering window slides across the image, processing the pixels within it.

The available filter dimensions range from **3 x 3** to **9 x 9** pixels. To select an appropriate size, magnify a background region of the image so that you can see the individual pixels. The filter size you select should be larger than the average noise feature but smaller than the data features.

Note: A smaller filter will alter the image less than a larger filter. Large filters can result in better suppression of noise, but can also blur desirable features in the image.

Step 3: Begin Filtering

After you have completed the selections, the filter name and size will be displayed at the bottom of the *Filter Wizard* dialog.

To being filtering, click on the **OK** button. Because filtering is an irreversible process, you will be prompted to filter the original image, filter a copy of the image, or cancel the operation.

If you choose **Copy and Filter**, enter a name and/or version number for the new copy in the pop-up box and click on **OK**.

3.11.b Selecting a Filter Directly

If you know the type and size of filter you want, you can select it directly from the *Image > Filter List* submenu. The submenu includes all the available filters.

The types of filters are:

- **Weighted Mean.** This filter is useful for reducing Gaussian noise. It calculates the weighted mean of the pixels within the filtering window and uses it to replace the value of the pixel being processed.
- **Out of Range Pixel.** This filter is useful for suppressing salt-and-pepper noise; its effect on Gaussian noise is minimal. This filter calculates the mean of the pixel values in the filtering window, including the pixel being processed. If the difference between the mean and the individual pixel value is above a certain threshold, then the individual value is replaced by the mean.
- **Median.** Also useful for suppressing salt-and-pepper noise, this filter calculates the median value of the pixels within the filtering window and uses it to replace the value of the pixel being processed. The median filter produces very little blurring if a small-sized window is selected.
- **Maximum.** This filter is useful for eliminating pepper noise in an image (it worsens the effect of salt noise). It replaces the value of the pixel being processed with the maximum value of the pixels within the filtering window.
- **Minimum.** This filter replaces the value of the pixel being processed with the minimum pixel value within the filtering window. This filter is useful for eliminating salt noise in an image (it worsens the effect of pepper).
- **MidValue.** This filter is useful for suppressing uniform noise within an image; however, it worsens the effect of pepper and salt. This filter replaces the value of

the pixel being processed with the mean of the maximum and minimum pixel values within the filtering window.

- **PowerMean.** This filter is useful for suppressing salt and Gaussian noise within an image (it worsens the effect of pepper noise). It replaces the value of the pixel being processed with the power mean of the pixel values within the filtering window.
- **ContraMean.** This filter is useful for suppressing pepper and Gaussian noise within an image (it worsens the effect of salt). It replaces the value of the pixel being processed with the contra-harmonic mean of the pixel values within the filtering window.
- **Adaptive.** This filter is useful for suppressing Gaussian noise and salt and/or pepper within an image. If the image contains a mix of salt and pepper, select this filter.

To begin filtering, select a filter type from the pull-down list. A pop-up box will prompt you to select a filter size.

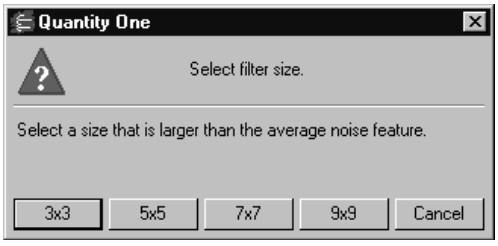


Fig. 3-23. Selecting a filter size.

Click on a button to select a size. (See, Step 2: Select Filter Size for guidance.) Because filtering is an irreversible process, you will be prompted to filter the original image, filter a copy of the image, or cancel the operation.

If you choose **Copy and Filter**, enter a name and/or version number for the new copy in the pop-up box and click on **OK**.

3.12 Invert Data

The **Invert** checkbox in the *Transform* dialog (see “Invert Display” on page 23) inverts the display of the image. However, in some cases you may need to invert the actual image data.

If the image has light bands or spots on a dark background (i.e., the signal intensity of the background is greater than the signal intensity of the sample), you need to invert the data before analysis.

Select **Invert Data** from the *Image* menu or toolbar. This function is reversible. You may need to use the **Transform** controls to adjust the appearance of the inverted image.

3.13 Text Overlays

To create and display textual notes directly on the image, select **Text Overlay Tools** from the *Edit* menu or main toolbar. This will open the *Text Overlay Tools* toolbar.

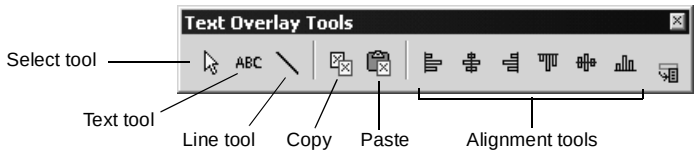


Fig. 3-24. Text Overlay Tools toolbar.

Creating a Text Overlay

To create a text overlay, click on the **Text Tool**, then click on the image at the spot where you want the text to appear. This opens the *Text Overlay Properties* dialog.

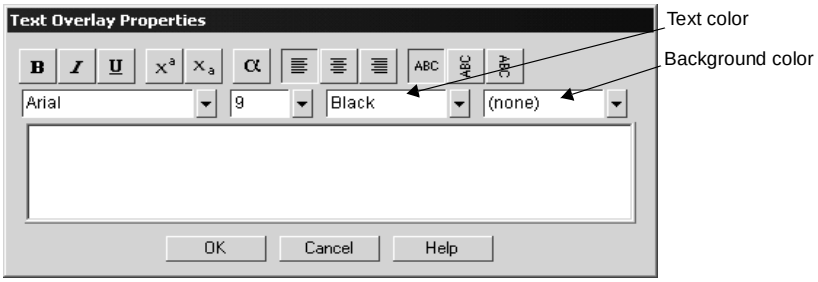


Fig. 3-25. Text Overlay Properties dialog.

To enter text, type in the main field. Use the buttons in the dialog to select the properties of the text, including format, alignment, and justification.

Select the font style, font size, color of the text, and color of the background in the text box using the pull-down lists.

After you have typed the text, click on **OK**. The text will appear on the image at the spot where you originally clicked.

Editing a Text Overlay

To edit a text overlay, make sure the **Text Tool** or **Select Tool** is selected, and then double-click on the overlay to open the *Text Overlay Properties* dialog. The existing text will be displayed and can be edited.

Line Tool

You can use the **Line Tool** to draw a line between text and an image feature, or between any two points of interest on the image.

Click on the **Line Tool** button, then drag on the image to create the line.

To resize or adjust a line, make sure the **Line Tool** or **Select Tool** is selected, and then position the cursor on one end of the line (marked by a circle) and drag.

To add arrowheads to a line, make sure the **Line Tool** or **Select Tool** is selected, and then double-click on the middle of the line. A dialog will pop up with options to add arrowheads to one or both ends of the line.

Moving and Copying Text and Lines

You can move, copy, or delete a single text overlay or line or a group of overlays and lines within an image. You can also copy and paste between images.

First, you must select the object(s). Click on the **Select Tool** button. To select a single overlay or line, click on it. To select multiple objects, either drag a box around them or hold down the **Shift** key and click on them individually. When dragging to select a group of objects, make sure that you completely surround all the objects to be selected.

Each selected overlay/line will have a green border.

- To *move* the selected object(s), position the cursor over the selection and drag.
- To *copy within an image*, hold down the **Ctrl** key while dragging the selected object(s). The copy will be created and dragged to the new position.
- To *delete* the selected object(s), press the **Delete** key.
- To *copy between images*, click on the **Copy to Clipboard** button, then open or select the image you want to copy to and click on the **Paste from Clipboard** button. The copied object(s) will be pasted into the new image in the same relative position they were copied from.

Note: If you are pasting into an image with a different pixel size (i.e., resolution), you will receive a message that the placement of the copy may not be exact. Click on **OK** to complete the paste, then position the pasted objects manually.

Viewing Previously Created Text Overlays/Lines

Previously created text overlays and lines will appear on the image when you open the *Text Overlay Tools* toolbar.

If you have concealed all overlays using the **Hide Overlays** command (section 3.3, Showing and Hiding Overlays), click on any of the buttons on the *Text Overlay Tools* toolbar to redisplay the text.

3.14 Erasing All Analysis from an Image

To delete all analysis and overlays from an image (including any lanes, bands, volumes, standards, text overlays, etc.), select **Clear Analysis** from the *Edit* menu. Since this process is irreversible, you will be prompted to confirm the selection.

3.15 Sort and Recalculate

To update, renumber, and recalculate all lane and band information, select **Sort and Recalculate** from the *Edit* menu.

3.16 Automation Manager

The **Automation Manager** allows you to save objects such as lanes, automated band detection, standard bandsets, volume overlays, and text and line overlays in a template file. These files can then be automatically applied to images either individually or in batches. To open the Automation Manager, select Analysis>Automation Manager.

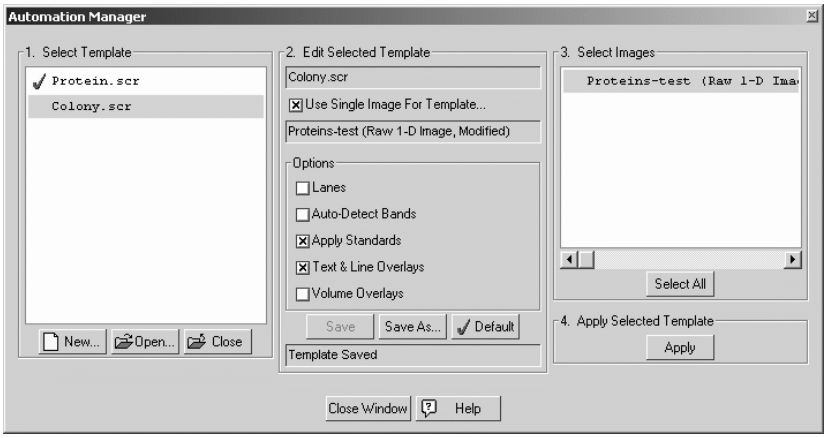


Fig. 3-26. Automation Manager dialog

3.16.a Step 1 - Select Template

The Select template field lists all the templates that are currently open. The Automation Manager remembers the files that were open the last time the application was open. To create a new template, click New. A new template appears in the list. See Section 3.16.b, Step 2 - Edit Selected Template for how to modify a new template. To open an existing template not currently in the list, click Open. This opens the standard Open dialog. To remove a template from the list, click Close.

3.16.b Step 2 - Edit Selected Template

Step 2, Edit Selected Template, lists the name of the currently selected template and the list of options available to the template. To edit the template, check the option(s) you would like to include and/or uncheck the option(s) you would like to remove from the template.

Use Single Image for Template

The Automation Manager allows you to specify a single image as the source for all options you select. Checking the *Use Single image for template* box opens the **Select Source Image for this Script item** dialog opens.

Fig. 3-27. Select an image

Make sure the image you select contains the object(s) you wish to apply to the destination images.

If you wish to use options from multiple images, then uncheck the box labeled, **Use single image for template**. As you check each option in step two you will be prompted to select a source image for that option.

Note: The source image must be open when the option check box is selected. Once you have finished checking options from a source, the source image can be closed.

When you are satisfied with your changes, click Save, or Save As to save it as a new template. If this is a new template, you must enter a new name for the template when you click Save. To change the name of a template, click Save As and enter a new name.

Default Automation

The default automation template is applied to the currently active image when you select Analysis>Apply Default Automation. To set a new default template, highlight the desired template in the Automation Manager and click Set as Default.

To run the default automation template on the currently active image, select Apply Default Automation from the Analysis menu.

Once a default template is chosen, The Automation Manager does not need to be open to be applied, nor do you need to select a default each time Quantity One opens as it remembers the default from the last open session of the application.

3.16.c Step 3 - Select Images

This portion of the Automation Manager lists the currently open images. Select the images to which you wish to apply the selected template. Use ctrl>click and shft>click to select more than one image, or click Select All to select all the images in the list.

3.16.d Step 4 - Apply Selected Template

Click **Apply** to apply the selected template in step 1 to the selected images in step 3.

4. Lanes

Before you can use many of the analysis functions, you must first define lanes and bands on the gel image. This chapter describes the tools for defining lanes.

Note: If you want to compare bands across lanes (using standards or band matching), the lane lines should be approximately the same length, with their starting points aligned across the top of the image. This is important for calculating the relative mobility of the bands. If gel wells are visible in the image, you should center the start points of the lane lines on the wells and position the ends of the lanes slightly below the last band for best results.

4.1 Defining Lanes

You can define lanes individually or as part of a frame. The functions for doing this are on the *Lane* menu and toolbar.

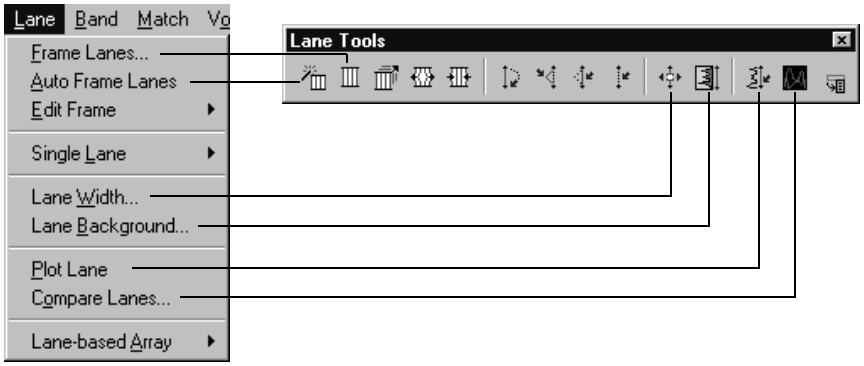


Fig. 4-1. Lane menu and toolbar.

In Quantity One, lanes are defined by red **lane lines** overlaid on a gel. The lane lines can be created individually (see section 4.1.c, Single Lanes), or they can be created as part of a **lane frame**.

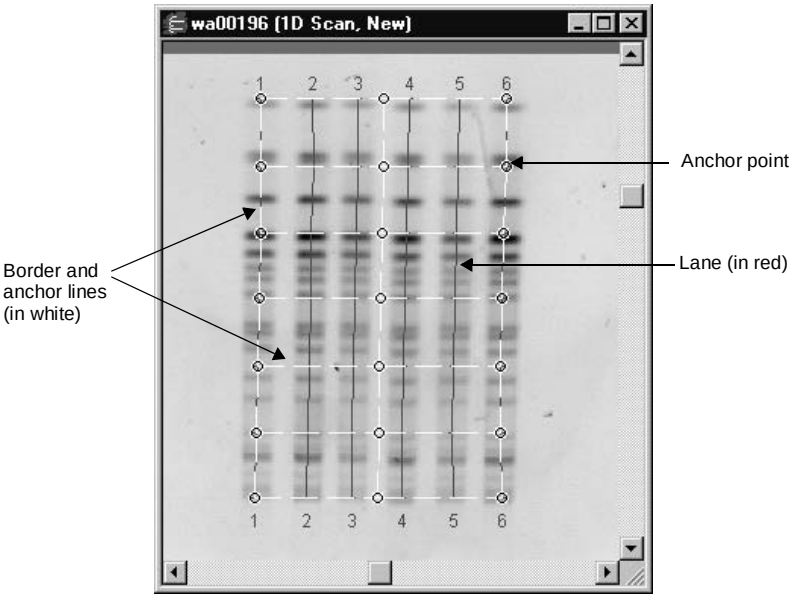


Fig. 4-2. Features of a lane frame.

4.1.a Lane Frames

The fastest way to define all the lanes on a gel is to create a lane frame using the **Auto Frame Lanes** command. If this command doesn't work well on your images, you can create and place a lane frame manually.

Auto Frame Lanes

Note: **Auto Frame Lanes** works best on gels with large numbers of clearly defined lanes and bands. Also, the lanes should be reasonably vertical and contain approximately the same amount of sample.

Select **Auto Frame Lanes** from the *Lane* menu or toolbar. The program will automatically detect the lanes and place a frame over them.

The lane frame contains individual lane lines numbered sequentially from left to right. The border and anchor lines of the frame are marked with dashed white lines, the lanes are solid red lines, and each anchor point (interior and corner) is marked with a circle.

The top and bottom of the frame are parallel with the top and bottom of the image. However, the interior anchor points and lines will “bend” the frame to follow the actual lanes in the gel, compensating for any curving or distortion in the gel.

If **Auto Frame Lanes** detects too few or too many lanes, you can add or delete lanes using the single lane commands (see section 4.1.c, Single Lanes).

If **Auto Frame Lanes** does not work on the image, you will be prompted to create a lane frame manually. To delete the lane frame, select **Clear Analysis** from the *Edit* menu.

Manual Frame Lanes

If **Auto Frame Lanes** does not work with your images, you can frame the lanes manually.

Select **Frame Lanes** from the *Lane* menu or toolbar. In the dialog, enter the number of lanes in the gel and click on **OK**.

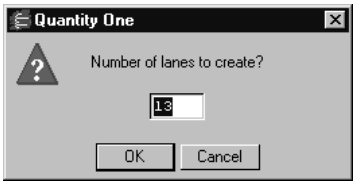


Fig. 4-3. Frame Lanes dialog.

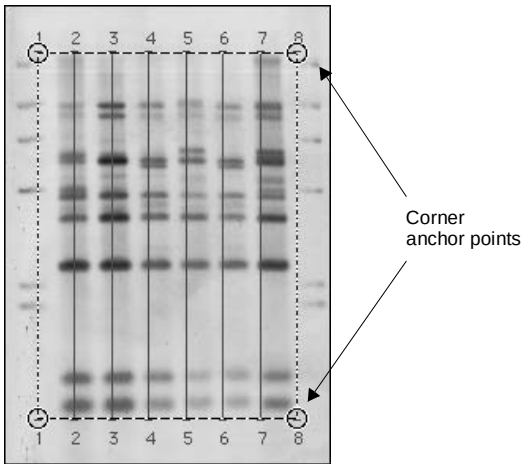


Fig. 4-4. Lane frame created using the Frame Lanes command.

The lane frame will be marked by corner anchor points, with no interior anchors. You can edit the frame as described below.

4.1.b Editing the Frame

If the frame is too large or small, or does not follow the lanes on the image, you can adjust it using the frame editing commands.

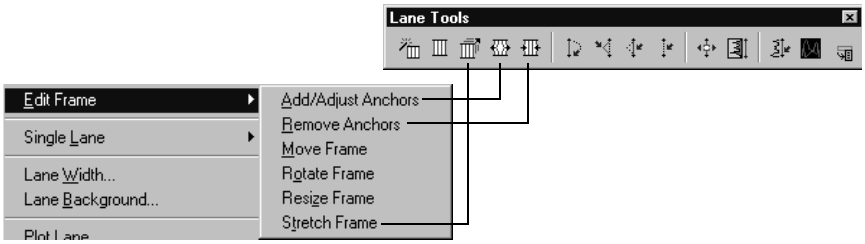


Fig. 4-5. Edit Frame tools.

Adjusting the Entire Frame

The following commands are located on the *Lane* > *Edit Frame* submenu:

- To *stretch* the frame in one direction (e.g., to encompass additional bands at the top or bottom of the image), select **Stretch Frame** from the submenu or *Lane* toolbar and drag an anchor point in or out. The opposite anchor point will remain fixed while the frame expands or contracts.
- To *move* the entire frame to a new position, select **Move Frame** from the submenu and drag an anchor point. The entire frame will move.
- To *rotate* the frame, select **Rotate Frame** from the submenu and drag an anchor point. The entire frame will rotate.
- To *resize* the entire frame, select **Resize Frame** from the submenu and drag an anchor point in or out. The frame will expand or contract from the center.

Adding and Adjusting Frame Anchors

To adjust a corner anchor point, select **Add/Adjust Anchors** from the *Edit Frame* submenu or toolbar and drag the anchor. This will move the anchor point and attached frame lines.

If the gel lanes are not straight, you can create additional anchor points along the frame to change the shape of individual lines.

Still using **Add/Adjust Anchors**, click anywhere on the frame lines. This creates interior anchor points both where you clicked and on the other side of the frame, connected by a frame line. Then drag the anchor points to bend the frame.

Add and adjust as many anchor points as you need to bend the lane lines to follow the lanes in the gel.

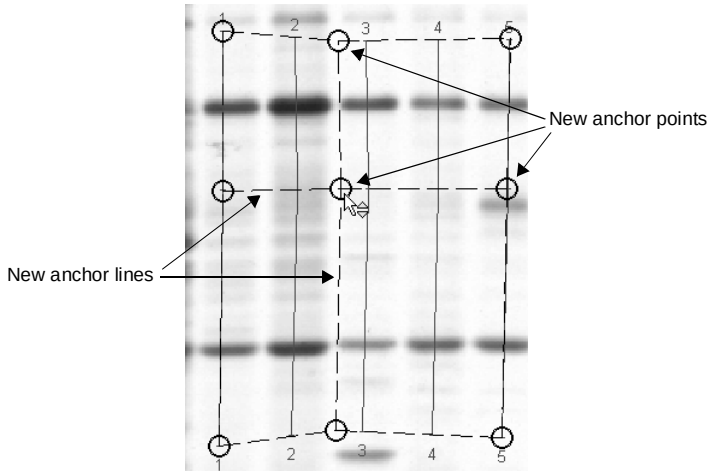


Fig. 4-6. Adjusting the anchor points of the lane frame.

Removing/Unadjusting Anchors

To remove an anchor point, select **Unadjust Anchors** from the *Edit Frame* submenu or toolbar and click on the anchor. The anchor will disappear and the adjusted lanes will straighten out.

4.1.c Single Lanes

You can define individual lanes using the single lane tools. These are located on the *Lane > Single Lane* submenu or on the *Lane* toolbar.

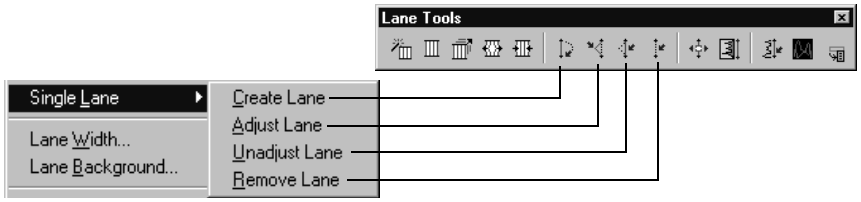


Fig. 4-7. Single Lane tools.

