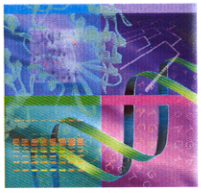
The Discovery Series™



Quantity One® 1-D Analysis Software

User Guide

BIO-RAD

Quantity One®

User Guide for Version 4.4
Windows and Macintosh

P/N 4000126-14 RevA

Bio-Rad Technical Service Department

Phone: (800) 424-6723

(510) 741-2612

Fax: (510) 741-5802

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

Notice:
No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording, or any information storage or retrieval system, without permission in writing from Bio-Rad.

Quantity One is a registered trademark of Bio-Rad Laboratories. **The Discovery Series** is a trademark of Bio-Rad Laboratories. All other trademarks and registered trademarks are of their respective companies.

WASTE text engine © 1993–1997 Marco Piovanelli

Limitations of Liability:
Bio-Rad is not responsible for the misinterpretation of results obtained by following the instructions in this guide. Whenever possible, you should contact the Technical Services Department at Bio-Rad to discuss your results. As with all scientific studies, we recommend that you repeat your experiment at least once before making any significant conclusions for presentation or publication.

Copyright © 2002 by Bio-Rad Laboratories. All rights reserved.

Table of Contents

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.

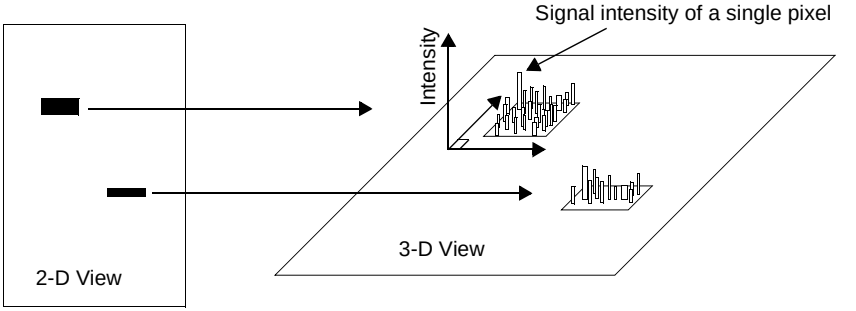


Fig. 1-1. Representation of the pixels in two digitally imaged bands in a gel.

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™ system, ChemiDoc™ 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, ChemiDoc, 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.

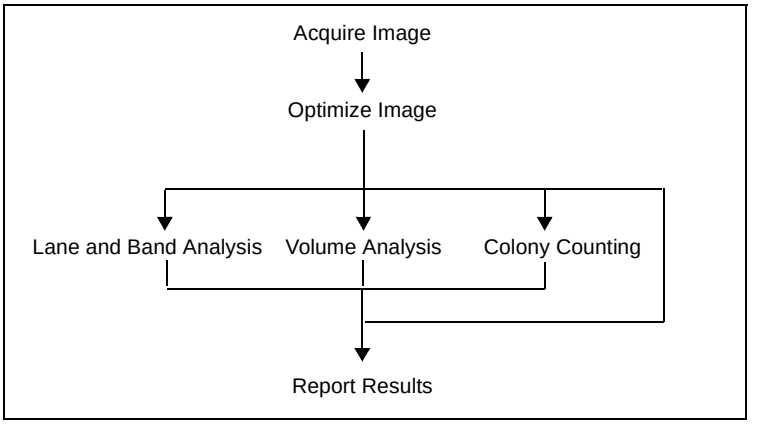


Fig. 1-2. Quantity One workflow.

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-700, GS-710, and GS-800 Imaging Densitometers; the Gel Doc, ChemiDoc, and ChemiDoc XRS gel documentation systems; the Fluor-S and Fluor-S MAX MultiImagers; 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 98, XP, NT 4.0, and 2000, or on a Macintosh PowerPC.

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 98 SE Windows NT 4.0 with service pack 6 Windows 2000 Windows XP
Processor:	Pentium ≥ 333 MHz

RAM:	≥ 128 MB or better for Gel Doc, ChemiDoc, ChemiDoc XRS, and VersaDoc systems.
	≥ 256 MB or better for Molecular Imager FX systems, Personal FX system, and GS-800 densitometer.
Hard disk space:	≥ 3 GB
Monitor:	17" monitor, 1024 x 768 resolution (absolutely required), True color.
SCSI:	Required for all Bio-Rad imaging devices except the Gel Doc, ChemiDoc, ChemiDoc XRS, and VersaDoc systems. Adaptec SCSI card recommended.
Printer:	Optional.

Macintosh

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

Operating system:	System 9.0 or higher, excluding Mac OS X.
Processor/Model:	PowerPC G3 processor or better.
RAM:	≥ 256 MB for all Bio-Rad imaging systems.
Hard disk space:	≥ 3 GB
Monitor:	17" monitor, 1024 x 768 resolution (absolutely required), Millions of colors.
SCSI:	Required for all Bio-Rad imaging devices except the Gel Doc, ChemiDoc, ChemiDoc XRS, and VersaDoc systems. Adaptec SCSI card recommended.
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

1.6.a Windows

Note: Windows NT and 2000 users: You must be a member of the Administrators group to install The Discovery Series software. After installation, members of the Users group must have “write” access to The Discovery Series folder to use the software.

Insert The Discovery Series **CD-ROM** into your computer. The installer will start automatically. (If the CD does not auto-start, use Windows Explorer to open the root directory on the CD-ROM and double-click on the **Setup.exe** file.)