Note: You can use the single lane commands on any lane within a frame; however, the lane will be detached from the frame.

To mark an individual lane, select **Create Lane** and drag a line from the top to the bottom of the gel lane. The lane line will be marked in red. Repeat this procedure to manually mark all the lanes you want to analyze in the gel.

Note: If the lane numbering gets out of sequence, select **Sort and Recalculate** from the *Edit* menu to renumber the lanes.

Adjusting Single Lanes

You can adjust the position of any lane line. Select **Adjust Lane** from the *Lane* > *Single Lane* submenu or the *Lane* toolbar and either drag one of the existing anchor points or click anywhere on the lane to create a new anchor point and drag it into position.

To undo any lane adjustments, select **Unadjust Lane** from the submenu or toolbar and click on an anchor point to remove it. If you remove the anchor points at either end of the lane line, you will delete the entire line.

Deleting Lanes

You can delete both single lane lines and lines from a lane frame.

Select **Remove Lane** from the *Single Lane* submenu or toolbar and click on the lane. You will be prompted to confirm the deletion.

Note: If you delete a lane from a group of lanes or a frame, select **Sort and Recalculate** from the *Edit* menu to renumber the remaining lanes.

4.1.d Lane Width

Lane width is important for band quantition. Only the region of the band within the lane sampling width is quantitated, so the defined lanes should be slightly *wider* than the actual lanes in the gel.

You can adjust the sampling width of all the lanes using the *Detect Bands* dialog (see section 5.2.a, Detection Parameters). See section 5.1, How Bands Are Identified and Quantified, for a full discussion of the effect of sampling width on band quantitation.

To adjust the width of a single lane, select **Lane Width** from the *Lane* menu or toolbar and click on the lane line. The *Sample Width* dialog will display the current width of the lane.

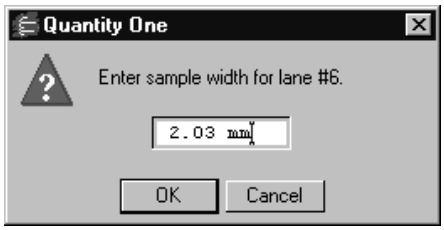


Fig. 4-8. Sample Width dialog.

Enter a new width in millimeters and click on the **OK** button.

4.1.e Lane Profile

After you have defined a lane, you can review the intensity profile of the lane. A lane profile provides a quick visualization of the intensity of your sample data, and is also useful for determining the level of background in the gel.

Select **Plot Lane** from the *Lane* menu or toolbar, then click on a lane. A lane profile graph will be displayed.

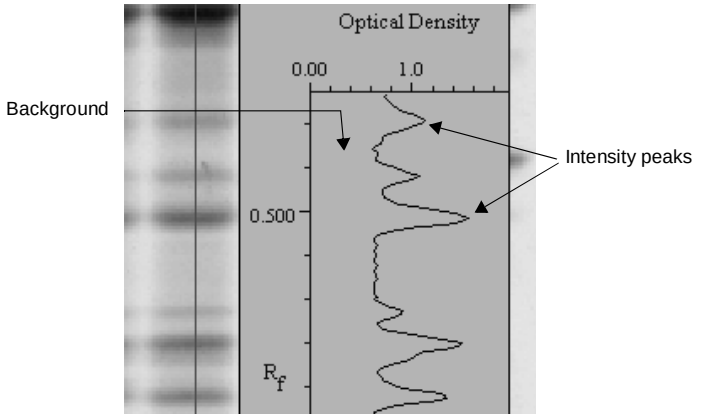


Fig. 4-9. Profile of a defined lane.

In the lane profile, bands are represented by peaks and background intensity is represented by the baseline region below the peaks. The profile is generated by calculating the average intensity of each horizontal row of pixels across the specified width of the lane.

To close the lane profile, click on **Hide Overlays** on the main toolbar.

4.2 Lane-Based Background Subtraction

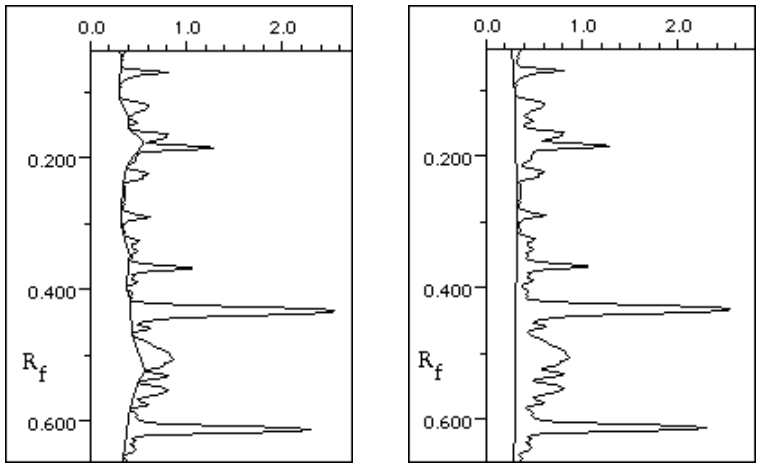
After defining lanes, we strongly recommend that you perform lane-based background subtraction. This is the best method for removing background intensity from lanes, and is required for Gaussian modeling of bands.

Lane-based background subtraction uses a “rolling disk” method of subtraction, named for a hypothetical disk that rolls along underneath the lane profile, removing different intensity levels along the length of the lane.

The size of the disk determines how much background will be subtracted. A large disk will follow the profile trace less closely, touching fewer points along the trace

and removing less background. A smaller disk will more closely follow the profile trace, removing more background.

A disk radius that is too large will result in poor removal of background. A disk radius that is too small may subtract actual data.



Small Radius **Large Radius**

Fig. 4-10. Examples of the background trace for small and large rolling disks. The small disk follows the profile trace more closely, resulting in more background subtraction.

Select **Lane Background** from the *Lane* menu or toolbar and click on a lane. The lane will be highlighted, the lane profile will be displayed, and the *Lane Background Subtraction* dialog will open.

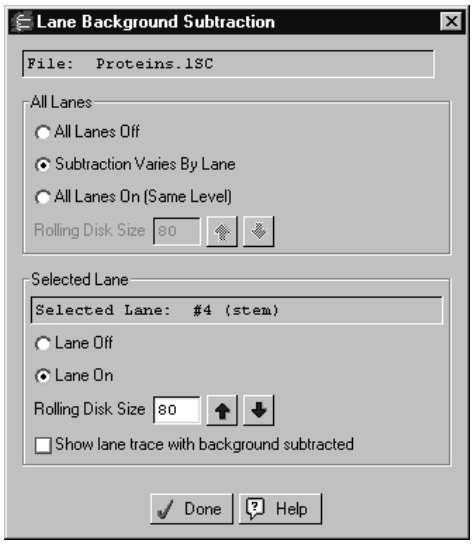


Fig. 4-11. Lane Background Subtraction dialog.

In the dialog, you can set the same subtraction level for all lanes or specify an individual subtraction level for the selected lane. Any changes you make will be automatically applied to the image. To close the dialog, click on **Done**.

4.2.a Profile Trace

When you make changes in the dialog, note that the profile trace of the lane also changes. In the standard view, the original "raw" trace of the line is shown in black, and the orange line represents the background beneath the peaks of the trace as defined by the rolling disk.

As you change the size of the rolling disk, the orange line changes, following the contours of the profile more or less closely as shown in Fig. 4-10.

To display the trace with the background removed, select **Show lane trace with background subtracted**. The orange line in the trace disappears along with the

background, and you can visually compare the relative intensities of the bands in the lane.

4.2.b All Lanes Off

To turn off lane-based background subtraction for all the lanes in the image, select the **All Lanes Off** button.

4.2.c All Lanes On (Same Level)

Select this option to set the same subtraction level for all lanes. The **Rolling Disk Size** field in the *All Lanes* section of the dialog displays the rolling disk radius in number of pixels. Enter a new value in the field, or use the arrows to change the value in 10 percent increments.

Typical rolling disk sizes range from 50 to 150. As you change the size, study the level of background subtraction in the lane trace.

4.2.d Subtraction Varies by Lane

You can set a different subtraction level for each lane in the gel using the controls in the *Selected Lane* area of the dialog. Any changes you make with these controls will only be applied to the selected lane; to select a different lane, click on it with the **Lane Background** command assigned.

Click on the **Lane Off** button to turn off subtraction for the selected lane. Select **Lane On** to turn on subtraction for the lane.

To adjust the subtraction level for the lane, change the value in the **Rolling Disk Size** field under *Selected Lane*. This field displays the rolling disk radius in number of pixels. Enter a new value in the field, or use the arrows to change the value in 10 percent increments.

Typical rolling disk sizes range from 50 to 150. As you change the size, study the level of background subtraction in the lane trace.

When you make changes to the selected lane, the **Subtraction Varies by Lane** button is selected. To turn off individual lane subtraction, choose either **All Lanes Off** or **All Lanes On**.

4.3 Compare Lanes

The *Compare Lanes* graph allows you to superimpose the intensity profiles of any number of lanes from any number of open images.

Select **Compare Lanes** from the *Lane* menu or toolbar, then click on the first lane you want to display. The *Compare Lanes* window will open.

Note: The image must have defined lanes for this command to work.

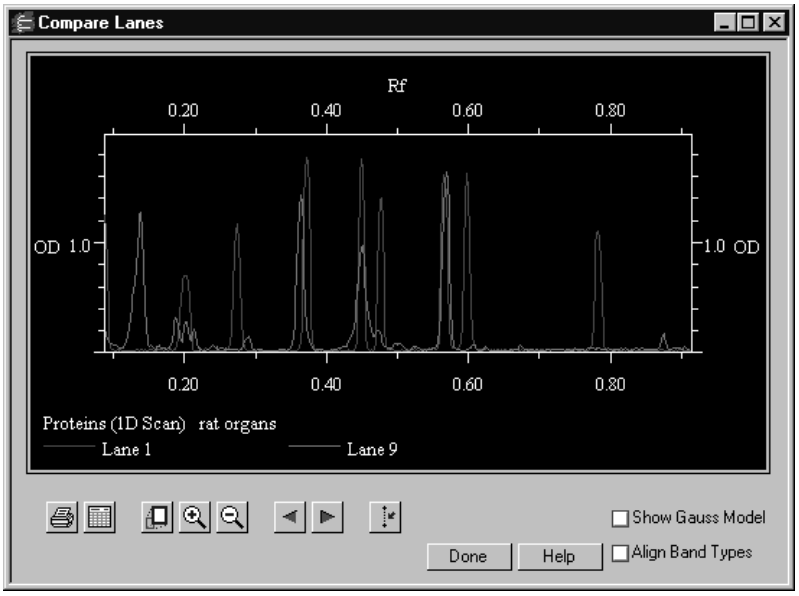


Fig. 4-12. The Compare Lanes dialog.

The X axis of the graph is the Rf value and the Y axis is the pixel intensity value at each point along the lane. *Compare Lanes* automatically "best fits" lanes within the display window to maximize the range of intensity values included in the graph. Rf values are displayed from 0.0 to 1.0.

Adding and Removing Lanes from the Graph

To add a lane to the graph, click directly on the lane in the image. The plot of the lane will appear in the graph.

Each lane you add will be displayed in one of eight colors, and will be identified by color in a legend underneath the graph. If you add more than eight lanes, the colors will repeat, but each lane will still be identified underneath the trace display. There is no limit to the number of lanes that can be displayed simultaneously.

To remove a lane profile, click on the **Remove Lane** button. A pop-up box will prompt you to select the lane to remove. If you remove a lane, the colors of the remaining lanes will change. Check the lane legend for an updated color code.

Magnifying the Graph

Use the **Zoom In** and **Zoom Out** buttons in the dialog to magnify regions of interest in the profiles. Alternatively, drag the cursor horizontally across the graph and release the mouse button to magnify the defined range.

Note: The magnifying functions in **Compare Lanes** only magnify the profile in the direction of the X axis. Therefore, the profile will appear to "stretch" without increasing in height.

The **Full View** button returns the graph to its default display.

If you have magnified part of the graph, the **Left arrow** and **Right arrow** scroll buttons can be used to pan left or right along the graph.

Show Gaussian Modeling

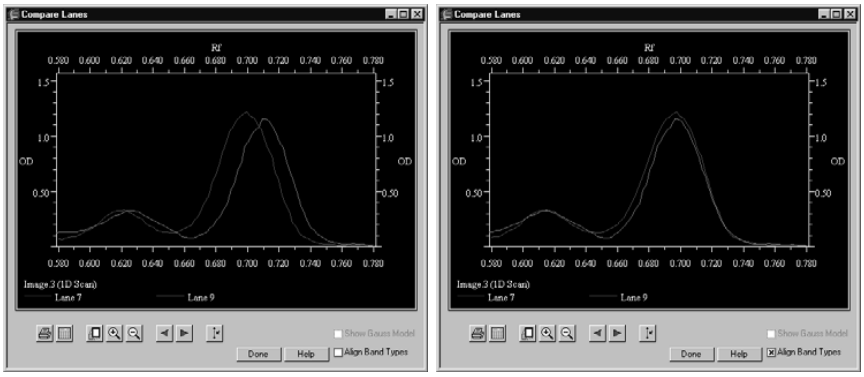
The **Show Gauss Model** checkbox is active if any of the selected lanes includes Gaussian modeling (see section 5.7, Gauss-Modeling Bands). If you select this checkbox, the Gaussian-fitted profiles will be superimposed on the regular lane profiles. The Gaussian profiles are displayed in white.

Align Band Types

The **Align Band Types** checkbox is active if any of the selected lanes includes defined band types (see section 6.2, Band Matching).

If this checkbox is selected, the profiles of all bands that have been identified as the same band type will be stretched and superimposed on one another, so their peaks align. This is useful if the same band appears as peaks in slightly different positions in different lanes, and you want to align the peaks to confirm that they are all the same band type.

Note: This command only changes the lane profiles as they are displayed in the *Compare Lanes* dialog, and will not affect image data in any way.



Unaligned Aligned

Fig. 4-13. Two band types as they appear in two lanes, before alignment and after.

Note that this function will not align band types from different band sets (e.g., Band Type 1 in Band Set A and Band Type 1 in Band Set B will not be aligned). However, the same band types from different images will be aligned.

Note: The Rf values in the X axis will no longer be accurate if **Align Band Types** is selected, since some band profiles will be stretched and their peaks shifted.

Printing and Exporting

Click on the **Print** button to print a copy of the *Compare Lanes* display.

Click on the **Export** button to export the data points in the graph to a spreadsheet. This will open the *Compare Lanes Export* dialog.

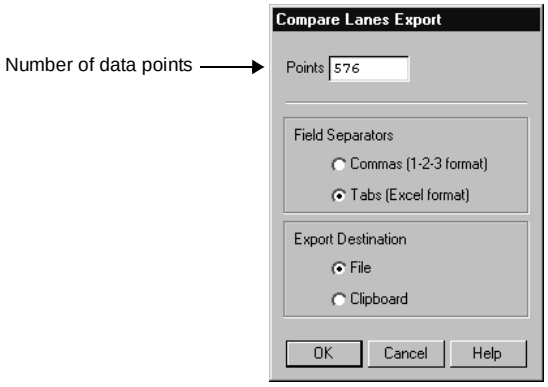


Fig. 4-14. Compare Lanes Export dialog.

This *Export* dialog includes a field for the number of data points to be taken along the length of each lane. The default value in this field is the maximum number of data points that are available for the lanes you are comparing.

Select the export format (tab or comma delimited) and destination (file or clipboard), then click on **OK**.

Note: The exported data will be different depending on whether you have checked **Align Band Types**, **Show Gauss Model**, or neither. If the **Show Gauss Model** checkbox is selected, each lane that has been Gaussian fitted will have two columns of data: one for the Gaussian-fitted profile and one for the regular profile. If the **Align Band Types** checkbox is selected, the exported values will reflect the stretched and shifted profiles of those lanes that have been aligned.

4.4 Lane-based Arrays

The lane-based array functions allow you to create a lane frame for the cells in an array. You can then specify the cell dimensions and quantitate them using the **Quantity Standards** function (see section 6.3, Quantity Standards).

Note: You can quantitate arrays outside of lanes using volume arrays (see section 7.7, Volume Arrays).

The first step in defining an array is specifying the number of columns and rows and creating an array frame.

Got to the *Lane* menu, open the *Lane-based Array* submenu, and select **Frame Array**.

Fig. 4-15. Lane-based Array tools.

Enter the number of columns in the array and click on **OK**.

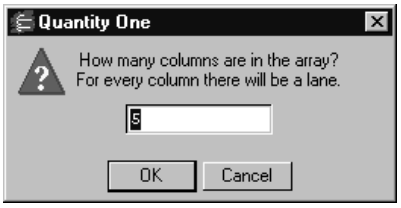


Fig. 4-16. Setting number of array columns.

In the next box, enter the number of rows and click on **OK**.

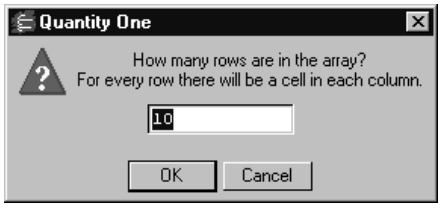


Fig. 4-17. Setting the number of array rows.

The array matrix will appear on the image. Each column will be marked by a red line, and each cell will be marked by top and bottom brackets.

Note: If the cells appear marked by lines instead of brackets, select **Band Attributes** from the *Band* menu and select **Brackets** in the dialog.

When you first create the array matrix, it will probably not be centered on the columns and cells in the actual image. In the next step, you will adjust the position of the matrix.

Adjusting the Array Matrix

The **Add/Adjust Anchors** tool will be automatically assigned to the mouse after you create the frame (otherwise, select it from the *Lane > Edit Frame* submenu). Position the cursor on the corner points of the frame and drag them into position so that the red lines run down the middle of the array columns and the top and bottom brackets are centered on the array cells (see section 4.1.b, Editing the Frame for guidance on adjusting frames).

If necessary, the **Adjust Lane** command (see “Adjusting Single Lanes” on page 7) and **Adjust Band** command (see section 5.3.b, Adjusting Bands) can be used to adjust the placement of columns and cells within the frame.

Reducing Background in the Array

After you have positioned the array, you should reduce lane background using the **Lane Background** command (see page 4.2, Lane-Based Background Subtraction). Lane background will affect quantitation of the cells.

Setting Array Cell Height and Width

Now you should adjust the cell brackets so that they completely enclose the cells in the array.

Select **Array Cell Height** from the *Lane > Lane-based Arrays* submenu and enter the height in millimeters of the array cells.

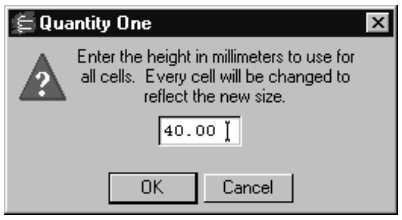


Fig. 4-18. Setting the array cell height.

When you click on **OK**, the cell brackets will adjust to the specified height. If you aren't sure of the exact height, you can experiment with different values.

Select **Array Cell Width** and enter the width in millimeters of all the cells in the array.

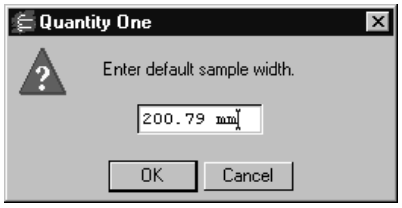


Fig. 4-19. Setting the array cell width.

When you click on **OK**, the cell brackets will adjust to the specified width. If you aren't sure of the exact width, you can experiment with different values.

Analyzing Array Data

When the brackets fully enclose each cell in the array, you are ready to analyze the data. You can display various measures of cell quantity on the image using the **Band Attributes** command on the *Band* menu. With the *Band Attributes* dialog open, select from **Peak Density, Average Density, Trace Quantity, Relative Quantity,** and other measures. You can also report these values by selecting **Lane Reports** from the *Reports* menu.

To use known quantities to calculate unknowns, you can use the **Quantity Standards** function (see section 6.3, Quantity Standards).

5. Bands

After you have defined the lanes on the gel image, you can automatically identify and quantitate bands using a set of adjustable parameters.

Note: You can quantitate bands, arrays, or other objects outside of lanes using volumes. See Chapter 7 for details.

The tools for band detection are located on the *Band* menu and on the *Band Tools* toolbar.

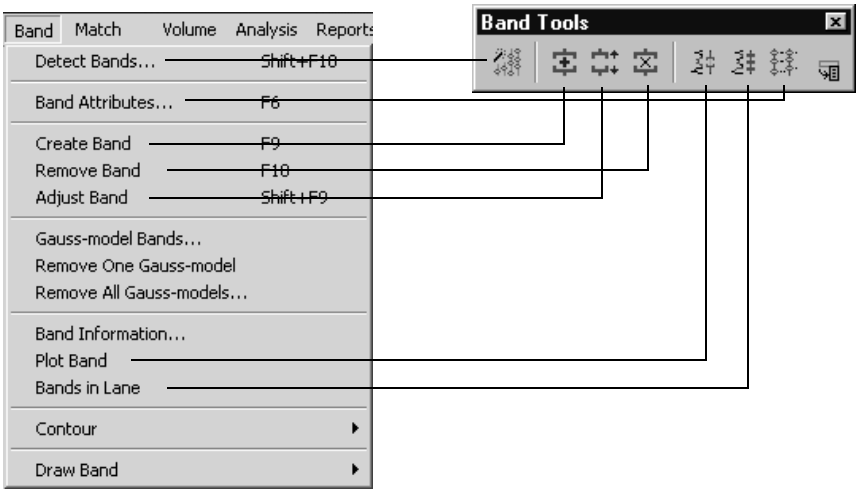


Fig. 5-1. Band menu and Band Tools toolbar.