The installer program will guide you through the installation. The installer will create a default directory under Program Files on your computer called Bio-Rad\The Discovery Series (you can select your own directory if you wish). The application program will be placed in the Bin folder inside The Discovery Series folder. Additional directories for storing user profiles and sample images will also be created

The installer will place an application icon on your desktop and create a folder named The Discovery Series under Programs on your Windows *Start* menu.

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

1.6.b **Macintosh**

Insert The Discovery Series **CD-ROM** into your Macintosh. The TDS-Mac folder will open on your desktop, displaying the installers for The Discovery Series applications. Double-click on the installer for your application.

Fig. 1-3. Installation program icon (Macintosh).

The installer will guide you through a series of screens. The installer will create a folder on your hard drive that contains the main application and associated sample images (you can select a different folder if you wish). The installation will also create a folder called The Discovery Series in the Preferences folder in your System folder; this contains the Help file and various system files.

Once installation is complete, the folder containing the application icon will appear open on your desktop.

1.7 Hardware Security Key (HSK)

Note: Initial installation of a network server does require 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 Licenses.

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.

1.7.a Windows

Fig. 1-4. PC Hardware Security Key

Before proceeding, please turn off your computer.

The HSK attaches to the parallel port on the back of your PC. If a printer cable is attached to this port, turn off the printer and disconnect it. After you have attached the HSK, you can attach the printer cable to the key itself and restart your computer and printer.

Note: Some parallel port devices such as zip drives may be incompatible with HSKs. Please check with your peripherals vendor.

The code for the PC hardware security key is EYYCY. This is printed on the key itself.

You will also need to install the system driver that allows the computer to recognize the HSK.

Note: Windows NT and 2000 users must be in the local administrator group to install the HSK driver.

To install the driver, open the Windows *Start* menu and select *Programs* > *The Discovery Series*. Select **Install HASP Hardware Security Key Driver** to begin installation.

Note: Windows 98 users must reboot their computer after installing the HSK. Windows XP, NT and 2000 users do not have to reboot.

1.7.b Macintosh

Fig. 1-5. Macintosh Hardware Security Key

Before proceeding, please turn off your Macintosh.

The Macintosh HSK must be inserted in the Apple Desktop Bus (ADB) path. The ADB port is located on the back of your Macintosh.

Fig. 1-6. Apple Desktop Bus icon on back of Macintosh.

The HSK can be inserted at any point in the ADB path—between the computer and the keyboard, between the keyboard and the mouse, between the keyboard and the monitor, etc. After you have attached the HSK, you can restart your computer.

The code for the Macintosh HSK is QCDIY. This code is printed on the key itself.

Note: If your Macintosh does not have an ADB, you may use an ADB-USB converter.

1.8 Starting the Program

The Hardware Security Key must be attached to the computer before you can start the software (unless you are using a network license).

1.8.a Windows

The installation program creates an application icon on your desktop. To start the program, double-click on this icon.

Fig. 1-7. Application icon.

You can also start the program from the Windows *Start* menu. Click on the *Start* button, select *Programs*, select *The Discovery Series*, and select the application name.

1.8.b Macintosh

After installation, the main application folder will be open on your desktop. To start the program, double-click on the application icon shortcut inside the folder. You can move this shortcut icon to your desktop.

1.9 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.

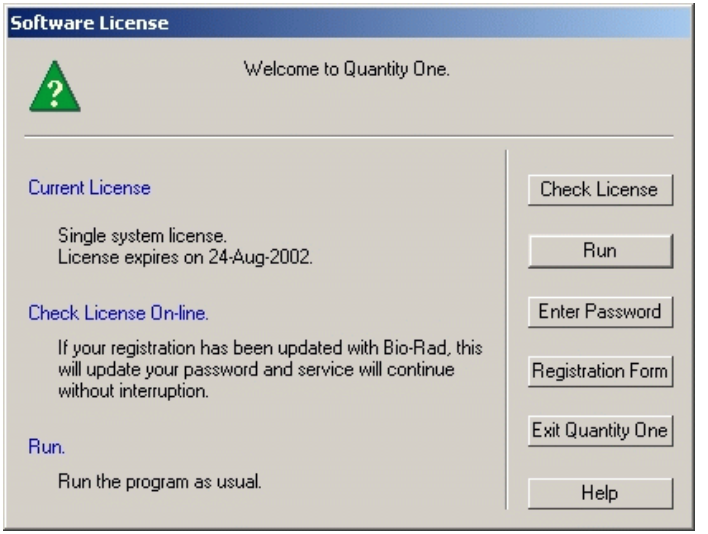


Fig. 1-8. Temporary license screen.

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.

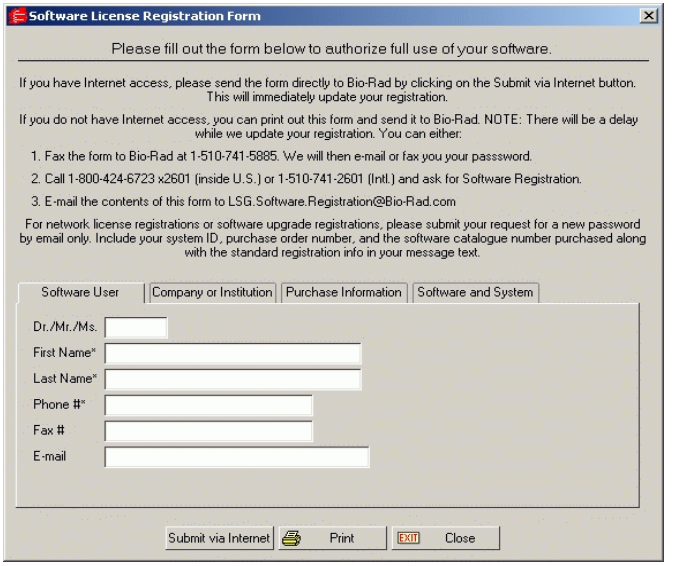


Fig. 1-9. Software License Registration Form.