Note: Before detecting bands, you should subtract background from the lanes using the **Lane Background** command (see section 4.2, Lane-Based Background Subtraction).

5.1 How Bands Are Identified and Quantified

You can automatically identify all the bands in an image using the **Detect Bands** command (see section 5.2, Band Detection), or you can mark them individually using the **Create Band** command (see section 5.3, Identifying and Editing Individual Bands).

Each identified band is defined by brackets above and below the band. The width of each set of brackets is determined by the lane sampling width (see section 5.2.a, Detection Parameters). The height of the brackets is determined automatically, using a band-finding formula together with parameters that you select.

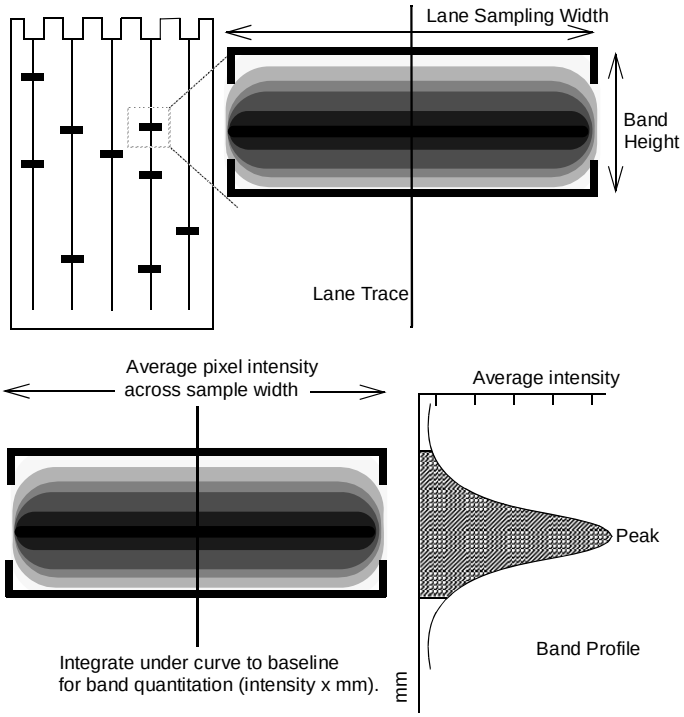


Fig. 5-2. Illustration of bracket quantitation.

When a band is quantitated, the average intensity of each horizontal row of pixels within the brackets is calculated. Next, the number of pixel rows between the top and bottom brackets is determined. Taken together, these result in an intensity profile for the band.

Finally, the area under the profile curve to the baseline is integrated, resulting in units of intensity x millimeters. This is the "trace quantity" of the band.

Note: Because the profile of an ideal band conforms to the shape of a Gaussian curve, band profiles can be "fitted" to a Gaussian model. The band quantity can then be quantitated from the area under the Gaussian curve. This is the best to resolve overlapping or closely spaced bands in images. See section 5.7, Gauss-Modeling Bands, for details.

5.2 Band Detection

The **Detect Bands** command automatically detects the bands in defined lanes, based on parameters that you select.

Note: Before detecting bands, you should subtract background from the lanes using the **Lane Background** command (see section 4.2, Lane-Based Background Subtraction).

Select **Detect Bands** from the *Band* menu or toolbar. The *Detect Bands* dialog will open.

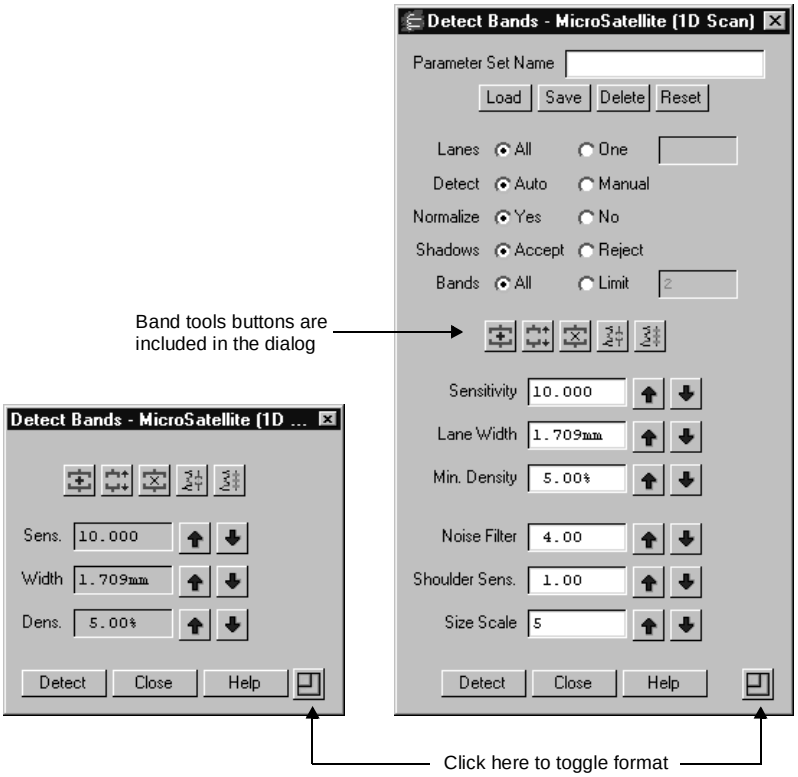


Fig. 5-3. Detect Bands dialog, short and expanded formats, with default values.

When you first open the *Detect Bands* dialog, all bands will be automatically detected based on the default parameters in the dialog, and the bands will appear marked on the image. The bands can be marked by either lines or brackets, depending on the settings in the *Band Attributes* dialog (see section 5.5, Band Attributes).

Note: If you have already manually identified bands (using **Create Band**, **Adjust Band**, etc.), the **Detect Bands** function will overwrite them. You should use **Detect Bands** first, and then manually add, adjust, or remove bands as needed.

The *Detect Bands* dialog has a short format and an expanded format; toggle between them by clicking the toggle box in the lower right corner.

To change a parameter in the dialog, you can either type in a new value or use the arrows to increase or decrease the setting by 10 percent. Experiment with different settings to find those best suited to your images.

5.2.a Detection Parameters

Lanes to Detect

If the intensities of the bands vary from lane to lane, you may need to use different detection parameters on different lanes. Specify whether you want to use the detection parameters for all the lanes or a single lane by selecting **All** or **One** next to the *Lanes* prompt. If you choose **One**, type the lane number in the field next to the **One** button.

When to Detect

If you select **Auto** next to the *Detect* prompt, band detection will occur immediately each time you change a detection parameter. You will not need to click on the **Detect** button located at the bottom of the form.

To change more than one parameter before detecting, choose **Manual**. With the **Manual** option, you can change parameter settings first, and then apply them by clicking the **Detect** button.

Normalization

Normalization is a way to compensate for differences in intensity between lanes. *It does not normalize for band quantitation.*

The intensity of each lane is determined by the darkest band in that lane. For example, suppose that in all but one of the lanes the darkest band has an intensity of 50,000 counts. In the one light lane, the darkest band is only 25,000 counts. With normalization, band detection will be twice as sensitive when processing the light lane, improving the detection of faint bands.

Select **No** next to the *Normalize* prompt to apply the band detection parameters in the same way to every lane in the gel image. Select **Yes** to normalize for the intensity of the lane.

Shadow Rejection

"Shadow bands" are common gel artifacts. Shadow bands are spaced at tandem repeat intervals and decrease in intensity as they progress further from a real band. The **Shadows** parameter is designed to limit the detection of shadow bands (see also **Band Limit**).

Select **Reject** to turn on the shadows filter. A band will detected only if it is darker than the one above it or spaced further than one tandem repeat unit from the previous band. This greatly reduces the number of shadows identified as real bands.

Select **Accept** to turn off the shadows filter.

Band Limit

If you know that all the lanes in a gel contain a specific number of bands, you can click on the **Limit** button next to the *Bands* prompt and type in the number of bands that you know are present. Only that number of bands will be detected in each lane, reducing the need for later editing.

Sensitivity

The **Sensitivity** setting determines the minimum signal intensity in the image that will be defined as a band. The higher the sensitivity value, the more bands will be detected.

If the sensitivity is set too high, background noise may be detected as bands. If the setting is too low, real bands may be missed.

The default sensitivity setting is 10.00. If the gel has faint bands (e.g., O.D. < 0.05, counts < 2,000), you may want to increase this value to 20.00.

Lane Width

The **Lane Width** setting determines the width along the lane lines that are sampled for band detection and quantitation.

When a band is detected, an average intensity for each horizontal row of pixels within the band brackets is calculated. The lane width determines the number of pixels in each row. Study the band lines in the image while you adjust the width. Select a width that is slightly *wider* than the bands in the gel.

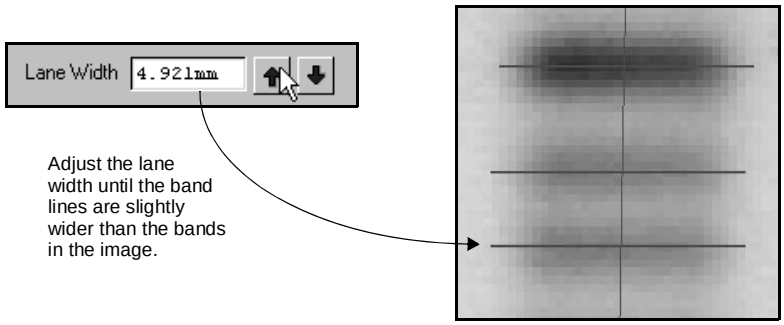


Fig. 5-4. Adjusting the lane width.

You can also change the widths of individual lanes using the **Lane Width** command (see section 4.1.d, Lane Width).

Minimum Density

When **Normalize** is turned off, the **Min. Density** defines the lowest signal intensity that will be counted as a band.

Before selecting a **Min. Density** value, use the **Plot Lane** command on the *Lane* menu to plot a trace of a lane that includes some faint bands. Then enter a value that is lower than the intensity of the peak of a faint band but is still above the background.

If faint bands are still undetected after adjusting this parameter, you may want to increase the **Sensitivity** setting.

If **Normalize** is turned on, **Min. Density** changes from an absolute value to a percentage. This percentage is the fraction of the signal intensity of the darkest band in the lane that will be detected as a band. For example, if the darkest band in a lane is 50,000 counts and the **Min. Density** is set to 25,000 counts, when you turn on **Normalize**, the **Min. Density** will switch to 50% (i.e., a band must be at least half as dark as the darkest band in the lane).

Noise Filter

The **Noise Filter** is used to minimize the number of small fluctuations in the image (i.e., noise) that are called bands while still recognizing larger features (i.e., real bands). This filter becomes especially important at higher **Sensitivity** levels.

The **Noise Filter** value refers to the size of the filter in pixels (e.g., a value of 2.50 equals a filter size of 2.50 x 2.50 pixels). Features smaller than the filter size will not be recognized as bands. Entering a noise filter size of zero turns it off completely. The default value is 4.00.

If band detection detects doublets as single bands, decrease the **Noise Filter** setting and/or increase the **Sensitivity**.

You can also try decreasing the **Size Scale** parameter instead of the **Noise Filter** to improve the detection of closely-spaced bands. However, if you decrease both the **Noise Filter** and the **Size Scale,** the fuzziness around bands may be mistakenly detected as separate bands.

Shoulder Sensitivity

Normally, band detection tries to distinguish shoulders as separate bands. When looking at a lane trace, these bands appear as flat or gently sloping abutments to darker, better-defined bands (i.e., there is no dip on the trace between the two bands).

Increasing the **Shoulder Sensitivity** will result in more shoulders being detected as bands. Changing this setting to zero will result in no shoulders being recognized as separate bands.

If band detection calls a doublet a single band, check the lane trace to see if there is a dip between the peaks of the two bands. If there is no dip, increasing the **Shoulder Sensitivity** value will help resolve the two bands.

Size Scale

The **Size Scale** field helps distinguish between trends in signal intensity and random intensity fluctuations. It is the number of pixels in a vertical column that are taken together to determine whether a band is present.

The **Size Scale** parameter is similar to the **Noise Filter** in that it uses the size of objects in the image to determine the nature of those objects. The default **Size Scale** setting of 5 pixels is optimal for most gel images. It can be set to any whole number greater than or equal to 3.

If a gel image has high levels of background noise, a larger **Size Scale** is appropriate. At low noise levels, a smaller value is preferable. You can also increase the **Size Scale** if the gel only has a small number of thick bands scanned at high resolution.

5.2.b Parameter Sets

You can save detection parameters for use on similar images. Enter a name for the set in the **Parameter Set Name** field, and click on the **Save** button.

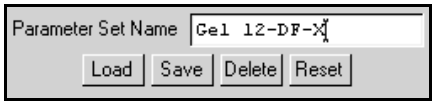


Fig. 5-5. Parameter set controls in the Detect Bands dialog.

To load a saved set of parameters, click on the **Load** button and select the set from the list. To remove a set of parameters, first load it, then click on **Delete**.

5.2.c Default Parameters

To return all detection parameters to their default values, click on the **Reset** button at the top of the form.

5.3 Identifying and Editing Individual Bands

To manually identify and edit individual bands in an image, use the **Create Band**, **Adjust Band**, and **Remove Band** commands on the *Band* menu and toolbar. (These are also included on the toolbar in the *Detect Bands* dialog.)

Fig. 5-6. Create, Adjust, and Remove Bands buttons.

Note: When editing individual bands, it is useful to display the band brackets. Select these in the *Band Attributes* dialog (see section 5.5, Band Attributes). If your bands are very closely spaced, defining them in brackets mode gives you greater control for more precise band definition. It also allows you to define overlapping bands.

5.3.a Identifying Individual Bands

With the bands displayed as brackets, select **Create Band** from the menu or toolbar, then click on either the top or bottom boundary of the band in the gel. An intensity trace of the lane will pop up next to the band.

Drag the cursor until the area of the band that you want to define—represented by the peak on the intensity trace—has been completely enclosed. The area of defined band will be highlighted on the trace.

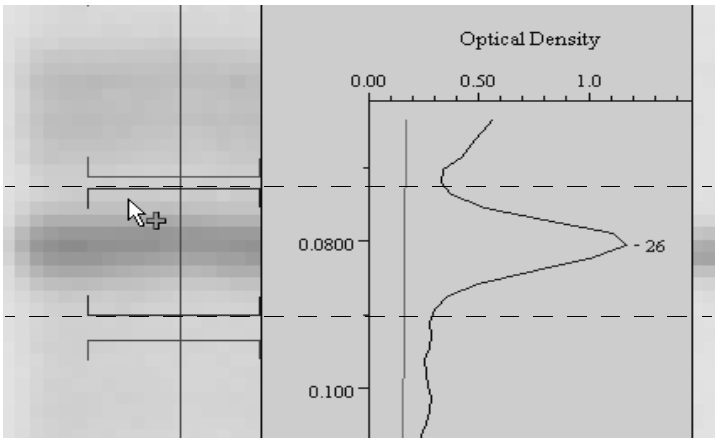


Fig. 5-7. Creating a band with brackets displayed.

When you release the mouse button, brackets will enclose the band on the image.

With the bands displayed as lines, select **Create Band** from the menu or toolbar, then click on the center of the band of interest. A line will appear in the center of the band.

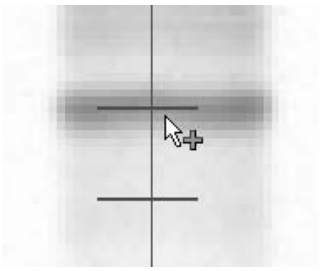


Fig. 5-8. Creating a band with lines displayed.

Note: After you have identified several bands, renumber the bands by selecting **Sort and Recalculate** from the *Edit* menu.

5.3.b Adjusting Bands

To reposition a band's boundaries, select **Adjust Band** from the menu or toolbar. If the bands are displayed as brackets, drag the upper or lower bracket of the band. If the bands are displayed as lines, drag *near* the upper or lower boundary of the band that you want to adjust.

When you drag the band boundary, a pop-up lane trace will appear, and the area of defined band will be highlighted on the trace. Drag the band boundary to the correct position.

5.3.c Deleting Bands

To undetect a band, select **Remove Band** from the menu or toolbar and click on the band. If the bands are displayed as brackets, a trace of the band will be displayed and a pop-up box will ask you to confirm the action. If the bands are displayed as lines, the band line will simply be deleted.

If the bands are displayed as lines, you can delete more than one band at a time. Select **Remove Band**, and drag a box around the bands to be removed.

If you undetect a band, it will no longer be counted as a band, but its intensity will still contribute to the total lane intensity.

Note: After you undetect bands, renumber the bands in the image by selecting **Sort and Recalculate** from the *Edit* menu.

5.4 Plotting Traces of Bands

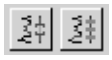


Fig. 5-9. Plot Band and Bands in Lane buttons.

Plot Band displays an intensity profile of a band. Select the command from the menu or toolbar and click on the band of interest.

Bands in Lane displays an intensity trace of a lane with the defined bands highlighted. Select the command from the menu or toolar and click on the lane of interest.

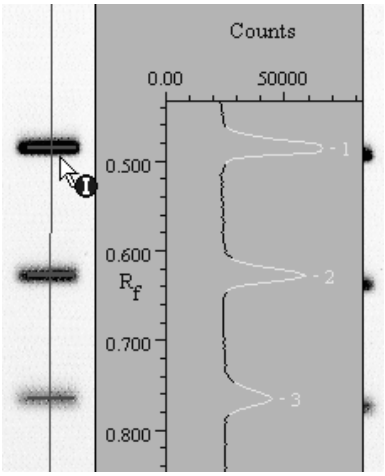


Fig. 5-10. Bands in Lane command.

5.5 Band Attributes

You can display different types of information about defined bands. Select **Band Attributes** from the *Band* menu or toolbar to open the dialog.

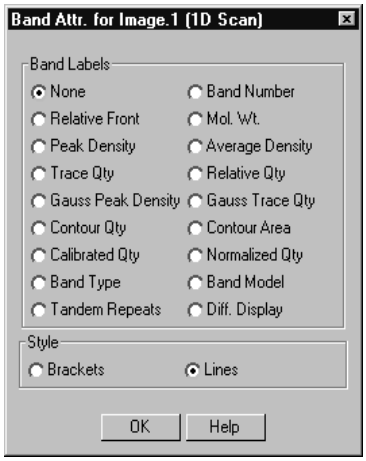


Fig. 5-11. Band Attributes dialog.

At the bottom of the dialog, select how you want to mark defined bands on the image—as **Brackets** or **Lines**. Lines are usually easier to read than brackets in gels with closely packed bands, while brackets are better for displaying and editing the boundaries of bands.

You can also select the band data to display on the image next to each band:

- **None**.
- **Band number**—The sequential number of a band in lane, as counted from the top of the lane.
- **Relative front—**The distance of a band from the top of its lane, divided by the total length of the lane. The lane length can be determined either by measuring a vertical line from the top of the lane to bottom or (if the lane is curved) by measuring along the length of the lane. Set the preferred measuring method in the *Preferences* dialog (see section 2.5.e, Application).

Note: Note that normalized Rf is derived from relative front; however, normalized Rf is calculated only for bands that have been modeled using standards or band sets, and can change based on the modeling.

- **Molecular Weight/Isoelectric Point/Base Pairs/other units**—This value is

determined by the type of standards defined for the gel, the band's position in the lane, and any modeling performed on the gel (using band matching or multiple lanes of standards) to compensate for gel distortion or smiling.

- **Peak density**—The intensity value of a band peak.
- **Average density**—The total intensity of the rows of pixels used to generate the profile of a band, divided by the number of rows.
- **Trace qty**—The quantity of a band as measured by the area under its intensity profile curve. Units are intensity x mm.
- **Relative qty**—The quantity of a particular band as measured by its intensity, expressed as a percentage of either the total intensity of all the bands in the lane or the total intensity of the lane (including the areas between bands). The calculation method (**% of Lane** or **% of Bands in Lane**) is set in the *Preferences* dialog (see section 2.5.e, Application).
- **Gauss Peak Density**—The intensity value of a band's Gaussian peak (after Gaussian modeling).
- **Gaussian Trace Quantity**—The quantity of a band as measured by the area under its Gaussian-fitted profile.
- **Contour qty**—The quantity of a band that has been identified using the **Contour** or **Draw Band** tools. It is the sum of the intensities of all the pixels within the band boundary multiplied by the area of each pixel. Units are intensity x mm^2.
- **Contour area**—The area (in mm^2) inside the boundary of a band that has been identified using the **Contour** or **Draw Band** tools.
- **Calibrated qty**—The quantity of a band as calculated from the trace quantity and quantity standards. (Note that this is different than quantity determined using volumes.) Units are user-defined.
- **Normalized qty**—The trace quantity of a particular band expressed as a percentage of the quantity of a selected band type that is present in the same lane.
- **Band type**—The band type number of a band that has been matched and placed in a band set.
- **Band model**—Displays the modeling lines across the gel that are generated by band matching, standards, or both. These lines are used to compensate for gel distortion or smiling.
- **Tandem repeats**—The number of repeated base-pair units in a band that has been analyzed using the **VNTR Calculations** function (see section 11.2, Variable

Number Tandem Repeats).

- **Differential Display**—If the band types have been normalized, this displays trends in increasing or decreasing expression of a band type across a gel based on its normalized quantity.

5.6 Band Information

The *Band Information* dialog displays information about each defined band. Select **Band Information** from the *Band* menu and click on a band.

An intensity profile of the band's lane will be displayed with the the selected band highlighted, and the dialog will open.

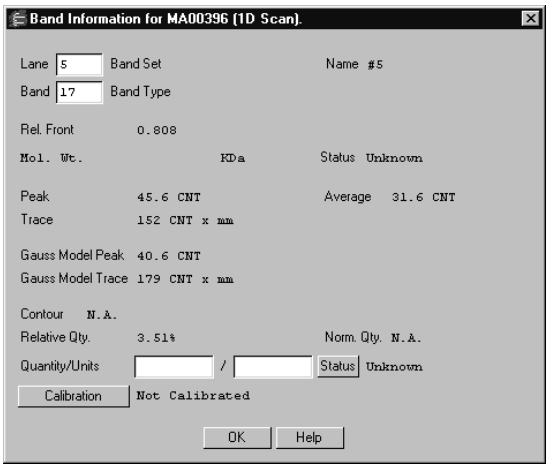


Fig. 5-12. Band Information dialog.