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.

1.9.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.)

1.9.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.

1.9.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.

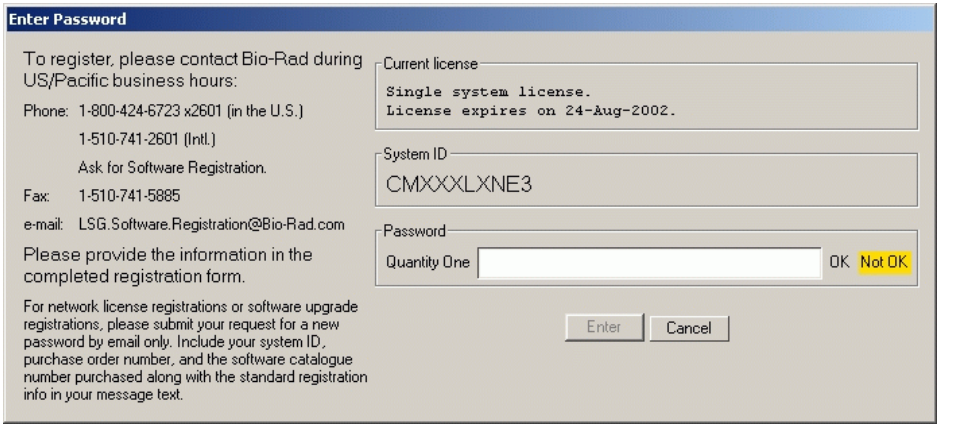


Fig. 1-10. Enter Password screen.

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.

1.10 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.

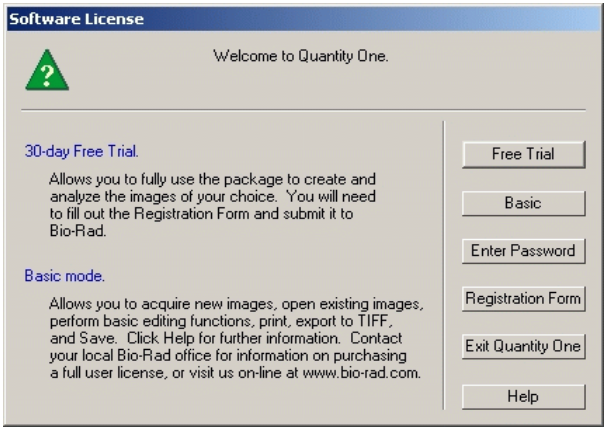


Fig. 1-11. Free Trial screen.

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.

1.11 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, Print, Export to TIFF, and Save.

1.12 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 pulldown 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.
- *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*).

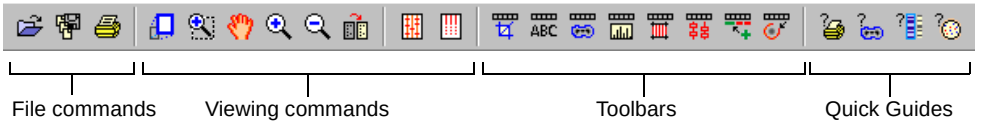


Fig. 2-1. Main toolbar.

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.

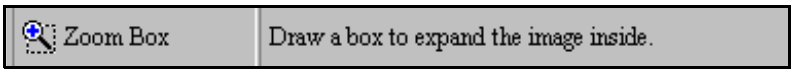


Fig. 2-2. Status boxes.

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.

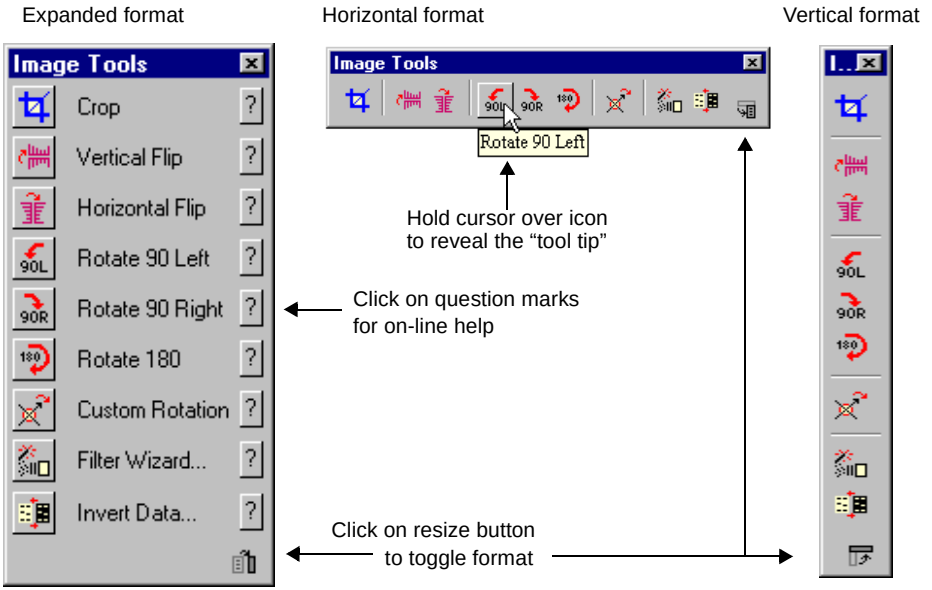


Fig. 2-3. Secondary toolbar formats and features.

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.

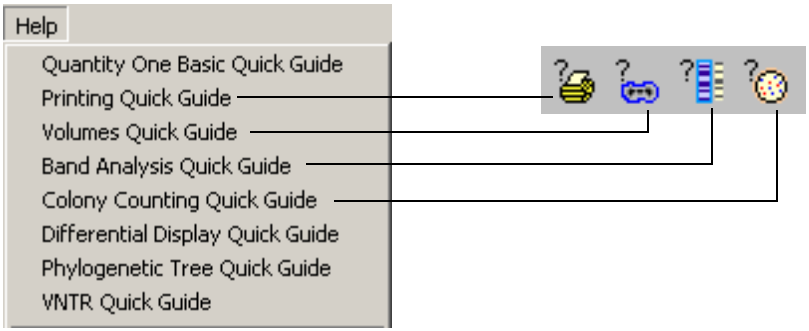


Fig. 2-4. Quick Guides listed on Help menu and main toolbar.

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.

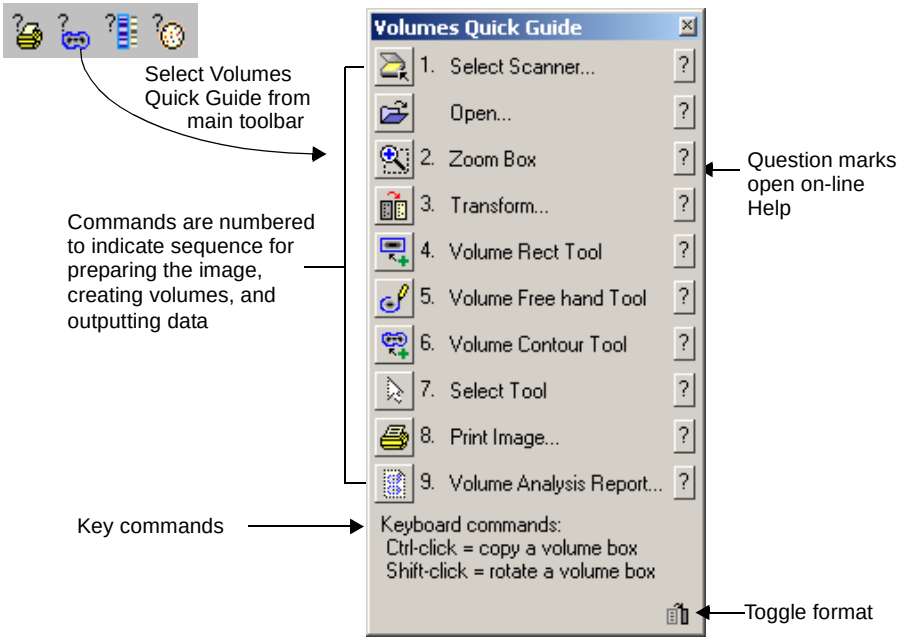


Fig. 2-5. Example of a Quick Guide: Volumes

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.

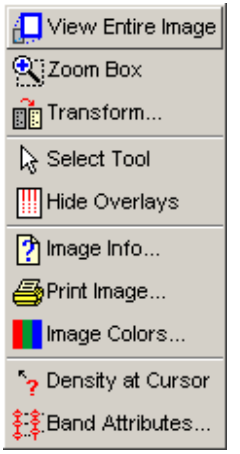


Fig. 2-6. Selecting Zoom Box from the right-click context menu.

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 pulldown 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.

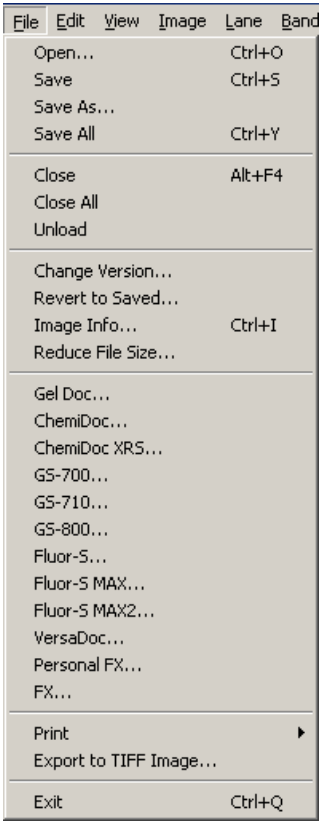
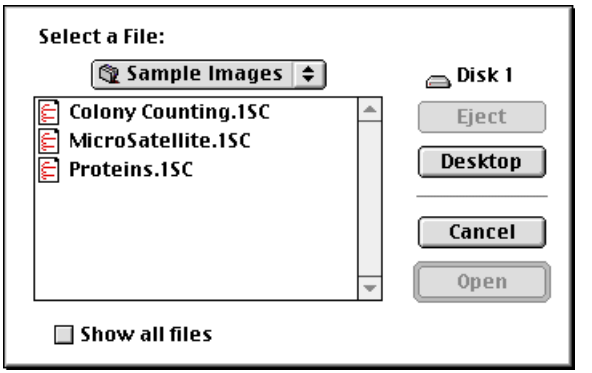


Fig. 2-7. File menu.