The lane and band number of the band you clicked on are listed at the top of the dialog. Enter new numbers in these fields to display information for a different band.

The band set and band type will be listed, if known. Other information includes:

- **Relative front—**The distance of the band from the top of its defined lane, divided by the total length of the lane. The lane length can be determined either by measuring a vertical line from the top of the lane to bottom or (if the lane is curved) by measuring along the length of the lane. Set the preferred measuring method in the *Preferences* dialog (see section 2.5.e, Application).

Note: Note that normalized Rf is derived from relative front; however, normalized Rf is calculated only for bands that have been modeled using standards or band sets, and can change based on the modeling.

- **Molecular Weight/Isoelectric Point/Base Pairs/other units**—This value is determined by the type of standards defined for the gel, the band's position in the lane, and any modeling performed on the gel (using band matching or multiple lanes of standards) to compensate for gel distortion.
- **Peak density**—The intensity value of the band's peak.
- **Average density**—The total intensity of the rows of pixels used to generate the profile of the band, divided by the number of rows.
- **Trace qty**—The band quantity as measured by the area under its intensity profile curve. Units are intensity x mm.
- **Gauss Peak Density**—The intensity value of the band's Gaussian peak (after Gaussian modeling).
- **Gaussian Trace Quantity**—The band quantity as measured by the area under its Gaussian-fitted profile.
- **Contour qty**—The quantity of a band that has been defined using the **Contour** or **Draw Band** tools. It is the sum of the intensities of all the pixels inside the band boundary multiplied by the area of each pixel. Units are intensity x mm^2.
- **Relative qty**—The quantity of a band as measured by its intensity, expressed as a percentage of either the total intensity of all the bands in the lane or the total intensity of the lane (including the areas between bands). The calculation method (**% of Lane** or **% of Bands in Lane**) is set in the *Preferences* dialog (see section 2.5.e, Application).
- **Normalized qty**—The trace quantity of the band expressed as a percentage of the quantity of a selected band type that is present in the same lane.

If the quantity of the band is known, you can enter the quantity and units next to the **Quantity/Units** prompt.

To calibrate the band against known quantities, click on the **Calibration** button and select the calibration curve. (See section 6.3.a, Creating and Applying a Set of Quantity Standards, for information on calibration curves.)

5.7 Gauss-Modeling Bands

If the bands are closely spaced or overlapping, Gaussian modeling can provide more accurate quantitation than regular band detection.

Gaussian modeling “fits” a Gaussian curve to each band profile and calculates band quantity from the area under the curve. Since the profile of a well-resolved, distinct band conforms to the shape of a Gaussian curve, this creates a band profile that is as close to ideal as possible.

Before:

After:

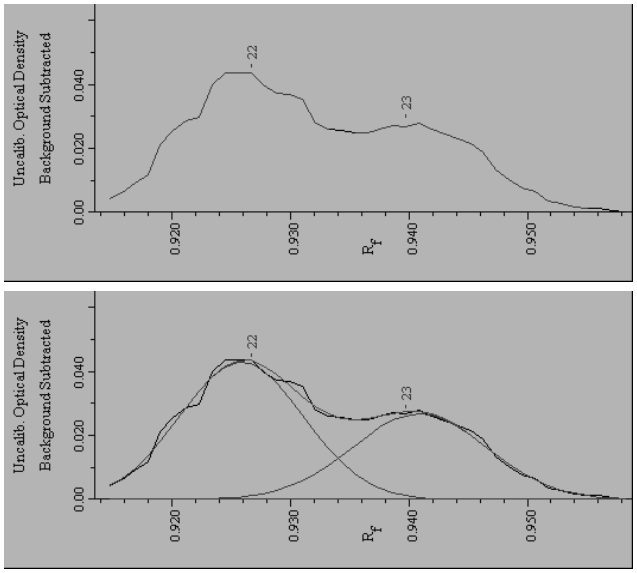


Fig. 5-13. Profiles of two overlapping bands, before (top) and after (bottom) Gaussian modeling. Modeling better resolves the band quantities.

For a band that overlaps with an adjacent band, Gaussian fitting provides the best way to resolve the area that overlaps. (This quantity would be lost with conventional band detection.)

Note: Gaussian modeling requires little or no background in lanes. Subtract lane background using the **Lane Background** command (see section 4.2, Lane-Based Background Subtraction) prior to modeling. Also, high-resolution images require significantly more time to model. To reduce image resolution, use the **Reduce File Size** command (see section 2.2.f, Reduce File Size).

To model bands using Gaussian fitting, first detect the bands, then select **Gauss-model Bands** from the *Band* menu.

Note: Gaussian modeling will not create bands or eliminate detected bands. It will simply apply a Gaussian curve to the profiles of detected bands.

In the pop-up box, select **All** to model all lanes, or **One** to model a single lane. If you select **One**, type the number of the lane in the field.

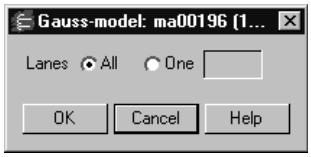


Fig. 5-14. Gauss-model Bands dialog.

Click on **OK**. A status box will display the progress of the modeling.

Reviewing the Results

Bands that have been Gauss-modeled will appear as normal bands in the image. To view the results of Gaussian fitting, magnify a few bands in a modeled lane using the **Zoom Box** tool, then select **Bands in Lane** or **Plot Band** from the *Band* menu and click on the lane.

Plot Band displays the Gaussian curve superimposed on the profile of the selected band. **Bands in Lane** displays the Gaussian profiles of all the bands in the lane, as shown in Fig. 5-13.

The *Band Information* dialog displays information about the Gaussian peak and trace quantity for modeled bands that you click on. In the dialog, you can compare the Gaussian values to the regular band detection values.

These quantities can also be displayed in the *Lane Report* and *All Lanes Report*.

Note: The quantities determined by Gaussian fitting cannot be used to in conjunction with **Quantity Standards** (see section 6.3, Quantity Standards). However, you can use the original trace quantities in calculating **Quantity Standards** after you have Gauss-modeled the bands.

Adjusting Bands in a Gauss-modeled Lane

If you use any of the individual band commands—**Create Band, Delete Band, Adjust Band**—in a lane that has been Gauss-modeled, the modeling will be automatically removed from that lane. The Gaussian models in a lane are interdependent, so changing a single band invalidates the modeling. After you have adjusted the bands, you can remodel the lane.

Incorrect Modeling

The **Gauss-model Bands** command will try to model all the bands in the selected lanes. Carefully review the results of modeling using the **Plot Band**, **Bands in Lane**, and **Band Information** commands as described above.

If the Gaussian curve does not adequately conform to the profile of a band, or if the Gaussian peak and trace quantities differ greatly from the normal peak and trace quantities in the *Band Information* dialog, it may be because there is too much lane background. Use the **Lane Background** command with a smaller rolling disk size to remove more background, then remodel the lane.

If Gaussian modeling does not work well with the bands in the image, simply remove the modeling. To remove the modeling from a particular lane, select **Remove One Gauss-model** from the *Band* menu and click on the lane. To remove the modeling from all lanes, select **Remove All Gauss-models**.

Removing Gaussian modeling will not affect band detection.

5.8 Irregularly Shaped Bands in Lanes

If some bands in lanes are irregularly shaped, you can use the contour or drawing features to define them. These functions give you more control over defining bands than either **Detect Bands** or **Create Bands**.

Note: These tools are similar to the **Volume Contour Tool** and the **Volume Freehand Tool**, except that they are lane-dependent. To quantify objects without defining lanes, see Chapter 7.

Contoured or hand-drawn bands are quantitated based on the signal intensity of all the pixels within the band boundary, using the following formula:

Quantity = Sum of the intensity of a pixel x pixel size for all the pixels in the boundary.

The intensity of a pixel is multiplied by the area of the pixel. This is done for all the pixels within the contour or drawn boundary. The area of the pixel is determined by the resolution of the image.

The resulting values have units of intensity x mm^2.

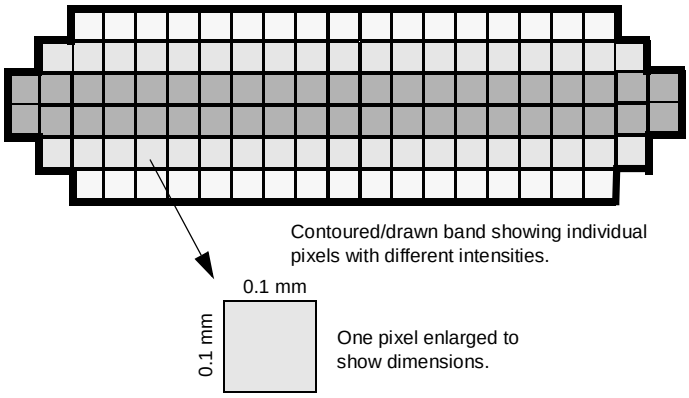


Fig. 5-15. A contoured band scanned at 100 x 100 micrometers.

The commands for contouring and drawing bands are located on the *Contour* submenu and *Draw Band* submenu of the *Band* menu, and on the *Contour Tools* toolbar.

Before using these commands, magnify the image so that the individual pixels in the band are clearly visible. This allows you to position the cursor more accurately.

5.8.a Contouring Bands

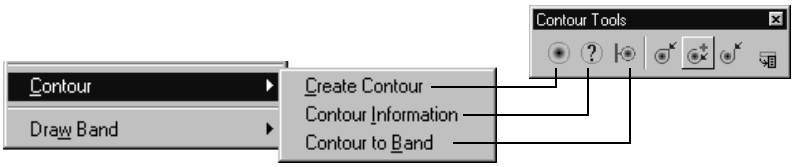


Fig. 5-16. Contour tools.

Creating Contours

Select **Create Contour** from the *Band* > *Contour* submenu or toolbar and click on a pixel at the edge of the band. This displays a contour that encloses pixels whose intensity is equal to or greater than that of the pixel at the cursor.

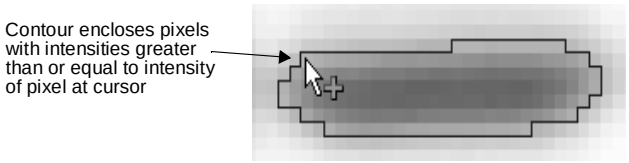


Fig. 5-17. Creating a Contour

If the contour does not encircle the band, reposition the cursor and click again. A new contour will be drawn in place of the old.

Contour Information

To display information for the selected contour, select **Contour Information**. A pop-up box will show the area, total intensity, and average intensity.

Converting a Contour into a Band

When you are satisfied with the contour, select **Contour to Band** from the menu or toolbar to redefine the contour as a band and assign it to the nearest lane.

Note: Before you can convert a contour into a band, you must define the lane containing the band.

The contour boundary will change color from yellow to red, and a band line will appear on the nearest lane.

Note: Gaussian modeling and the **Plot Band** command do not work on contoured bands.

To list areas and quantities of contoured bands in reports, select the **Contour Area** and **Contour Qty** report formatting options. Display this information on the image using the *Band Attributes* dialog.

5.8.b Drawing Tools

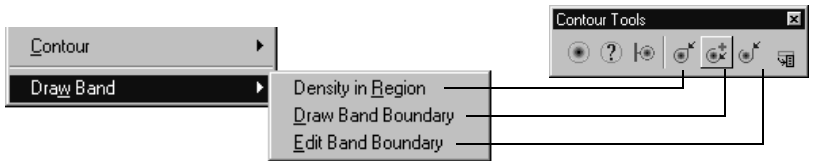


Fig. 5-18. Drawing tools.

Density in Region

Density in Region displays intensity information for any area on an image.

Select **Density in Region** from the *Band > Draw Band* submenu or *Contour Tools* toolbar, and use the cursor to draw a line around a region of interest.

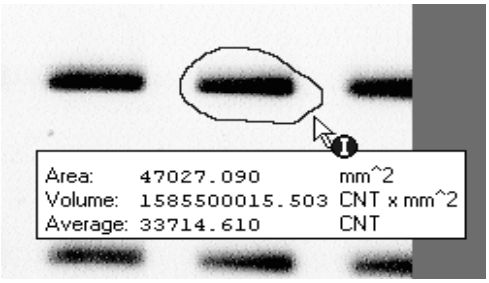


Fig. 5-19. Density in Region tool.

When you close the border, a pop-up box will display information about the enclosed area. For very small regions, magnify the region before using this command.

Drawing Band Boundaries

Note: To use the drawing tools, at least one lane must be defined on the image. Also, magnify the region you want to draw in using **Zoom Box**.

Use **Draw Band Boundary** to draw the boundary of a band manually. Select the command from the *Band > Draw Band* submenu or *Contour* toolbar, and drag the cursor around the region that you want to define as a band. A boundary line will appear.

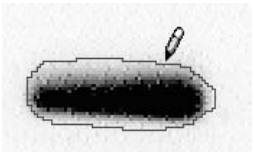


Fig. 5-20. Draw Band Boundary tool.

If you make a mistake and need to retrace part of the band boundary, backtrack with the cursor; the path will be erased and you can redraw it.

Note: Be sure to magnify the image before drawing a boundary. If you try to draw a very small boundary, the software will think that you are backtracking and erase the boundary.

When the cursor crosses the line, the color of the line will change to indicate that it is a band boundary, and a band line will appear on the nearest lane.

If you keep drawing, each time the line crosses itself a new band will be created, replacing the old band.

Note: Gaussian modeling and the **Plot Band** command do not work on drawn bands.

To list areas and quantities of drawn bands in reports, select the **Contour Area** and **Contour Qty** report formatting options. Display this information on the image using the *Band Attributes* dialog.

Editing Band Boundaries

To change a drawn band boundary, select **Edit Band Boundary** from the *Band* > *Draw Band* submenu or *Contour* toolbar and drag the cursor across the previously defined boundary. A line will appear. When you recross the old boundary, the line will change colors and the new boundary will be created.

6. Standards and Band Matching

After you have defined the lanes and bands in a gel, you can identify the standard lanes, enter the values of the standards, and determine the values of the experimental bands using those standards. You can also compare sample similarity by matching bands across lanes.

Finally, you can identify bands of known quantity and use these to generate a calibration curve for quantitating unknown bands.

These tools are found on the *Match* menu and *Match Tools* toolbar.

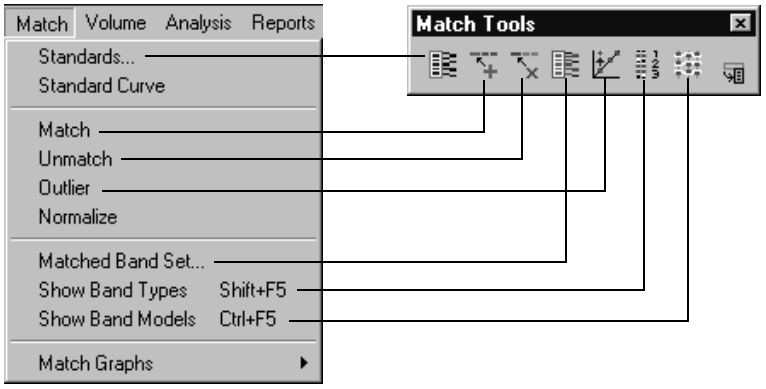


Fig. 6-1. Standards and matching tools.

6.1 Standards

If your gel includes lanes of standards, you can enter the values of the standards and automatically calculate the values of the unknown bands in the gel.

Note: In general, the more standard lanes in a gel, the greater the accuracy of the calculated band values. We recommend a minimum of two standard lanes per gel. Also, the modeling algorithm works best with standard lanes spaced evenly across the gel, as shown in the following figure. Multiple standard lanes also facilitate comparisons of sample similarity.

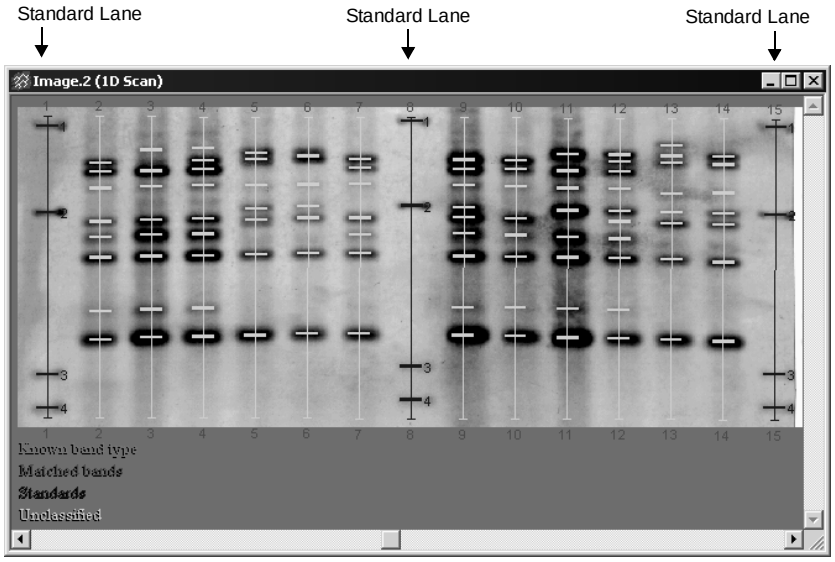


Fig. 6-2. Standard lanes as defined on a gel image.

With the image open and lanes and bands defined, select **Standards** from the *Match* menu or toolbar. A dialog will open, listing any saved standards as well as predefined Bio-Rad standards.

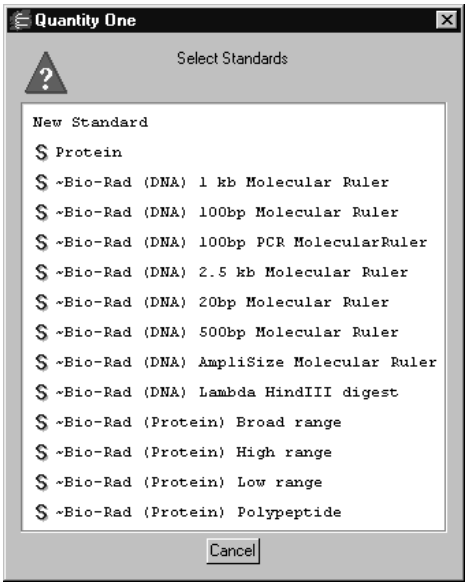


Fig. 6-3. Selecting a set of standards.

Sets of Bio-Rad molecular weight and base pair standards are installed with the software.

6.1.a Selecting Predefined Standards

If you are using Bio-Rad or other predefined standards, select them from the pop-up list. The *Standards* dialog will open (see section 6.1.c, Standards Dialog), displaying the values of the standards.

6.1.b Creating New Standards

To create a new set of standards, select **New Standards**. A dialog will pop up in which you can specify the units.

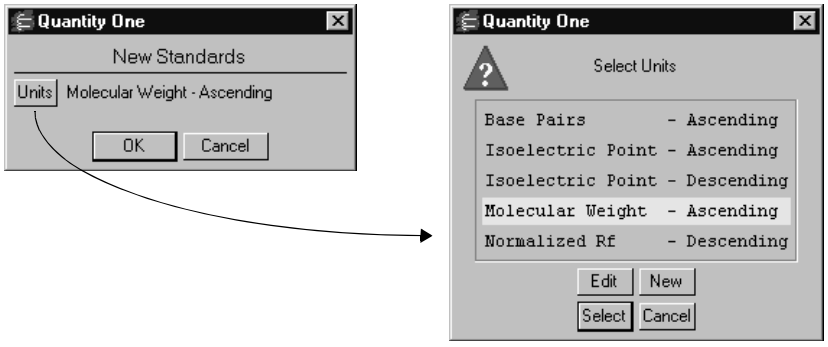


Fig. 6-4. Specifying units.

Click on the **Units** button to specify the units. This opens a dialog in which you can select from a list that includes **Base Pairs, Isoelectric Point, Molecular Weight,** and **Normalized Rf**.

Note: Rf (relative front) expresses the distance a band has traveled down a lane as a fraction of either the total length of the lane or the vertical distance from the top of the lane to the bottom (the calculation method can be specified in the *Preferences* dialog).This provides a generic measure of the positions of bands in lanes. Normalized Rf is derived from relative front, and includes the results of modeling across the gel that comes when multiple lanes of standards are defined on the image. Such modeling is designed to take into account any distortion or smiling across a gel.

To specify a set of units not on the list, click on the **New** button and specify the unit's parameters in the dialog. To edit a set of units, select them and click on **Edit**.

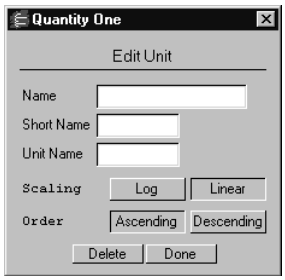


Fig. 6-5. Units dialog.

Note: "Ascending" means that bands of higher molecular weight or isoelectric point are at the top of the gel image, and bands of lower molecular weight or isoelectric point are at the bottom of the gel image.

To select a set of units, select them from the list and click on **Select**, then click on **OK**. The *Standards* dialog will open. Here you can enter values for the standards, apply them to the bands on the image, and save them as a set for future use.

6.1.c Standards Dialog

The *Standards* dialog contains the values of the standards, and includes tools for applying them to the standard lanes in the image and displaying and adjusting the standards regression curve.

The dialog opens with a default name for the standards. For new standards, this is a generic name (e.g., Standards 1). You can enter a new name in the field.