2.2.a Opening Images

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

Macintosh version:



Windows version:

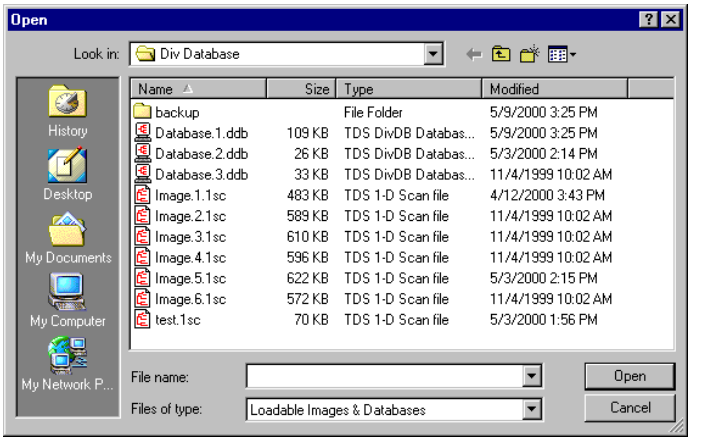


Fig. 2-8. Open dialog box.

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.

An image created in the Windows version of Quantity One can be opened in the Macintosh version, and visa versa. However, you must add a **.1sc** extension to your Macintosh files to open them in Windows.

Note: This version of Quantity One will open any image created with an earlier version of Quantity One.

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

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.

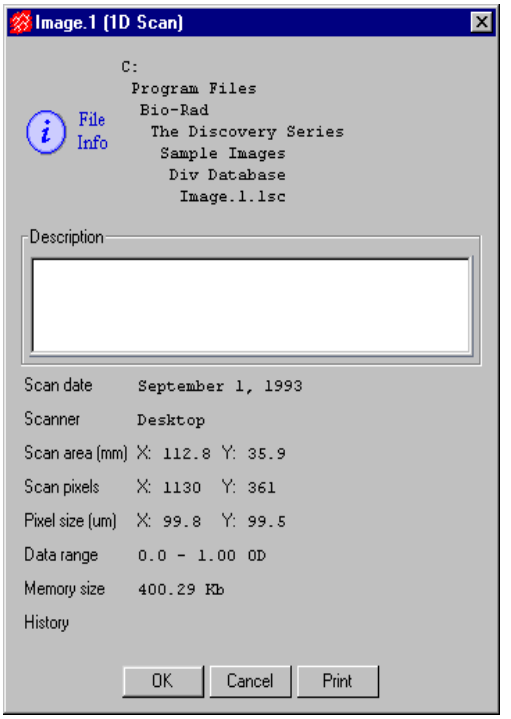


Fig. 2-9. Image Info box.

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.

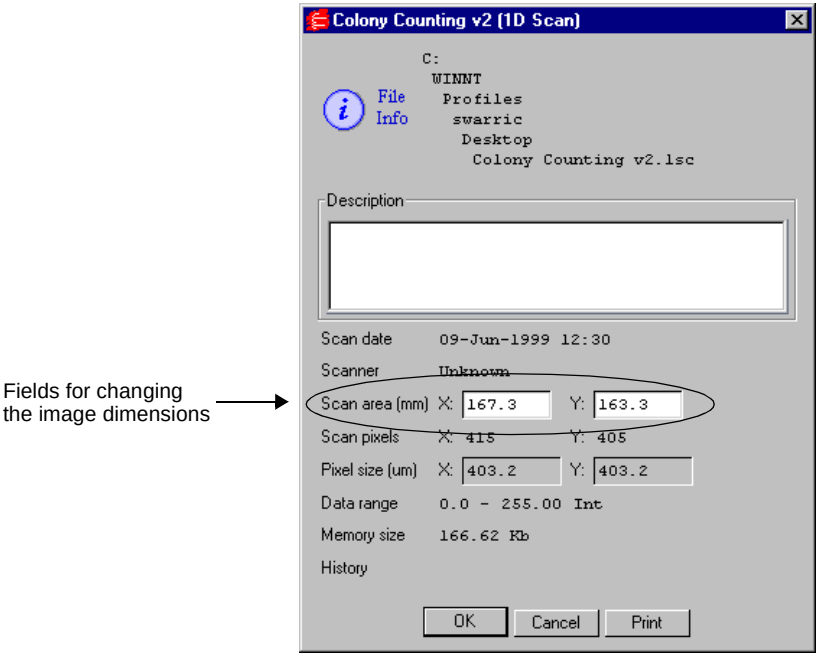


Fig. 2-10. Image Info dialog box with fields for changing the image dimensions.

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.

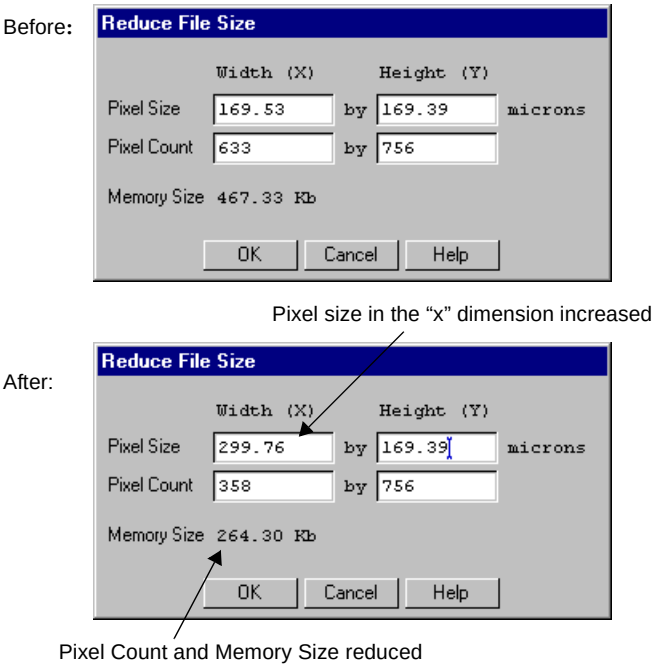


Fig. 2-11. Reduce File Size dialog box, before and after pixel size increase.

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.

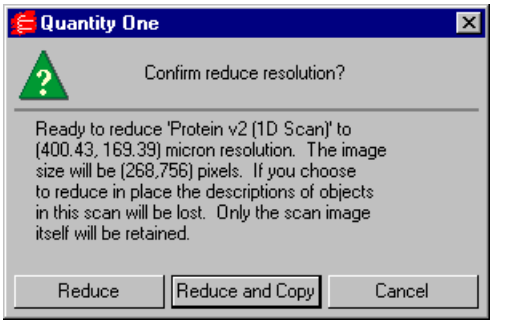


Fig. 2-12. Confirm Reduce File Size pop-up box.

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
2. ChemiDoc
3. ChemiDoc XRS
4. GS-700 Imaging Densitometer

5. GS-710 Imaging Densitometer
6. GS-800 Calibrated Densitometer
7. Fluor-S MultiImager
8. Fluor-S MAX MultiImager
9. Fluor-S MAX2 MultiImager
10. VersaDoc
11. Personal Molecular Imager FX
12. Molecular Imager FX

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.

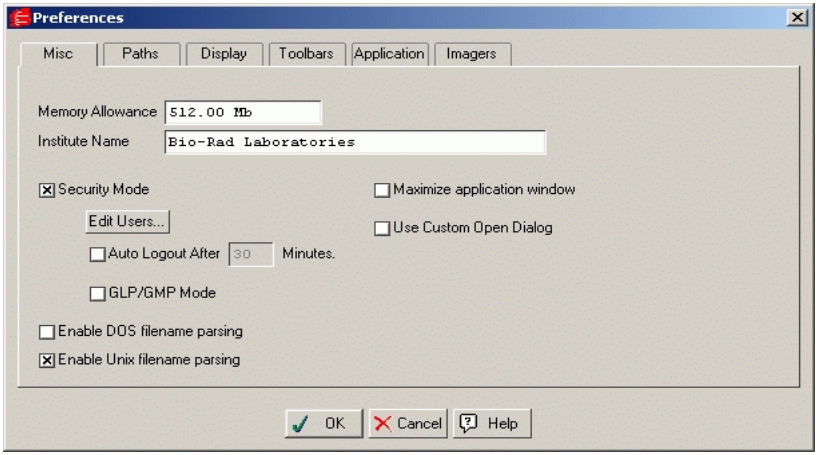


Fig. 2-13. Preferences dialog box.

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.

Institute Name

Enter the name of your institution in this field.

Security Mode

Security Mode allows you to set up a list of users who can activate Quantity One functions on the local machine. Security Mode allows you to track any changes made to images. If Security Mode is active, select File>Image Info... to view the list of changes to the file and who made the changes.

To activate Security Mode, check the box labeled Security Mode. Once checked, you will be prompted for a new Security Mode password which will be required for making changes, adding or removing users, or for disabling Security Mode.

With Security Mode active, a user must enter a user name and password to activate Quantity One commands. Security Mode is machine specific, so any images residing on a network or shared drive can be accessed from another machine that does not have Security Mode active. Although changes made on other machines are recorded in the Image info dialog, no user name appears. The same is also true for changes made to images while Security Mode is inactive.

Auto Logout

To have Quantity One automatically log out the current user after a period of inactivity, check the box labeled Auto Logout after and enter a number of minutes in the field. After the time has expired, the user will have to log in again to resume using Quantity One.

Adding and Removing Users

Once you activate Security Mode, you can add users to your list. Click Edit Users to open the Security Mode Users dialog.

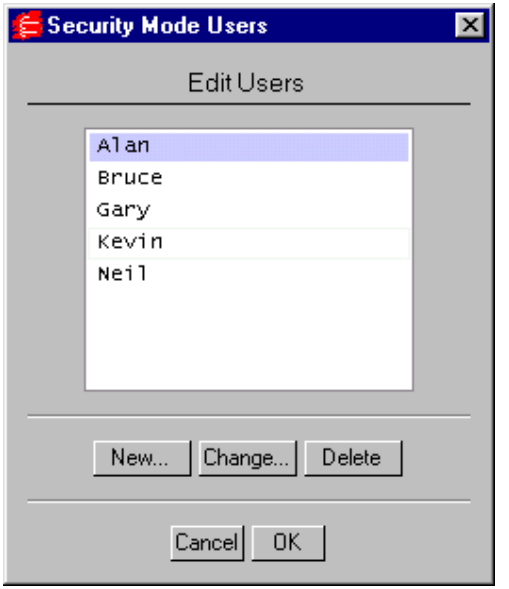


Fig. 2-14. Security Mode Users Dialog

Click **Add** to add a new user. Enter a user name and password. To remove a user, highlight the name in the list and click **Remove**. This permanently removes the user from the list. However, any changes made by this user remain in the history section of the Image Info dialog.