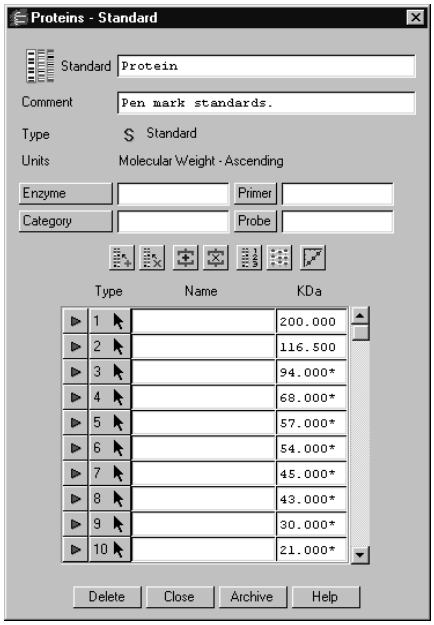


Fig. 6-6. Standards dialog.

You can enter additional information next to the **Comment** prompt. The **<category>** buttons and fields can be used to further define the standards.

Entering Standard Values

If you are creating new standards, enter the values of the standard bands in the table in the middle of the dialog. Predefined standards already have these values entered.

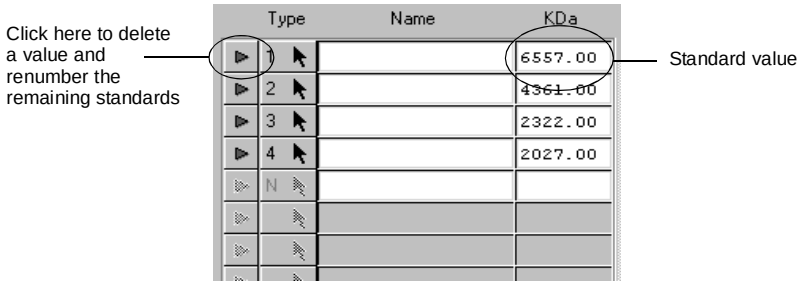


Fig. 6-7. Entering the values of the standards.

The table has three columns, labeled **Type**, **Name**, and the units you previously selected (e.g., KDa, pI, Rf). In the units column, type a value for the first standard band and press the **Enter** key. The cursor will skip to the field below, and you can enter a value for the second standard band. Repeat this process until all the standard values have been entered.

Note: The values do not need to be entered sequentially. They will automatically sort themselves in ascending or descending order, depending on how you specified the units.

You can enter a name for each standard band in the **Name** column. This will appear in subsequent reports and printouts.

To remove a standard value, click on the triangle button at the beginning of the row and select **Delete**. The remaining standards will be renumbered.

Applying Standard Values to Lanes

To apply the values to the standard lanes on the image, click on the **Apply to Lane** button and click on a lane.

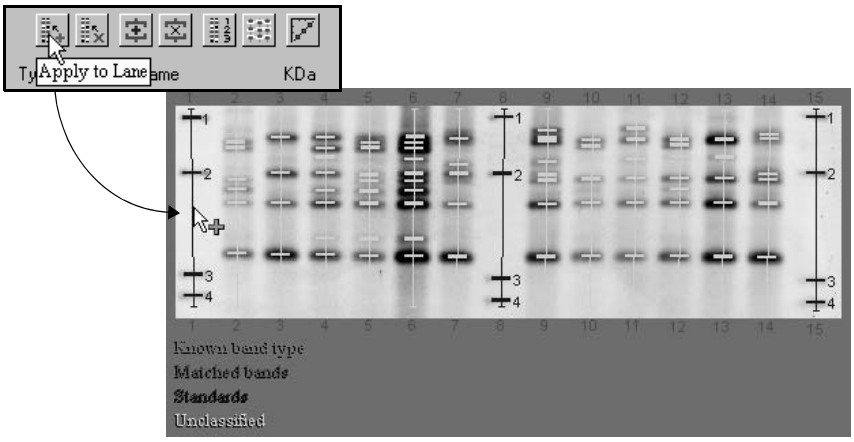


Fig. 6-8. Click on the Apply to Lane button and click on the lanes containing standards.

The values will be applied to the bands in the lane you select. Click on any remaining standard lanes to apply the same values to them.

Note: In general, the more evenly spaced the standard lanes, the greater the accuracy of the calculated band values. We recommend a minimum of two standard lanes per gel. Modeling lines that connect the standard bands in different lanes are used to compensate for any smiling or distortion across the gel.

You can also click on the **Arrow** button next to a standard value to apply that value to a particular band in a lane on the image. Click on the button, then click on the standard band. The remaining bands in the lane will be numbered sequentially based on the initial assignment.

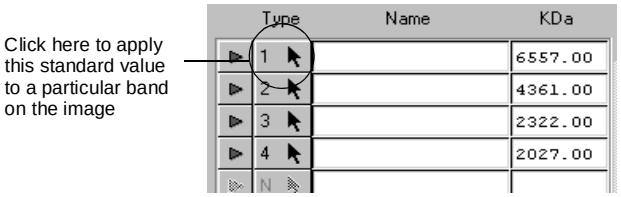


Fig. 6-9. Applying a single standard value.

After you have applied the standard values to a lane, the bands in that lane will change color to blue, indicating that they are now standards.

You are now ready to select the standards regression curve to use for calculating the unknowns.

Removing Standard Values from Lanes

The **Clear from Lane** command removes all the standard values from the lane(s) you select. Click on the button, then click on the lane or lanes from which you want to delete the standard values.

Showing the Modeling Lines

Click on the **Show Modeling Lines** button in the **Standards dialog** to display lines across the gel connecting the same standard bands in different lanes. Unknown bands that fall along these lines have the same values as the standards.

To redisplay only the band numbers with no modeling lines, click on the **Show Band Types** button.

6.1.d Standards Regression Curve

After you have applied the standard values to the image, you are ready to select the regression model to use to calculate the values of the unknown bands. (Note: You must apply the values to a lane before you can view and adjust the regression curve.)

Click on the **Standard Curve** button in the *Standards* dialog, then click on a lane. A graph of the standards regression curve is displayed on the image, and the **Standard Curve Options** dialog is displayed as well.

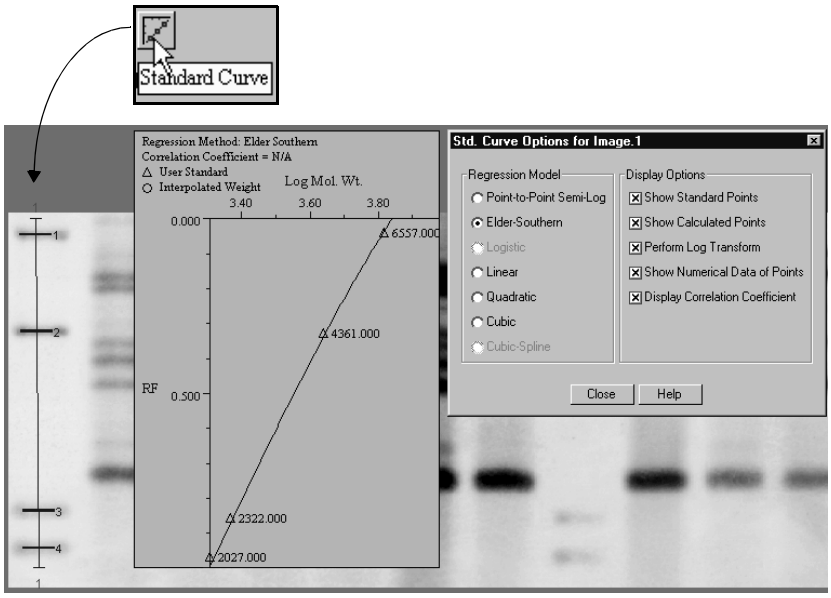


Fig. 6-10. Displaying the standard curve and options dialog.

As you click on different lanes, the curve is displayed for each lane in turn. The X axis is the standard value and the Y axis is the Rf value.

Use the *Std Curve Options* dialog to change the regression model for the curve, as well as various display options.

Standards Regression Models

Select different regression models in the *Std Curve Options* dialog while you study the standard curve with the standard points displayed. Then choose a curve that best

fits the data points. (The correlation coefficient provides another measure of curve fit.)

Note: Note that point-to-point semi-log is the only method available if you perform band matching on the image, because band matching adjusts the positional values of bands in localized areas based on your identification. Point-to-point semi-log is appropriate for this kind of localized variation, whereas the other methods are not. Therefore, you should select point-to-point semi-log if you intend to perform band matching (required for similarity analysis) on the gel.

Point-to-point semi-log. This and the Elder-Southern method are especially useful for describing band migration in static-field electrophoresis gels. This is the only method available if you perform band matching on the image (see note above).

Elder-Southern. This and the point-to-point semi-log method are especially useful for describing band migration in static-field electrophoresis gels. At least three standard points are required to use this method.

Linear. This method of least-squares polynomial fits is useful for modeling pulsed-field electrophoresis gels.

Quadratic. At least three standard points are required to use this method of least-squares polynomial fits.

Cubic. At least four standard points are required to use this method of least-squares polynomial fits.

Logistic. At least five standard points are required to use this method of nonlinear least-squares curve fitting.

Cubic-Spline. At least five standard points are required to use this beta-cubic-spline method.

Display Options

The following options will change how the curve graph is displayed:

Show Standard Points displays the standard data points on the graph. The standard points in that lane will be marked on the graph as triangles. Note that known band types will appear marked as standards.

Show Calculated Points displays the calculated points on the graph. The calculated points in the lane will be marked on the graph as circles.

Perform Log Transform changes the shape of the curve from linear to log. This will not change the calculated values.

Show Numerical Data of Points displays the value of each band on the graph next to its corresponding point.

Display Correlation Coefficient displays the correlation coefficient for the linear, quadratic, and cubic regression models.

Note: The correlation coefficient is a measure of how well the regression model fits the data. It is the square root of the proportion of total variation that can be explained by the regression model. A correlation coefficient of 1.000 would indicate 100 percent certainty of fit.

6.1.e Displaying Calculated Values

To view the calculated values of all the bands in the gel image, select **Band Attributes** from the *Band* menu, then select the value to be displayed (molecular weights, base pairs, etc.). Values can be displayed and printed in report format using the lane and match reports on the *Reports* menu.

6.1.f Saving, Opening, and Deleting Standards

Standards are saved with the image; you can also save copies of them in a separate archive that is available to all images.

To save the standard values with the image, click on the **Close** button to close the Standards dialog, then save the image. When you open the image again, the standards will be available when you select **Standards**.

To save these standards for use on other images, click on the **Archive** button. The standards will be saved in an archive file separately from the image.

To use a set of archived standards, open any image, then choose **Standards** from the menu or toolbar and select the archived standards. They will be imported into the image.

To delete a set of standards you have created, open them, then click on the **Delete** button at the bottom of the dialog. A pop-up box will ask you to confirm that you want to proceed with the deletion before completing the action. This will delete the standards from both the image and the archive.

To modify a set of standards you have created, open them, make your changes, then save the image and (if desired) archive the new standards.

Read-Only Standards

You can make archived standards read-only. Read-only standards cannot be deleted from or modified in the archive using the methods described above; they can still be deleted from or modified in the image.

To make standards read-only, insert a tilde character (~) in front of the name of the standards, then click on the **Archive** button. These standards will always be available under that name in the list of standards.

All Bio-Rad standards are read-only.

Disabling/Deleting the Archive

To delete the archived standards (including Bio-Rad standards), remove the **oneprefs.dbs** database from the **fixed.prm** folder on your hard drive. Under Windows, this folder is located in the Bio-Rad/Program Files/The Discovery Series directory. On the Macintosh, this file is located in The Discovery Series folder in the Preferences folder in the System Folder.

After you remove this file, a new, empty **oneprefs.dbs** database will be automatically created the next time you open an image. You can use this to begin a new archive.

6.2 Band Matching

To compare the similarity of samples in a gel (using the phylogenetic tree, similarity matrix, etc.), you must match bands across lanes using the commands on the *Match* menu and toolbar.

Note: If you have run standards on the gel, you should define them before proceeding as described in the previous section. Multiple lanes of standards will facilitate the band matching program; however, they are not required.

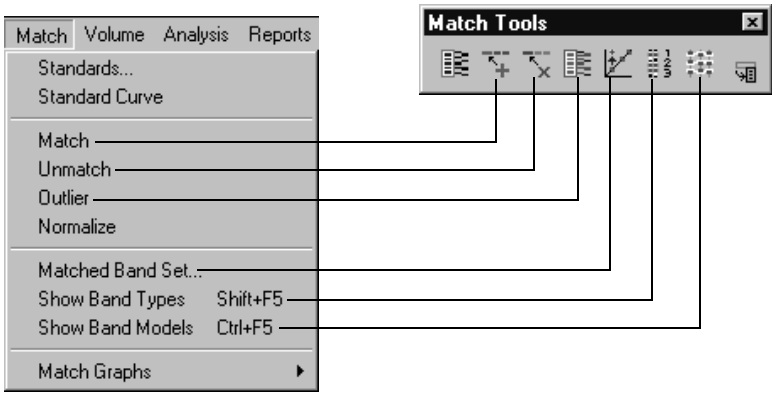


Fig. 6-11. Matching tools.

Select **Match** from the menu or toolbar and click on a representative experimental lane in the gel image. This may be a lane that contains most or all of the bands that you are interested in, and/or a lane in which the bands are particularly well-resolved. Each band in this lane will be designated as a different "band type."

Note: If you have defined standards in the gel, a pop-up box will warn you that the regression model for calculating band values will be restricted to point-to-point semi-log. If you selected a different regression model when defining standards, it will be changed.

The first time you click on an experimental lane with the **Match** command, a pop-up box will prompt you to specify the matching tolerance.

Fig. 6-12. Query box: Apply matching to the whole lane?

Tolerance is the minimum spacing that the matching model expects to find between unique bands. It is expressed as a percent of lane height. You can enter a value between 0.2 and 10 percent. If the bands are very close together, enter a tolerance of 2.5 percent or less.

After you select a tolerance, click on **Yes** to automatically match all bands across the lanes. (Click on **No** to match only the specific band you clicked on.)

When you click on **Yes**, the bands in the lane you selected will change to green, indicating that they are known band types that have been identified by you. A band type number will appear next to each band.

The automatic matching mechanism will attempt to match the bands in the other lanes to the known band types. Matched bands are labeled in red, with the number of the band type appearing next to each band. These matched bands are connected by modeling lines.

Yellow bands are bands that the software cannot accurately match. The matching algorithm is deliberately conservative to avoid incorrect labeling, so a number of yellow bands may appear on the image.

Your next step will be to identify the yellow bands as either new band types, or existing band types that could not be automatched.

To summarize:

- **Green band**s are known band types.

- **Red bands** are bands that have been automatically matched with the known band types.
- **Yellow bands** are bands that have not been matched and are unclassified.

Displaying Band Types and Modeling Lines

To display the band type numbers on the image, select **Show Band Types** from the *Match* menu or toolbar.

Band type modeling lines reveal the path along the gel image that the software uses to match bands of the same type in different lanes. These lines are based on the positions of the known (green) bands and any standards you may have defined.

To display the band type modeling lines, select **Show Band Models** from the *Match* menu or toolbar.

6.2.a Editing the Results of Band Matching

After you have matched bands automatically, you can manually indentify the remaining unknown bands as new or existing band types. Use the tools on the *Match* menu and toolbar to manually identify new bands, unmatch bands, or identify bands as outliers from the band set.

Note: The modeling lines are designed to give you guidance on identifying new bands.

- To identify an unknown (yellow) band as a new band type, select **Match** and click on the unknown band.
- To match a band to a particular band type, select **Match** and first click on the identified red or green band. Then click on the red or yellow band you want to match. The band will appear green (known) and the modeling line will change to reflect the match.
- If a matched (red) band in a lane is in fact a new band, select **Match**, hold down the **Shift** key, and click on the red band.
- To change a matched band to unknown (yellow), select **Unmatch** and click on that band.
- To remove a green band from the modeling, select **Outlier** and click on that band. An X will appear through the band, indicating that the software is ignoring it when auto-matching bands across the gel image.

Manually match bands until all the bands in the image are identified. The gel should have no yellow (unknown) bands, and the modeling lines should intersect at or near the middle of the bands across the gel. If the modeling lines are not parallel, the lanes may be warped or distorted and thus difficult to compare using the automatic analysis features.

6.2.b Band Set Dialog

The *Band Set* dialog contains the values of all the experimental bands in a gel, a tool bar for band matching, and other information about the bands.

To open the dialog, select **Matched Band Set** from the *Match* menu or toolbar and click anywhere on the image.

The dialog will open with a default name for the band set (e.g., Band Set 1). Enter a new name at the top of the dialog, and add any comments or category/attribute information you want to associate with the band set.

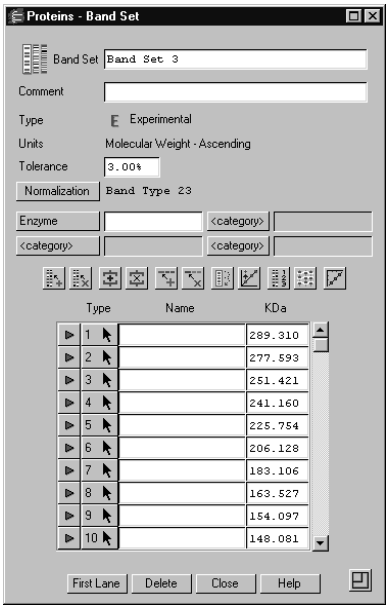


Fig. 6-13. Band Set dialog.

List of Band Types

The values of the individual bands are listed in the dialog. These values are based on any standards you have defined and the band matching. If you have not defined standards, normalized Rf units are used.

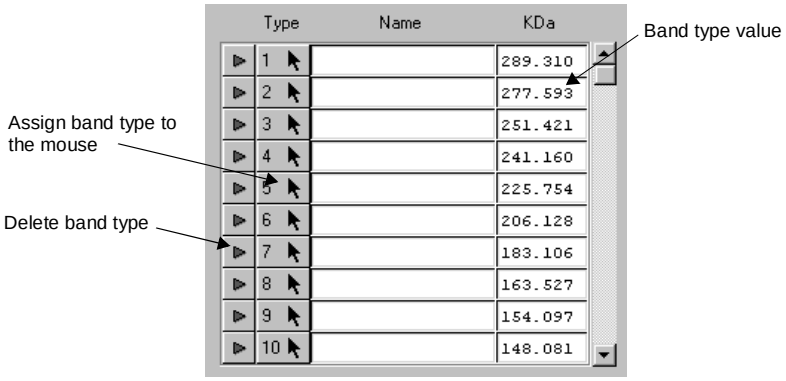


Fig. 6-14. Applying and editing band type values.

If you change any of the values in the list, that will be reflected in the matching on the image.

Click on the numbered **Arrow** button next to a band value and click on a band in the gel to identify that band.

You can enter names for the band types in the **Name** column. These will appear in subsequent reports and print-outs.

To remove a band from the set, click on the triangle icon next to the band value. Confirm the deletion, and the remaining band types will be renumbered.

Band Set Toolbar

The toolbar in the **Band Set** dialog contains all the commands needed for matching.

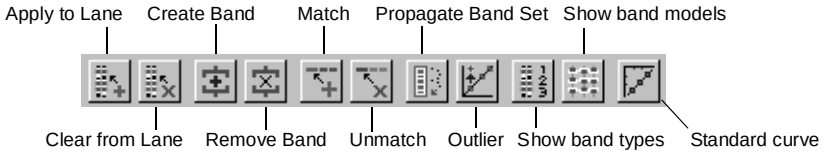


Fig. 6-15. Band set toolbar.

Apply to Lane applies the values in the band set to any lane in the gel. Click on the button, then click on the lane. The bands in that lane that can be matched will change to red.

Clear from Lane removes the identified values from any lane in the gel. Click on the button, then click on the lane to be cleared. The bands in the lane will change to yellow.

Create Band and **Remove Band** are standard band commands that have been included in the toolbar for convenience (see section 5.3.a, Identifying Individual Bands).

Match is used to identify a new band. Click on the button, then click on the unknown (yellow) band to identify it and add it to the set.

Unmatch removes the identification of a band. Click on the button, then click on the matched band to change it to unknown.

Propagate Band Set applies all the values in the set to the bands in a lane, based on a few identified bands in the lane. Click on this button, then click on the lane. The bands in that lane that can be matched will change to green to indicate their known status.

Outlier excludes a known (green) band from the band set model. However, the band will still be marked as known.

Show Band Types displays all the red, green, and yellow bands on the image, with the band type numbers next to the matched bands.

Show Band Models displays the modeling lines across the gel image.

Standard Curve displays the *Standards Regression Curve* (see section 6.1.d, Standards Regression Curve). Click on the button, then click on any lane in the image.

Other Band Set Functions

The units of the bands are displayed in the top half of the dialog, as is the matching tolerance used. Tolerance is the minimum spacing between unique bands that you specified when you created the band set.

The **Normalization** button allows you to pick a specific identified band that appears in all the lanes to normalize the relative quantities of the other bands against (see section 11.1, Differential Display for more information).

Click on the resize button in the lower right corner to reconfigure the dialog to its smaller, palette version, which displays only the tool buttons and band type buttons.

To delete the band set, click on the **Delete** button.

To close the band set, click on **OK**.

Note that the band set is saved when you save the image.

6.2.c Tips for Gels Without Standards

If you are not using standards, we recommend that you load at least two lanes per gel with a reference sample containing many if not most of your experimental bands of interest.

Select the **Match** command from the menu or toolbar, and click on this reference sample lane to create a new band set. Then select **Matched Band Set** from the *Match* menu to open the *Band Set* dialog, and apply that band set to other reference sample lanes using the **Propagate Band Set** command.

Propagate Band Set is a feature that not only simplifies identifying bands, it allows the software to do some optimizations that will significantly speed up modeling. Choose another reference sample lane that you want to apply the band set to. Start by using the **Match** command to identify one or two bands in the lane, then click on the **Propagate Band Set** button and click on the lane to assign the remaining band types to the lane.

Once the reference sample lanes have been modeled, identify any unknown bands using the methods outlined in the previous sections.

6.2.d Normalizing for Quantity

You can normalize the quantities of the bands in a gel to the quantity of a particular identified band that appears in all lanes. This is useful if you have loaded different amounts of sample in each lane.

Note: Quantity normalization is required for calculating Differential Display.