GLP/GMP Mode

The **GLP/GMP Mode** checkbox allows you to prevent changes to an image that would change the raw image data. In GLP/GMP mode, the following commands and submenus will be disabled:

- **Reduce File Size** (*File* menu)
- **Subtract Background** (*Image* menu)
- **Custom Rotation** (*Image* menu)
- **Filter Wizard** (*Image* menu)
- **Filter List** (*Image* menu)

- **Invert Data** (*Image* menu)

If you attempt to use any of these functions in GLP/GMP mode, you will receive a message that the function is not available.

To set GLP/GMP mode, click on the checkbox. You will be prompted to enter the Security Mode password.

To disable GLP/GMP mode, click on the checkbox to deselect it, then enter the Security Mode password for confirmation.

Note: GLP/GMP mode is only available if Security Mode is active.

Use Custom Open Dialog

By default, Quantity One uses the standard *Open* dialog box for the operating system you are using (Windows and Macintosh). Quantity One also has a customized **Open** dialog box, which includes some navigational features that are specifically tailored to The Discovery Series software. Select this checkbox to display this custom dialog box.

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.

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.

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, click on **Browse** and select a new directory. To return to the default TMP directory, click on the **Default** checkbox.

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.

Jump Cursor on Alert (Windows only)

Select **Jump Cursor on Alert** to set the cursor to automatically jump to the **OK** button in a pop-up dialog box.

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.

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.)

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 Volumes Quick Guide

If this checkbox is selected, the *Volumes Quick Guide* will open automatically when you open the program.

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 Imagers

Click on this tab to specify the imaging devices that you want to appear on the *File* menu. By default, all supported imaging devices will be included; deselect the checkboxes of the imagers that you *do not* want to include on the *File* menu.

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.*

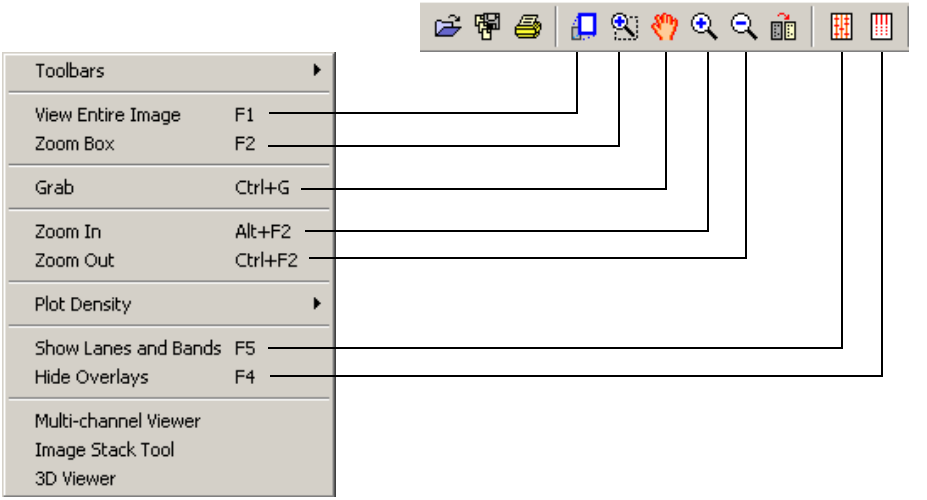


Fig. 3-1. Viewing functions on View menu and main toolbar.

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.

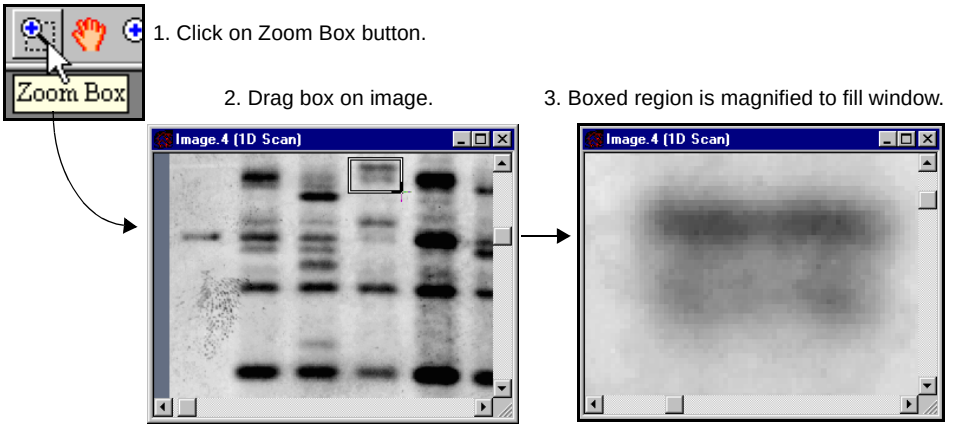


Fig. 3-2. Zoom Box tool.

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.