Select **Normalize** from the *Match* menu and click on a matched band that appears in *every lane*. (If the band is not present in a lane, the normalized quantity for that lane will be zero.) The quantity of that band will be set to 100 in each lane, and the quantities of the other bands will normalized to that band.

You can view the normalized quantities using the *Band Attributes* dialog or in various reports.

6.2.e Graphs of Match Data

You can display graphs of different kinds of data associated with the matched bands. The commands for displaying these are located on the *Match > Match Graphs* submenu.

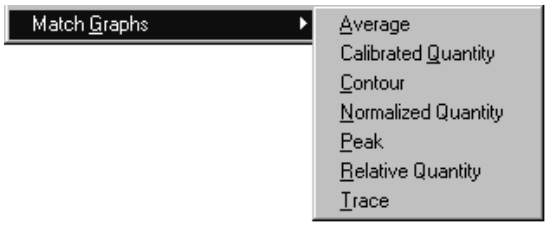


Fig. 6-16. Match Graphs submenu.

From the *Match Graphs* submenu, select a type of graph and click on a matched band. The bands in the matched group will be displayed along with the selected graph.

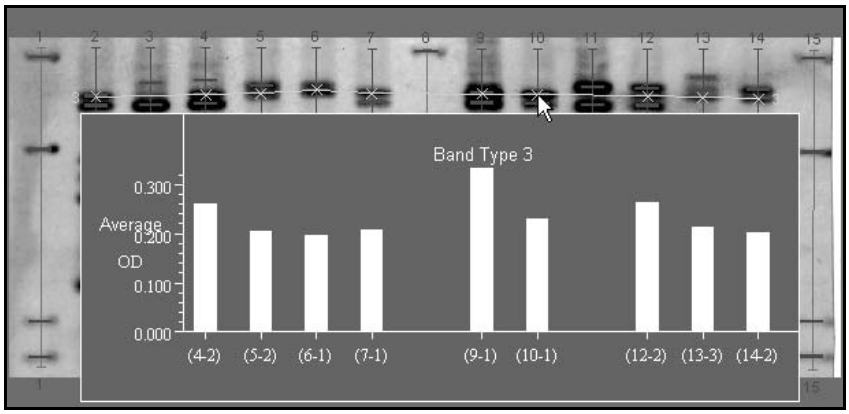


Fig. 6-17. Example of a match graph.

- **Average** displays a histogram of the average densities of the bands in a band type.
- **Calibrated Quantity** displays a histogram of the quantities of the bands in a band type as calculated from a calibration curve (see section 6.3, Quantity Standards).
- **Contour** displays a histogram of the intensity of each contoured band in a band type. This function only works with contoured bands.
- **Normalized Quantity** displays a histogram of the normalized quantities of the bands in a band type. See section 6.2.d, Normalizing for Quantity, for instructions on how to normalize for quantity.
- **Peak** displays a histogram of the peak intensities of the bands in a band type.
- **Relative Quantity** displays a histogram in which each bar represents the quantity of the band in a lane as a percentage of either (1) the total intensity data in the band's lane, or (2) the total intensity of all the bands in the band's lane. The calculation method (**% of Lane** or **% of Bands in Lane**) is set in the *Preferences* dialog.
- **Trace** displays a histogram of the trace quantities of the bands in a band type group.

Each bar of the histogram is labeled on the X axis with the lane number, and (where space permits) the band number. The Y axis is labeled with quantitative values. If the bands in the match group span the entire image window, the histogram will not include bars for the bands in the left-most lanes because of the space required for labeling the axis.

To avoid this problem, decrease the size of the image using **Zoom Out** until there is blank space between the left side of the window and the first band of the match group. Redisplay the histogram. If one or more bars are still not included, continue decreasing the magnification until you can see all the data.

6.3 Quantity Standards

From bands of known quantity, you can generate a calibration curve for determining the quantities of all the bands in lanes or cells in lane-based arrays. (To quantitate bands outside of lanes, see Chapter 7.)

Using the *Quantity Standards* dialog, you plot the quantities of the known bands against their intensities to generate a calibration curve. You then apply this curve to unknown bands in the current gel as well as other gels.

To create a calibration curve, the quantities of at least two bands must be known. The greater the number of known bands and the wider the range of their values, the more accurate the calibration curve will be.

Note: The band intensities calculated by Gaussian fitting (see section 5.7, Gauss-Modeling Bands) cannot be used in conjunction with **Quantity Standards**. However, you can continue to use the trace intensity (i.e., the area under a band's intensity profile) to calculate **Quantity Standards** after you have Gauss-modeled your bands.

6.3.a Creating and Applying a Set of Quantity Standards

Select **Quantity Standards** from the *Analysis* menu. A pop-up box will prompt you to create a new curve or load a saved calibration curve.

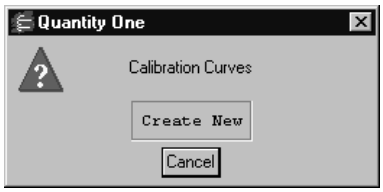


Fig. 6-18. Loading a quantity calibration curve.

Select **Create New** to open a blank *Quantity Standards* dialog.

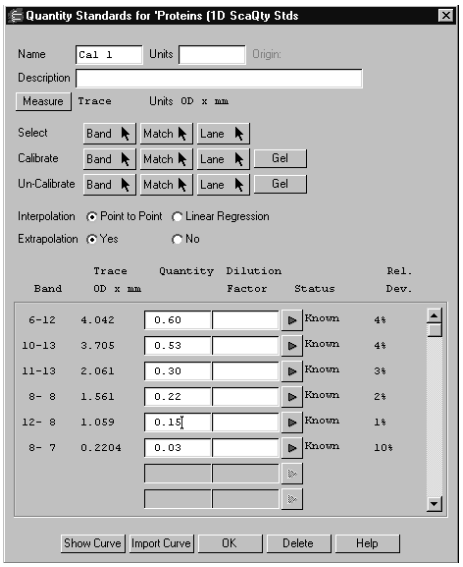


Fig. 6-19. Quantity Standards dialog.

The dialog will open with a default name for the quantity standards (e.g., Cal 1). Enter a new name, specify the quantity value units (e.g., µg), and enter any descriptive information in the appropriate fields at the top of the dialog.

The calibration curve is generated by plotting the quantities versus the intensities of the known bands. The intensities of the bands can be measured in several different ways. Click on the **Measure** button to display a list.

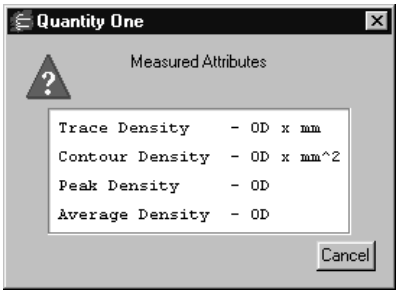


Fig. 6-20. Band attributes to measure for relative quantity.

The options in the *Measure* dialog are:

- **Trace density**—The intensity of a band as measured by the area under its intensity profile curve. Units are intensity x mm.
- **Contour density**—The intensity of a band that has been defined using the **Contour** or **Draw Band** tools (see section 5.8, Irregularly Shaped Bands in Lanes). It is the sum of the intensities of all the pixels inside the band boundary multiplied by the area of each pixel. Units are intensity x mm^2.
- **Peak density**—The intensity value of a band's peak.
- **Average density**—The total intensity of the rows of pixels used to generate the profile of a band, divided by the number of rows.

Identifying the Known Bands in the Gel

The dialog includes three buttons that can be used to select the known bands in the gel:

- To select bands one at a time, click on the **Band** button, then click on each band of known quantity. Each band will be highlighted.
- If all of the known bands are the same band type, click on the **Match** button, then click on one of the bands. The matched group of bands will be highlighted.

- If all of the known bands are in one lane, click on the **Lane** button, then click on the lane with the known quantities. The entire lane will be highlighted.

Fig. 6-21. Buttons for selecting bands of known quantity.

In the lower part of the *Quantity Standards* dialog, the lane and band numbers of the selected bands will appear in the **Band** column. The intensity of each band will also be listed.

Entering the Quantities

Enter the quantity of each known band in the **Quantity** column. When you enter a quantity, the value of the band in the **Status** column will change from **Unknown** to **Known**.

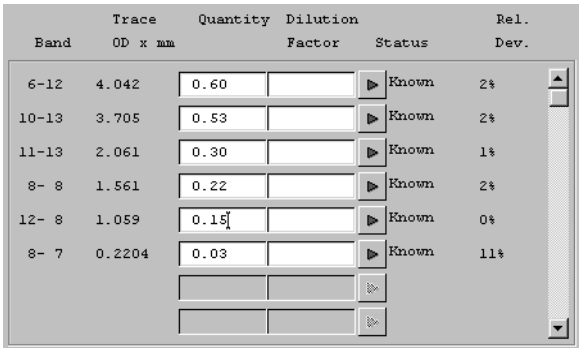


Fig. 6-22. Entering the known quantities.

After you have entered a few quantities, the status of the remaining bands in the list may change to **O.R.**, meaning that the remaining bands are out of the current range of values (based on their intensities and what you have already entered).

Alternatively, the software may automatically calculate an unknown quantity (if it is between two known quantities) and enter a value for it; in this case the **Status** column will indicate that the quantity has been calculated (**Calc.**)

In either case, you can type a quantity directly into the **Quantity** column and the status will change to **Known**.

Relative Deviation of Known Quantities

After three values have been entered, the relative deviations of those bands are automatically calculated and displayed in the **Rel. Dev.** column. The relative deviation is calculated from the known value that you entered and the back-calculated value from the calibration curve.

If the deviation value is too high, you can exclude a band from the calibration curve. Click on the arrow button next to the problem band. In the pop-up box, select **Remove** to remove the band from the *Quantity Standards* dialog. All the information about that band will be deleted.

Alternatively, select **Outlier** from the pop-up list to retain the information about the band in the calibration file but exclude it from the calibration curve.

6.3.b Calibration Curve

Interpolation and Extrapolation

There are two methods for calculating the calibration curve:

- **Point to Point** generates a curve in which each data point is connected directly to the next, regardless of the shape of the resulting curve.
- **Linear Regression** (using the method of Least Squares) generates a smooth curve that is the “best fit” of the values you provided.

Select the preferred option next to **Interpolation** in the dialog.

Next, indicate whether the curve should be extrapolated beyond the highest and lowest known values by selecting **Yes** or **No** next to the **Extrapolation** prompt. Note that values extrapolated from the **Point to Point** curve may be unreliable.

Displaying the Calibration Curve

To display the calibration curve, click on the **Show Curve** button at the bottom of the *Quantity Standards* dialog. A graph of the known quantities versus measured intensities will be displayed in a separate window.

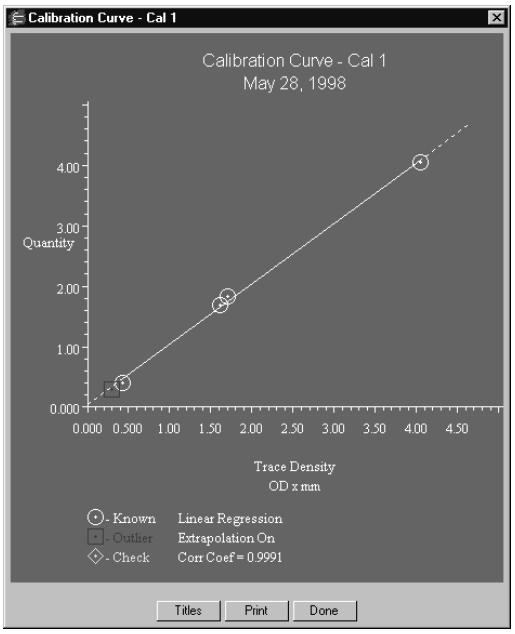


Fig. 6-23. Calibration curve with linear regression and extrapolation.

Known values used to calculate the curve are marked by circles. Values identified as outliers are marked by squares.

- To change the status of a point on the graph, click on it. The status will toggle between **Known** and **Outlier**.
- To display a legend at the bottom of the graph, click on **Titles**.
- To print the graph, click on **Print**.
- To close the graph window, click on **Done**.

6.3.c Applying the Calibration Curve

After you have generated a calibration curve, you are ready to calculate the quantities of the unknown bands. Select a button next to **Calibrate** to calculate the quantity of an individual band, match, lane, or the entire gel.

Fig. 6-24. Buttons for calibrating bands of unknown quantity.

If you select **Band, Match**, or **Lane**, click on the button and then click on the object in the gel. Click on **Gel** to calculate the entire gel. Bands of calculated quantity are highlighted in the image.

Unapplying the Calibration Curve

To undo the quantity calculation for a band, match, lane, or gel, use the appropriate button next to **Un-Calibrate**.

6.3.d Generating Standard Bands via a Dilution Series

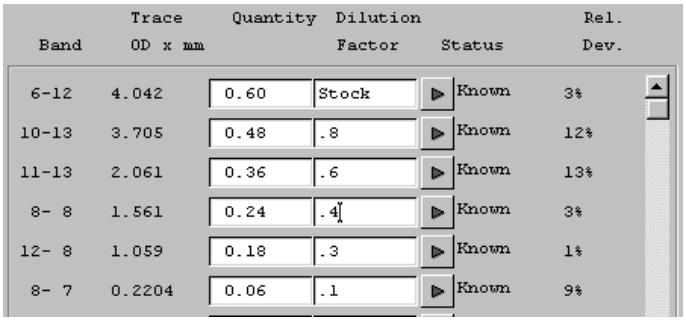
One way to generate bands of known quantity is to start with a stock solution and make several dilutions. Different dilutions can be loaded into different lanes, resulting in a dilution series.

Quantity One will calculate the values in a dilution series if you enter the known quantity and a dilution factor (e.g., for a solution that has been diluted to 10 times the volume, type 1/10 or 0.1).

In the *Quantity Standards* dialog, next to the band from the undiluted stock solution, enter the known quantity.

In the **Dilution Factor** column, type “stock.”

Next to the remaining bands in the series, enter the appropriate dilution factors in the **Dilution Factor** column.



Band	Trace OD x mm	Quantity	Dilution Factor	Status	Rel. Dev.
6-12	4.042	0.60	Stock	Known	3%
10-13	3.705	0.48	.8	Known	12%
11-13	2.061	0.36	.6	Known	13%
8- 8	1.561	0.24	.4	Known	3%
12- 8	1.059	0.18	.3	Known	1%
8- 7	0.2204	0.06	.1	Known	9%

Fig. 6-25. Entering a dilution series.

The quantity of each band will be automatically calculated.

6.3.e Importing a Calibration Curve

A calibration curve created for one gel can be applied to other gels.

Make sure that the new gel you want to quantitate and the gel with the existing calibration curve are both open.

Click on the new gel to quantitate. Select **Quantity Standards** from the *Analysis* menu and click on **Create New**, as previously described.

In the new *Quantity Standards* dialog, click on the **Import Curve** button, and select the existing calibration curve from the list. When you make the selection, the values for the curve will be displayed in the new *Quantity Standards* dialog. Each standard value will be labeled **Import** in the **Band** column.

Checking the Imported Curve

If the quantity of one or more bands in the new image is known, you can verify the accuracy of the imported calibration curve. Go to **Select** in the *Quantity Standards* dialog, click on **Band**, then click on a known band. Its lane number, band number, and intensity will be displayed in one of the standard values fields.

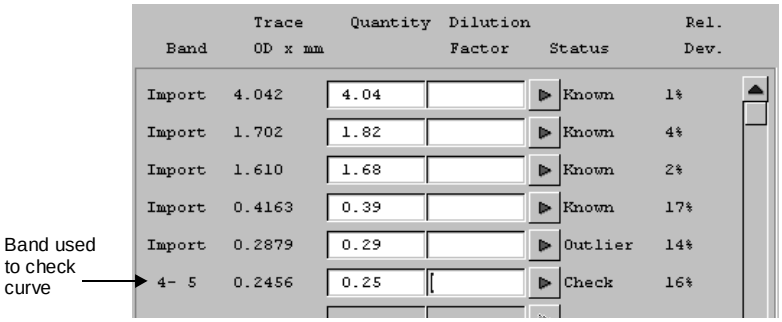


Fig. 6-26. Checking an imported calibration curve.

When you enter the band's value, its status will change to **Check**, indicating that it is used to verify the accuracy of the calibration curve and is not used in calculating the curve itself.

Click on the **Show Curve** button to display the graph, and note that the **Check** bands are enclosed in diamonds. If the **Check** bands *do not* fall on or very near the calibration curve, we recommend that you *do not use* the imported standards for this gel.

7. Volume Tools

You can use the **Volume** tools to quantitate bands, spots, arrays, and other image data.

What is a Volume?

A volume is the total signal intensity inside a defined boundary drawn on an image. To measure the amount of a particular object (e.g., a band or spot), you draw a volume rectangle, contour, free hand, or circle around the object and compare the intensity data inside the boundary with the data of other objects or a standard using the **Volume Analysis Report** and **Volume Regression Curve** (see section 12.6, Volume Analysis Report).

Volume = Sum of the intensities of the pixels within the volume boundary x pixel area

Volume units = intensity units x mm^2

Volumes are similar to band contours (see section 5.8.a, Contouring Bands), except that they are not dependent on lanes and bands.

7.1 Creating a Volume

To create a volume, select **Volume Tools** from the main toolbar or the *Edit* menu. These commands are also located on the *Volume* menu.

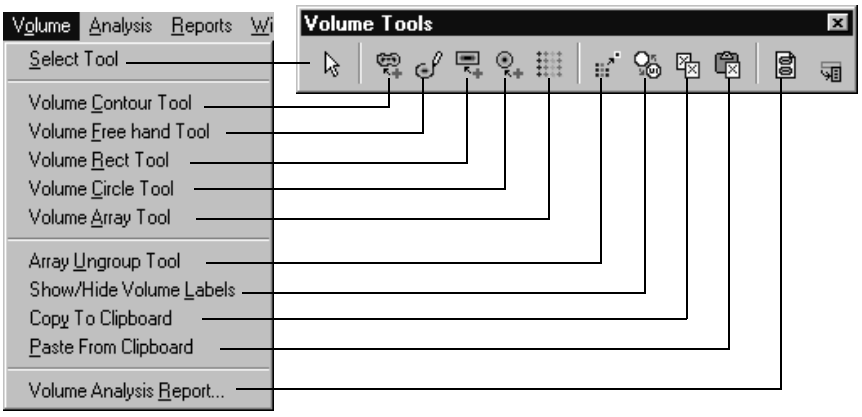


Fig. 7-1. Volume tools.

Note: When using any of the following tools, be careful to completely surround the data you want to quantitate. You should also adjust for background intensity (see section 7.6, Volume Background Subtraction). You may want to experiment with several different volumes drawn around the same object before selecting the one that gives you the best quantitation data.

Volume Contour Tool

Use the **Volume Contour** tool to quickly create a volume boundary that follows the outer edge of the object you want to quantify. To use this tool, first magnify the object, then click on the **Volume Contour** button. Using the tool:

- *Click* on a pixel at the edge of a band or other object to create a contour that encloses pixels of equal or greater intensity.
- *Drag* to create a contour that changes as you move over pixels of different intensity. Drag from inside the object outward until the contour follows the outer edge of the object. When you release the mouse button, the volume is created.

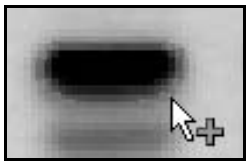


Fig. 7-2. Volume contour.

The contour should completely surround the data you want to quantify.

To edit the contour, position the cursor on the border. The cursor will change to a pencil tool. Drag across the line; a new white line will appear. When you recross the old line, a new contour will be created.

Volume Free Hand Tool

Use the **Volume Free hand Tool** to manually draw a volume boundary. First magnify the band or other object (you must be able to see the individual pixels). Then click on the **Volume Free hand** button and use the cursor to draw a line around the object. When the line crosses itself, a free hand volume is created.

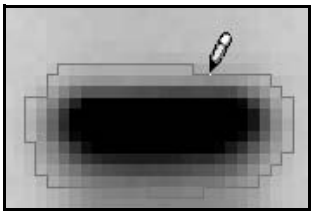


Fig. 7-3. Volume free hand.

If you make a mistake while drawing, backtrack with the mouse. The line you draw should completely surround the data you want to quantify.

To edit the volume, position the cursor on the border and drag across the line; a new white line will appear. When you recross the old line, a new free hand volume will be created.

Volume Rect Tool

Use the **Volume Rectangle Tool** to create a volume box around an object. Click on the **Volume Rect** button, then drag a box around the object to be quantified. When you release the mouse button, the volume is created.

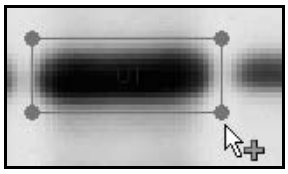


Fig. 7-4. Volume rectangle.

To *resize* the box, click on it to select it, then position the cursor on one of the corner anchor points and drag.

To *rotate* the box, click on it to select it, then hold down the **Shift** key while dragging an anchor point. The volume will pivot around its center. This is useful if the object is lying at an angle—for example, if the gel is smiling.

Volume Circle Tool

Use the **Volume Circle Tool** to create a circular boundary around an object (such as a spot). To use this tool, click on the **Volume Circle** button, then position the cursor at the center of the object to be quantified and drag outward. As you drag, a circle will appear. When you release the mouse button, the volume is created.

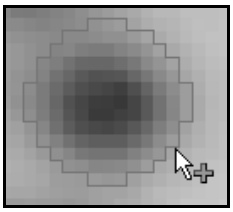


Fig. 7-5. Volume circle.

The volume circle should completely surround the data you want to quantify.

To resize the circle, click on it to select it, then position the cursor on the circle border and drag.

7.2 Volume Labels

Volume labels help to identify the type of the volumes you create. As you create each volume, it is given a default Auto label. The default labels are: U1, U2, U3, etc. The "U" stands for unknown, as distinguished from standard (Std) and background (B) volumes. The number indicates the sequence in which the volume was created. To change the volume type, double click the volume to open the Volume Properties dialog box.

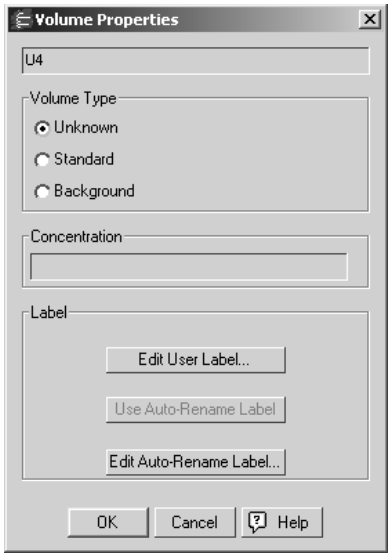


Fig. 7-6. Volume Properties dialog box.

At the top of the Volume Properties dialog box is the current label for the selected volume. Volume Type lists the types of volumes: Unknown, Standard, and Background. The type of the current volume is indicated by the selected radio button. Choose a new type by clicking a different radio button. If you choose Standard as the new volume type, you must enter a concentration greater than zero in the Concentration field.

Note: If you change a volume's type (e.g., change an unknown to a standard), any subsequent volumes of the original type will be renumbered. For example, if you create volumes U1 and U2, and then designate U1 as a background volume, U2 will be renumbered U1.

7.2.a Editing Volume Labels

In the **Volume Properties** dialog box you can choose to give the selected volume a User label as well as modify the style of the Auto labels.

Editing User Labels

To create or edit a User label for the selected volume, click **Edit User Label** to open the **Text Overlay Properties** dialog box. In the **Text Overlay Properties** dialog box you can change the, font, size, color, orientation, and background of the selected text. The text field displays the current volume label. To change the text of the label, type a new label in the text field.

Note: A change to the text of the label will not cause a renumbering of unedited volume labels. However, if you change the type for a particular volume, this will renumber the subsequent volumes without changing the text or style of the User label.

If you want to remove the User label and return the Auto label for the selected volume, click Use Auto Label. Note that changes do not take effect until you click Done. So if you find you want to keep the User label, click Cancel instead.

Editing Auto Labels

To modify the style of the Auto labels, click Edit Auto Labels. Like the Edit User Label tool, this opens the **Text Overlays Properties** dialog box. Use the options in

the **Text Overlays Properties** dialog box to modify the style of the Auto labels. Note that you must first select the text in the text field before making any changes.

Note: Editing the Auto labels affects only the style of the labels, not the text or numbering of the Auto labels.

Modifying the style of your Auto labels does not affect the style of any existing User labels. To modify the styles for your User Labels, you must modify each individually. See Editing User Labels for further information.

When you first create volumes, they appear labeled. Click **Show/Hide Volume Labels** on the Volume menu or toolbar to hide the labels.

7.3 Volume Features

Each new volume you create initially has a green border, which indicates that the volume is selected. If you click elsewhere on the image, the border will change to blue, indicating that the volume is deselected.

To reselect the volume, click on it again. If you move the cursor over the volume, selected or not, the border changes to gold.

After you create a volume, you can view the volume data (area, density, etc.) by selecting the **Volume Analysis Report** from the *Reports* menu.

Tips

The volume you draw should completely surround the data you want to quantitate. You should also adjust for background intensity

You may want to experiment with several different volumes drawn around the same object before selecting the one that gives you the best quantitation data.

Displaying Volumes

To display previously created volumes after opening an image, select **Volume Tools** from the *Edit* menu or main toolbar.

If you have concealed all overlays using **Hide Overlays**, click on any button in the *Volume* toolbar to display the hidden volumes.

7.4 Moving, Copying, and Deleting Volumes

You can move, copy, or delete a single volume or group of volumes within an image. You can also copy and paste volumes between images.

First, select the volume(s). Click on the **Select Tool** button on the *Volume* toolbar. To select a single volume, click on it. To select multiple volumes, either drag a box around them or hold down the **Shift** key while you click on them one at a time. When you drag to select a group of volumes, make sure that you completely surround all the volumes.

Each selected volume will have a green border.

- To *move* the selected volume or volumes, position the cursor over the selection and drag.
- To *copy within an image,* hold down the **Ctrl** key while dragging the selected volume or volumes. The copy will be created and dragged to the new position.
- To *delete* the selected volume or volumes, press the **Delete** key.
- To *copy between images,* click on the **Copy to Clipboard** button on the *Volume* toolbar, then open or select the image you want to copy to and click on the **Paste from Clipboard** button. The copied volume(s) will be pasted into the new image in the same relative position it was copied from.

Note: If you are copying to an image with a different pixel size (i.e., resolution), you will receive a message that the placement of the copy may not be exact. Click on **OK** to complete the paste, then position the pasted objects manually.

7.5 Volume Standards

You can use volumes of known concentration to calculate the concentrations of unknown volumes.

To classify a particular volume as a standard, double-click on it. This will open the *Volume Properties* dialog.

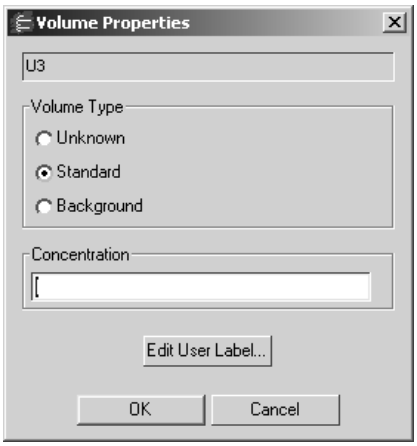


Fig. 7-7. Volume Properties dialog.

Select the **Standard** option button, then enter the concentration in the **Concentration** field. (Do not include units.) Click on **OK** to close the dialog.

Standard volumes have the default names S1, S2, S3, etc., based on their creation sequence. Display or hide volume names using **Show/Hide Volume Labels** command as previously described.

After you have identified two or more standards, you can use the **Volume Regression Curve** (see section 12.7, Volume Regression Curve) under the *Reports* menu to calculate the concentrations of the unknown volumes.

Note: Volumes that fall above the greatest standard value, or below the lowest standard value, cannot be guaranteed for accuracy.

To change a standard back to an unknown, double-click on it, then select the **Unknown** button.

7.6 Volume Background Subtraction

When you draw a volume, you will probably include some background pixels inside the volume. These background pixels will usually have an intensity value that you do not want to include in the volume quantitation. There are two ways of calculating this background intensity: local and global.

The background subtraction method is selected in the *Volume Report Options* dialog (see section 12.6.a, Volume Report Options).

Local Background Subtraction

Local background subtraction calculates a separate background intensity for each unknown and standard volume you create. For each volume, the intensities of the pixels in a 1-pixel border around the volume are added together and divided by the total number of border pixels. This gives an average intensity for the background around each volume, which is then subtracted from the intensity of each pixel inside the volume.

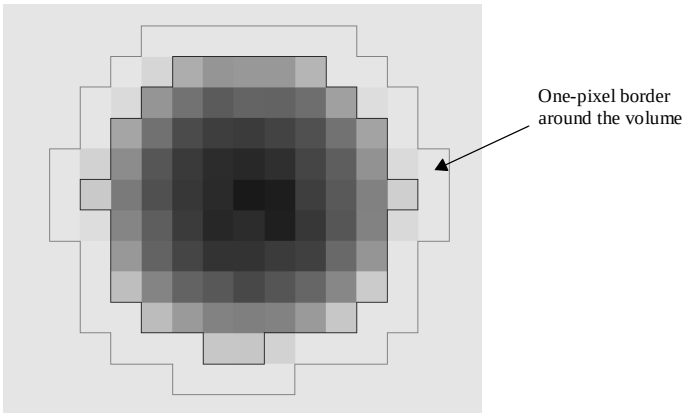


Fig. 7-8. Local background is calculated from a one-pixel border around the volume

Any pixels inside the volume that have the same intensity as the background pixels will be reduced to zero, thereby eliminating them from the quantitation.

Global Background Subtraction

Note: If you select **Global Background Subtraction** in the *Volume Report Options* dialog, but do not define a background volume as outlined below, you will effectively select no background subtraction.

Global background subtraction calculates a single background intensity for the entire gel. This average background intensity is then subtracted from all the volumes in the gel. The steps for calculating global background subtraction are:

1. Create a volume using one of the volume tools in a representative background region of the image (i.e., a region where there is no data and where the average pixel intensity appears to be the same as the background intensity surrounding your data).

2. Double-click on the volume to open the *Volume Properties* dialog, and select the **Background** option button.

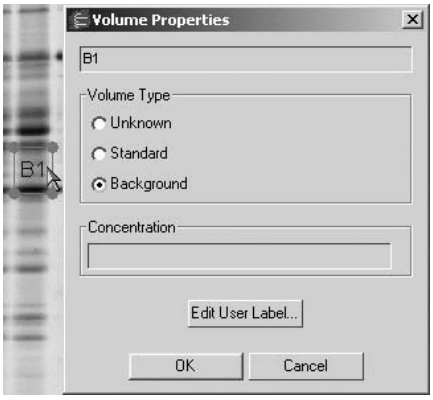


Fig. 7-9. Defining a background volume object.

The average intensity of the pixels in the background volume will be calculated and subtracted from each pixel in all standard and unknown volumes. Any pixels inside the volumes that have the same intensity as the average background will be reduced to zero, thereby eliminating them from the quantitation.

If you create more than one background volume, all the pixels in those background volumes will be used to calculate the average background.

Background volume(s) will have default names B1, B2, etc., based on their creation sequence. You can display/hide volume names using the **Show/Hide Volume Labels** command.

Note: If the region you identified as background has a higher average intensity value than a data object, that object will have a negative adjusted volume in the *Volume Analysis Report*. If this happens, select a new background region that has less intensity than the data object.

Displaying the Results of Background Subtraction

The *Volume Analysis Report* (see section 12.6, Volume Analysis Report) will display both the unadjusted volume and the volume with background subtracted (adjusted volume) of standards and unknowns, so you can see exactly how much intensity was subtracted.

7.7 Volume Arrays

The **Volume Array Tool** on the *Volume* menu and toolbar can be used to quantitate dot blots, slot blots, and other arrays.

Note: You cannot create a volume array in an image with asymmetric pixels (i.e., different dimensions in x and y). If you want to create a volume array in such an image, select **Reduce File Size** from the *File* menu to change the pixel dimensions of the image (see section 2.2.f, Reduce File Size).

What Is a Volume Array?

A volume array is a matrix of volume circles or rectangles that can be sized/positioned as a group and overlaid on images of blots, wells, or cells for easy quantitation. The individual cells in the array have the same functionality as standard volumes. You can define cells as background volumes, standards, and/or unknowns, as described in the sections above.

You report the array data as you would standard volumes, using the **Volume Analysis Report**.

Creating a Volume Array

On the *Volume* menu or toolbar, select the **Volume Array Tool**. This will open the *Build Volume Array* dialog.

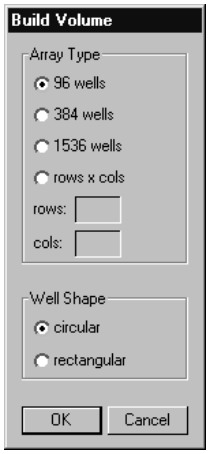


Fig. 7-10. Build Volume Array dialog.

In the dialog, you can select a standard microtiter plate dimension (**96 wells**, **384 wells**, or **1536 wells**) or select **Rows x Cols** and enter the number of rows and columns in the array in the appropriate fields.

Select the shape of the wells/cells (**Circular** or **Rectangular**) and click on **OK**.

The array overlay will be created and displayed on the image.

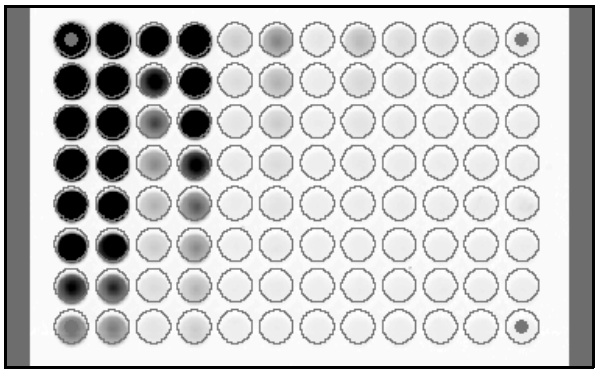


Fig. 7-11. Array overlay.

Like regular volumes, array volumes are initially displayed without labels. To show the labels of the individual wells/cells, click on the **Show/Hide Volume Labels** button on the toolbar. Like regular volumes, array volumes are initially labeled U1, U2, U3, etc.

Note: If large volume arrays are slow to display or edit on your computer and the volume labels are showing, try hiding the volume labels using the **Show/Hide Volume Labels** command. This will increase the processing speed considerably.

When you create an array overlay, it is automatically selected (the cells will be displayed with green borders) and the **Select** tool is assigned to the mouse. You can then move the array overlay so that it is properly centered on the image, resize the cells so they fit the blots/wells in the image, and resize the overlay so the four corners fit over the four corners of the array on the image.

To delete the entire array overlay, select it and click on the **Delete** key.

Moving an Array

To reposition an array overlay, move the cursor over any individual cell until the cursor changes to a multidirectional arrow and the cell border turns yellow. Then hold down the cursor and drag the entire array to a new position.

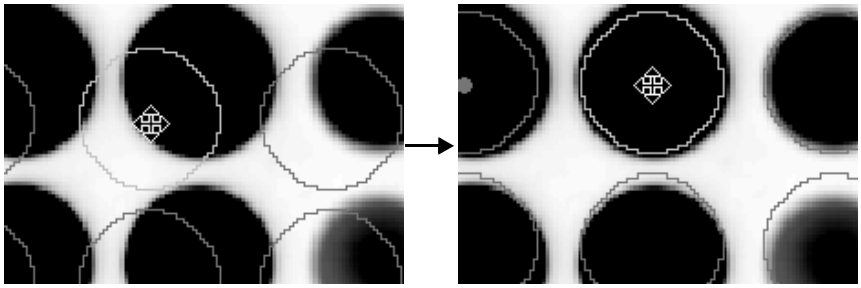


Fig. 7-12. Moving an array.

Resizing an Array

To resize an array overlay, make sure it is selected, then position the cursor over the dot at the center of one of the corner cells. Green lines will appear connecting the array frame at the four corners.

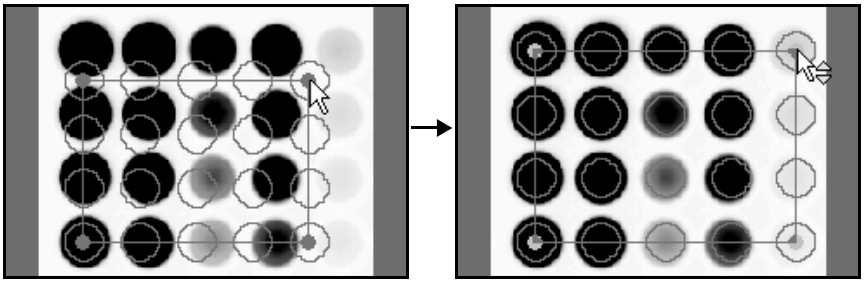


Fig. 7-13. Resizing an array.

Hold down the mouse button and drag the array frame in or out to compress/expand the array.

Resizing the Array Cells

To resize the individual cells in the array, magnify any individual cell and move the cursor over the cell border (or corner anchor point, in the case of rectangles) until it changes to a cursor with an adjustment symbol. Hold down the mouse button and drag to move the border in or out. All the cells in the array will be resized accordingly.

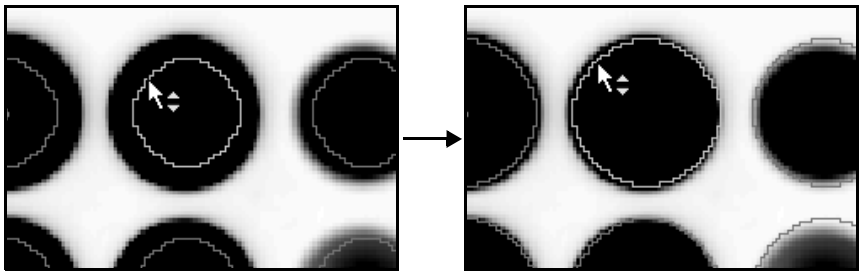


Fig. 7-14. Resizing an array cell.

Copying an Array

To copy an array within the same image, select it, then hold down the **Ctrl** key while dragging it. The copy will be created and dragged to the new position.

To copy an array between images, select it, then click on the **Copy to Clipboard** button on the *Volume* toolbar. Open or select the image you want to copy to and click on the **Paste from Clipboard** button. The copied array will be pasted into the new image in the same relative position it was copied from.

Note: If you are pasting into an image with a different pixel size (i.e., resolution), you will receive a message that the placement of the copy may not be exact. Click on **OK** to complete the paste, then position the pasted array manually.

Ungrouping an Array

You can ungroup the individual cells in an array, so they behave like normal, stand-alone volumes.

With the array selected, select the **Array Ungroup** command from the menu or toolbar. This command cannot be undone, and you will be prompted to confirm the action.

The ungrouped array will appear deselected (i.e., displayed in blue). You can then move the cells individually, and perform all normal volume operations on the individual cells.

8. Colony Counting

You can use Quantity One to automatically count the number of white, blue, or plaque colonies in a Petri dish.

Note: For best results, when capturing the image of a Petri dish, the dish should fill the imaging window. Also, images with colonies *should not* have asymmetric pixels. (Asymmetric pixels can be generated by densitometers and the **Reduce File Size** command.) The colony counting function will not work properly on images with asymmetric pixels.

Select **Colony Counting** from the *Analysis* menu to open the *Colony Counting* dialog.

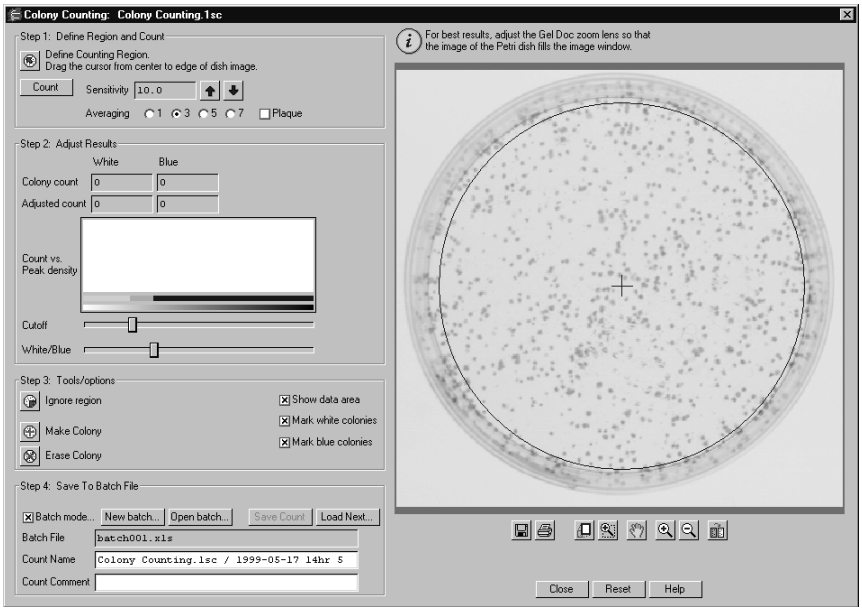


Fig. 8-1. Colony Counting dialog.

The dialog has been arranged from top to bottom to guide you through the procedure.

8.1 Defining the Counting Region

First, you must define the region you want to count in the Petri dish image.

Click on the **Define Counting Region** button in the dialog and position the cursor at the center of dish image. Drag the cursor outward. As you drag, a blue circle will expand on the image—this defines the border of the counting region.

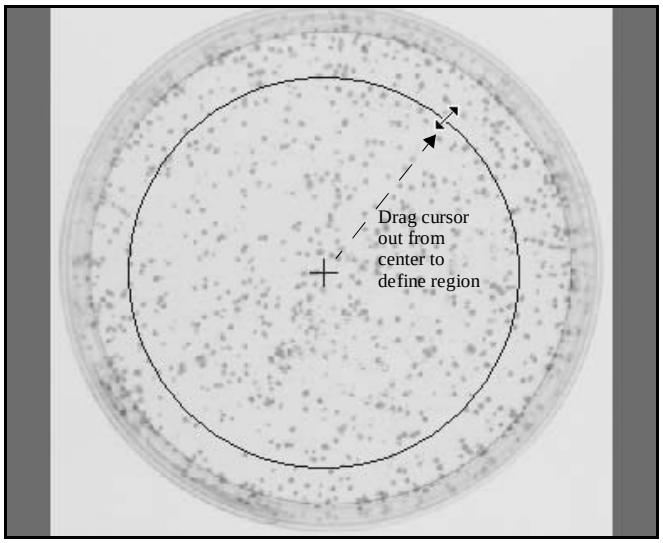


Fig. 8-2. Defining a counting region.

If you make a mistake in defining the counting region, click on the **Reset** button at the bottom of the dialog to start over.

Position the blue border until it is just inside the interior edge of the Petri dish.

Note: If the border disappears when you release the mouse button after dragging, check to make sure that the **Show Data Area** checkbox is checked. This checkbox is located at the bottom of the dialog.

If the circle is slightly off-center, you can reposition it by positioning the cursor on the center "target" of the circle. The cursor will change to a multidirectional arrow, and you can drag the entire circle.

To resize the counting region circle, position the cursor on the outer edge of the circle. The cursor will change to a bidirectional arrow and you can drag the border in or out.

8.2 Counting the Colonies

After you have positioned the circle, you are ready to detect colonies.

If you are counting plaques, click on the **Plaque** checkbox. (Because plaques appear as clear circles on a darker background, this checkbox must be selected for proper detection.)

Before counting, you may want to adjust the **Sensitivity** and **Averaging** parameters described below.

When you are ready to count, click on the **Count** button.

Sensitivity

The **Sensitivity** setting determines the minimum signal intensity in the image that will be counted as a colony. (This is based on the slope of the signal's peak.) The higher the sensitivity, the more colonies will be detected.

If the sensitivity is set too high, background noise will be erroneously detected as colonies. If the setting is too low, real colonies may be missed.

The default sensitivity setting is 10.00. If the image has faint colonies (e.g., O.D. < 0.05, counts < 2,000), you may want to increase this value to 20.00.

Averaging

Averaging is designed to prevent random signal noise (such as salt or pepper) in the image from being detected as colonies. If the image is noisy, you should select the highest value that still results in good separation of colonies (default = 3).

A low averaging value may result in noise being detected as colonies. A high averaging value may result in two closely spaced colonies being counted as one.

8.3 Displaying the Results

When you click on **Count**, the number of detected colonies will appear in the **Results** section of the dialog in the **White** column.

Fig. 8-3. Example of a dish with white colonies.

The colonies will also appear marked as gold triangles on the image itself.

Note: If the colonies are not marked on the image, make sure that the **Mark White Colonies** checkbox at the bottom of the dialog is checked.

A text box on the image will indicate how many colonies were detected on the image.

Doing a Recount

To recount using different parameters, change the **Sensitivity** and/or **Averaging** settings. This will erase the old count. Then click on **Count** again to recount.

Redrawing the counting region circle or clicking on the **Reset** button will also erase the count.

8.4 Making and Erasing Individual Colonies

If automatic colony detection has missed or erroneously detected some colonies, you can manually mark or unmark them directly on the image using the buttons under **Tools/Options**.

Fig. 8-4. Colony counting tools.

To mark a colony, click on the **Make Colony** button, then click on the spot on the image that you want to identify as a colony.

To unmark a colony, click on the **Erase Colony** button, then click on the colony on the image that you want to unmark.

The colony count will change accordingly.

8.5 Using the Histogram to Distinguish Colonies

The histogram in the *Colony Counting* dialog is a graphical representation of the signal data in the image. You can use the histogram and associated sliders to reduce the number of incorrectly identified colonies and/or distinguish between white and blue colonies in the image.

Colonies Versus Background Noise

If there is a clear peak on the left end of the colony counting histogram, it is probably due to background intensity or noise in the image. (For information on subtracting background from entire images, see section 3.10, Whole-Image Background Subtraction; for information on filtering noise from images, see section 3.11, Filtering Images.)

If background is being detected as colonies, you can use the histogram and the **Cutoff** slider to correct this.

Drag the **Cutoff** slider to the right until it is centered on the right edge of the background peak.

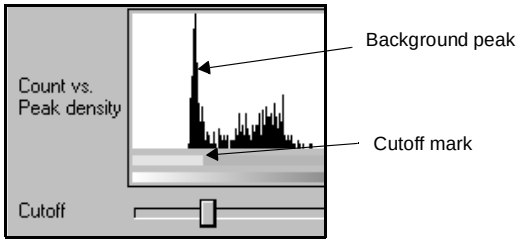


Fig. 8-5. Using the Cutoff slider.

The yellow portion of the bar beneath the histogram marks the range of image data has been designated as background noise, and is not being considered for colony counting purposes. The gold portion of the bar marks white colony data range.

The colony count displayed in the dialog and on the image should decrease. On the image, you should also see the incorrectly identified colonies disappear as you drag the slider.

White and Blue Colonies

If you know you have white and blue colonies in the image, and there are two clear peaks on the histogram to the right of the background peak, you can use the histogram to distinguish between these types of colonies.

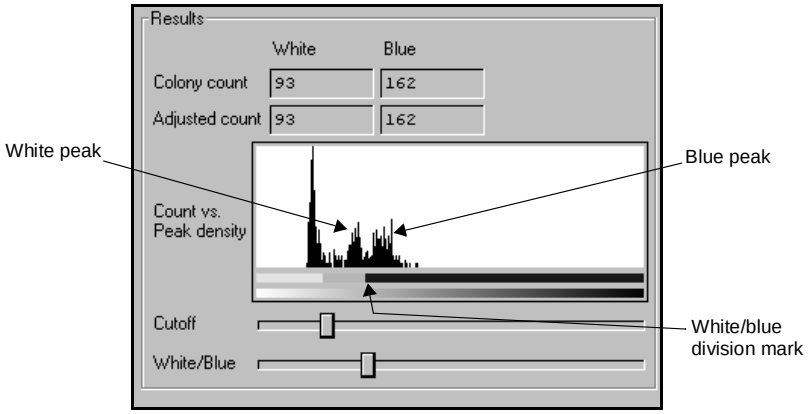


Fig. 8-6. Using the White/Blue slider.

Drag the **White/Blue** slider to the left until it is positioned between the two peaks. The white colony data range is indicated by gold on the bar beneath the histogram, and the blue colony data range is marked with blue.

As you drag the slider, the numbers of white and blue colonies will change in the dialog and in the text box on the image. Also on the image, you should see the marked white colonies (gold triangles) change to blue colonies (blue squares).

Note: If the blue colonies are not marked on the image, check to make sure that the **Mark Blue Colonies** checkbox at the bottom of the dialog is checked.

8.6 Ignoring a Region of the Dish

If a particular region of your Petri dish is damaged and you do not want to consider the colonies (if any) that appear there in the final count, you can exclude that region of the dish from the calculations.

Click on the **Ignore Region** button, then position the cursor on one edge of the region you want to ignore. Drag the cursor on the image, defining the full region you want to ignore.

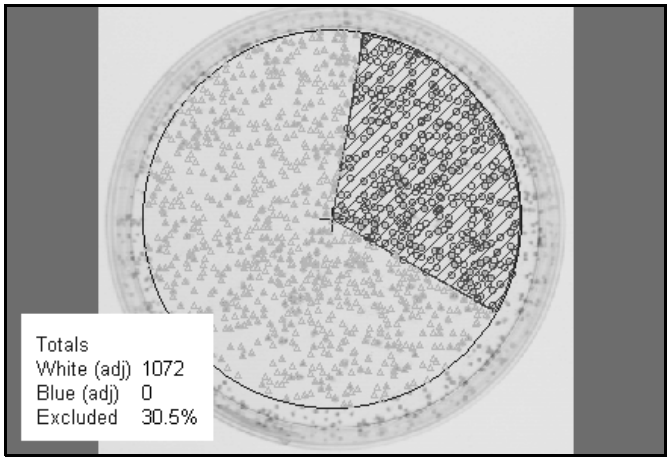


Fig. 8-7. Marking a region to ignore.

As you drag, you will create a “pie slice” marked with red cross-hatching. Any colonies in this region will not be considered in the final count.

When you have defined the region, release the mouse button. If you want to change the size of the ignored region, position the cursor on the edge of the pie slice near the rim of the blue circle. The cursor will change to a bidirectional arrow, and you can drag the edge of the pie slice.

Colony Count and Adjusted Count

After you have defined a region to ignore, two different counts will appear in the dialog: the colony count and the adjusted count.

The **Colony Count** is the number of colonies that appear in the defined circle minus those in the ignored region.

The **Adjusted Count** is an estimate of the total colony count in the Petri dish; it uses the known colonies to extrapolate the number of colonies that might have appeared in the ignored region if it had not been damaged. The adjusted count is calculated based on the area of the ignored region and the density distribution of colonies in the rest of the circle.

8.7 Saving/Resetting the Count

A colony count can be saved to the image and/or a separate spreadsheet file.

Saving to the Image

Any count you perform is automatically stored with the image. To save the count with the image, exit the *Colony Counting* dialog by clicking on the **Close** button, and use the **Save** commands under the *File* menu to save the image.

To view the count data again, simply open the image and open the *Colony Counting* dialog.

To save a count or multiple counts to a spreadsheet file, see the following section.

Resetting the Count

Click on the **Reset** button to clear the *Colony Counting* dialog and any changes you have made to the image. This command cannot be undone.

8.8 Saving to a Spreadsheet

The **Batch File** controls allow you to export colony data from an image or multiple images to a Microsoft® Excel spreadsheet for review and comparison. To activate these controls, click on the **Batch Mode** checkbox.

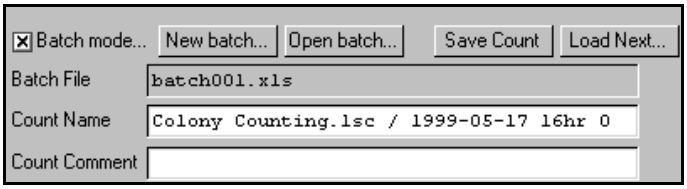


Fig. 8-8. Batch Mode controls.

Creating/Opening a Batch File

To create a new batch file, click on the **New Batch** button. This will open a dialog in which you can specify the name and location of the spreadsheet you want to create.

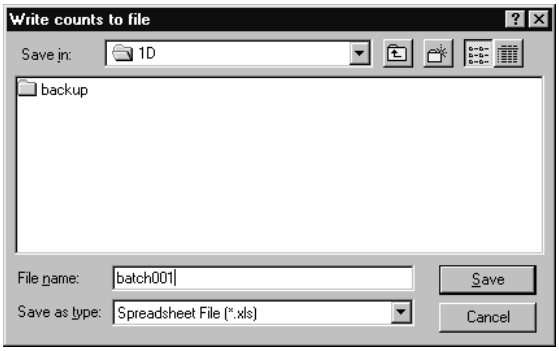


Fig. 8-9. Creating a batch file.

When you click on **Save**, the new batch file name will be displayed in the *Colony Counting* dialog.

To open an existing batch file, click on the **Open Batch** button. This will open a similar dialog. Select the Excel file you want to open from the appropriate directory.

Naming/Saving a Count

Enter a name for the count you want to save in the **Count Name** field, or use the default name (the file name plus a time stamp). Enter any comments in the **Count Comment** field. This data will be included in the spreadsheet.

To save the currently displayed count to the batch file, click on the **Save Count** button. The number of colonies, as well as associated count settings, will be added to the spreadsheet. After you have saved the current count, the **Save Count** button will become deactivated. If you adjust the count in any way, the button will become active again and you can add the adjusted count to the spreadsheet.

Loading Another Image

After you have saved the count(s) for the current image, you can open another dish image by clicking on the **Load Next** button. This will open a standard *Open* dialog from which you can select the image.

The new image will be loaded into the *Colony Counting* dialog.

Note: The image will only be loaded into the *Colony Counting* dialog; it will not open in a separate image window in Quantity One.

After you have saved the count(s) for the new image to the batch file, you can either load another image using the **Load Next** command or click on **Close** to close the *Colony Counting* dialog.

9 ReadyAgarose™ 96 Plus

ReadyAgarose 96 plus gels are pre-cast agarose gels that accommodate 96 samples and standards. These high throughput gels allow loading samples from 96 well microtiter plates. The gels are 4 and 12 multi-channel pipet compatible, and the tip-to-tip spacing of the multi-channel pipetors results in adjacent samples from microtiter plates being loaded in alternating wells on the ReadyAgarose 96 plus gel.

The ReadyAgarose 96 plus feature of Quantity One allows you to manipulate the gel image to rearrange the lanes from samples run on the ReadyAgarose 96 plus gels and put them in a 96 well microtiter plate format, thus simplifying sample tracking for analysis. This allows easy comparison and analysis of gel data based on the microtiter plate format.

When acquiring the gel image, zoom in such that there is minimum blank space around the gel. This is extremely important with low resolution imagers. Once the gel image is acquired, the ReadyAgarose 96 plus feature uses a step-by-step approach to analyze the image. The First step is to align the gel image. Quantity One then places lane overlays over each lane. You can then make slight adjustments to the size and position of the lane overlays. The final step allows you to determine the lane format. There are three lane formats from which to choose, 8 x 14, 4 x 28 (default), or 2 x 56 format (includes standard lanes).

9.1 ReadyAgarose Wizard

Once you have acquired your image, select ReadyAgarose 96 plus from the Analysis menu. This opens the ReadyAgarose 96 plus Wizard.

Step 1 - Positioning the Image

The first panel of the wizard displays your image with four red crosshairs.

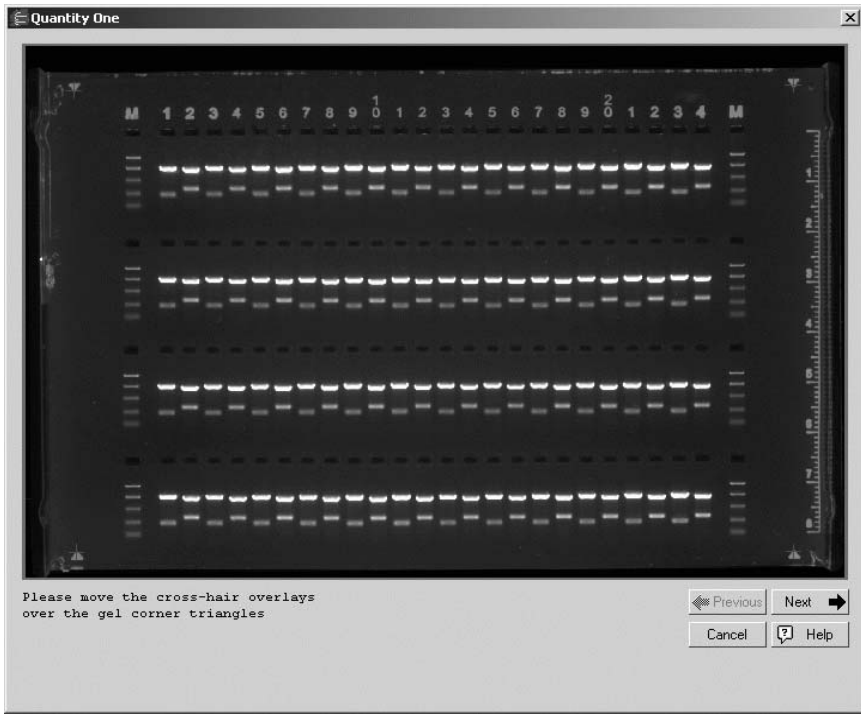


Fig. 9-1. Positioning the Image

Click and drag each crosshair to place them over the four triangles visible in the gel holder. The ideal position is at the tip of the triangle. When you have correctly positioned the crosshairs, click the Next button.

Step 2 - Resizing, Repositioning, and Spacing the lanes

In the next panel, the image displays 96 lanes based on the position of the crosshairs in the previous panel.

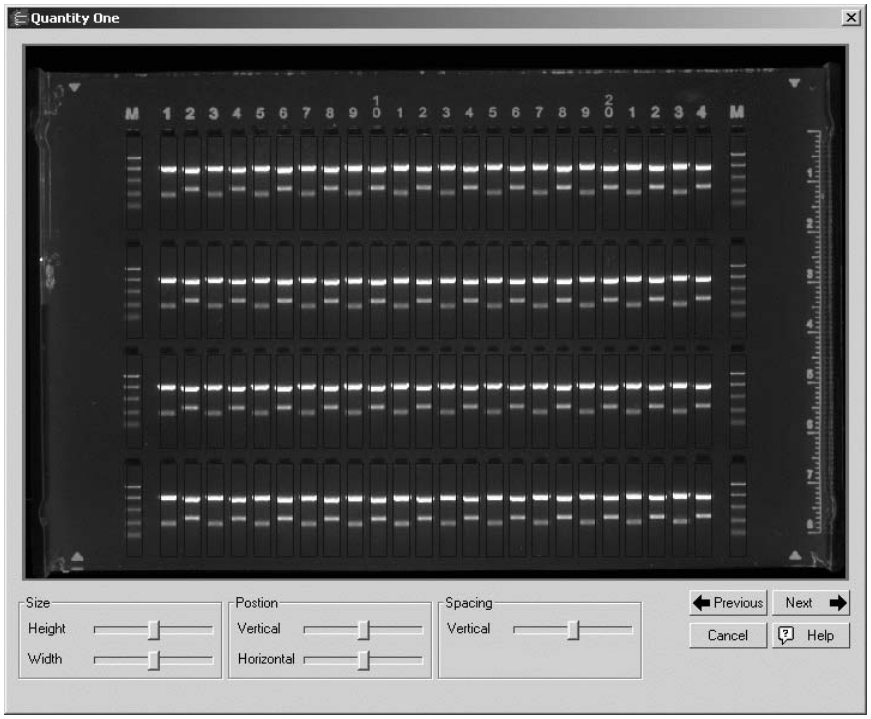


Fig. 9-2. Resizing and Repositioning the Lanes

Use the slider bars at the bottom to change position, resize the lanes, or modify the vertical spacing of the lanes. Note that the sliders move, resize, or space all the lanes at once. When you are satisfied with the location of the lanes, click Next.

Step 3 - Arranging the Lane View

This panel places the 96 lanes in a new view. The default view is 4 x 28.

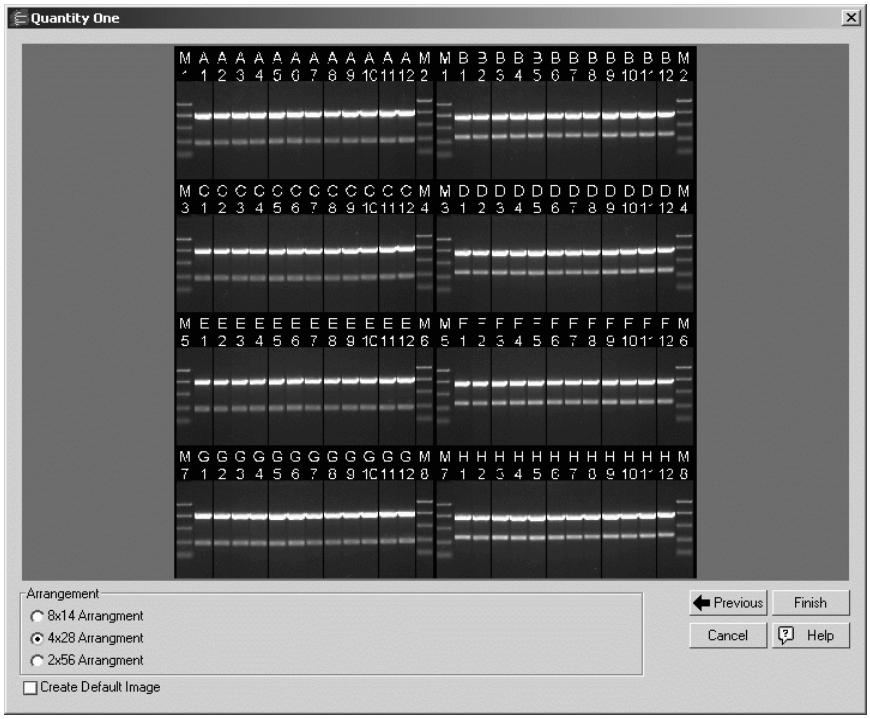


Fig. 9-3. Arranging the Lanes

To arrange the lanes in a different view, select the appropriate view at the bottom of the panel. If you would like Quantity One to create an additional default image with the lanes in their original orientation, check the box labeled, "Create Default Image". When you are satisfied with the gel arrangement, click Finish to create your new image.

10. EXQuest Spot Cutter

The EXQuest Spot Cutter is a precision instrument that accurately locates and excises protein spots from 1D gels or blots, and loads them into microplates for down-stream processing and analysis.

Fig. 10-1. EXQuest Spot Cutter

The following sections describe how to calibrate the EXQuest Spot Cutter and excise spots using Manual Excision or lane/Volume Excision.

Finally, enter a name for your application in the Name field and click on OK to implement your changes.

After you have created an application, you can select it from the application tree by selecting Custom and the name you created. You can delete the application by selecting Custom, Delete, and the name of the application.

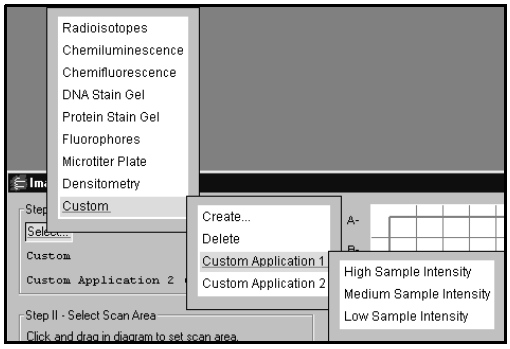


Fig. G-7. Selecting a custom application.

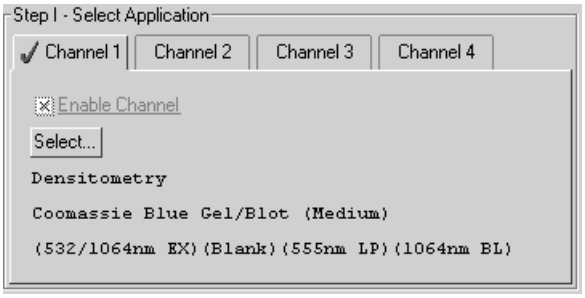


Fig. G-8. Application selection and settings.

G.3 Step II. Select Scan Area

To select a scan area, drag your mouse within the scanning window. (In the scanning window, your cursor appearance will change to a cross.) The border of the scan area you are selecting is marked by a frame.

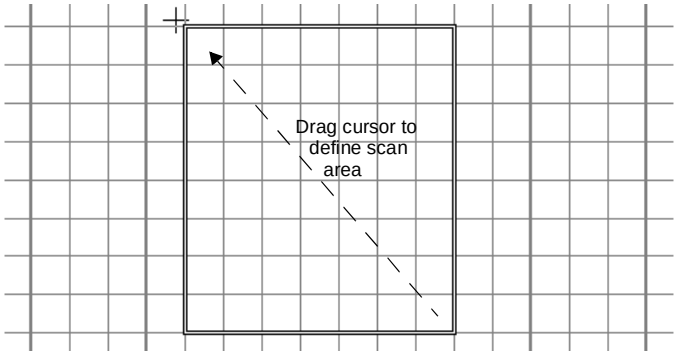


Fig. G-9. Selecting a scan area.

If you are in quadrant mode, note that the frame “locks” onto the next quadrant as you drag. When you release the mouse button, the border changes to a dashed blue line, indicating a selected area.

- To *reposition* the scanning box you have selected, position your cursor inside the box and drag. The entire box will move.
- To *resize* the box, position your cursor on a box side and drag. The side you have selected will move.
- To *redo* the box entirely, position your cursor outside the box and drag. The old box will disappear and a new box will be created.