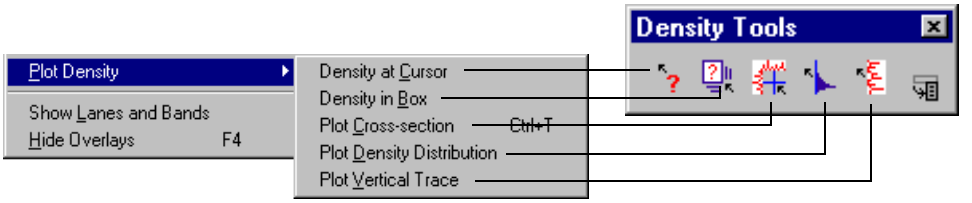


Fig. 3-3. Density tools on the menu and toolbar.

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.

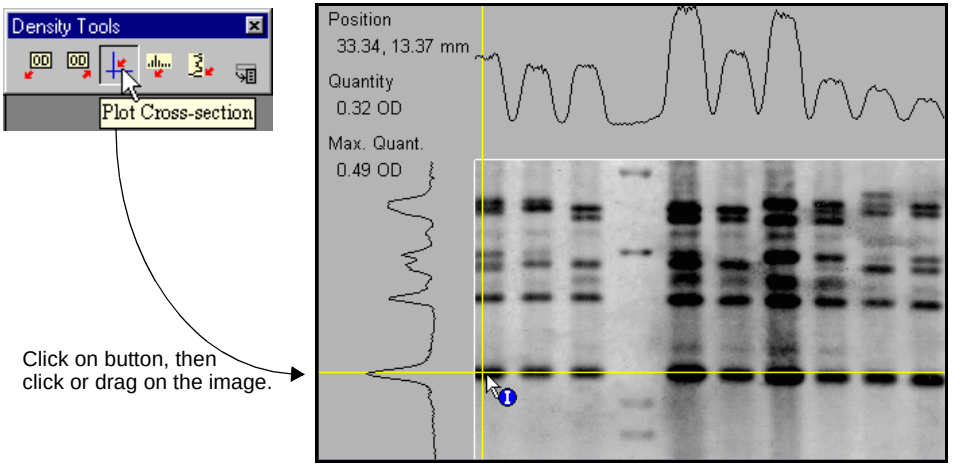


Fig. 3-4. Plot Cross-section tool.

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.

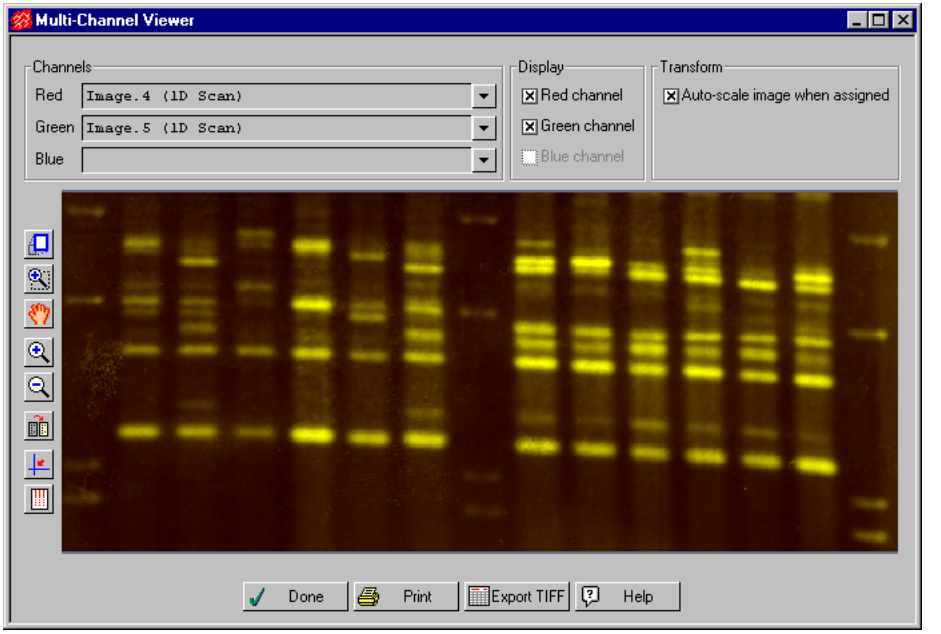


Fig. 3-5. Multi-Channel Viewer.

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 pulldown button next to the **Green** or **Blue** name field. Select the image name from the pulldown list. Add a third image using the same procedure.

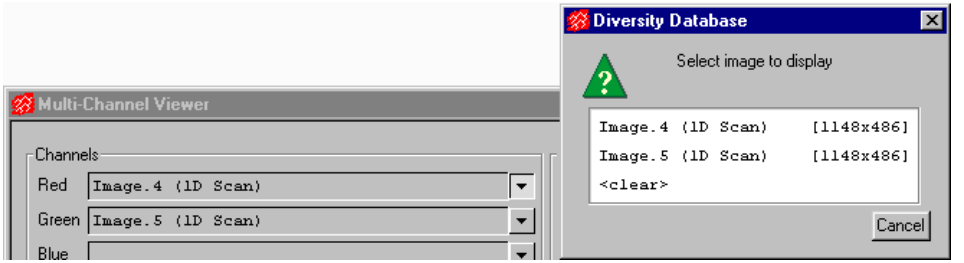


Fig. 3-6. Selecting images to display in the viewer.

To reassign the different images to different channels, use the pulldown buttons to the right of the name fields. Select **<clear>** from the pulldown 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 11.5, 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.

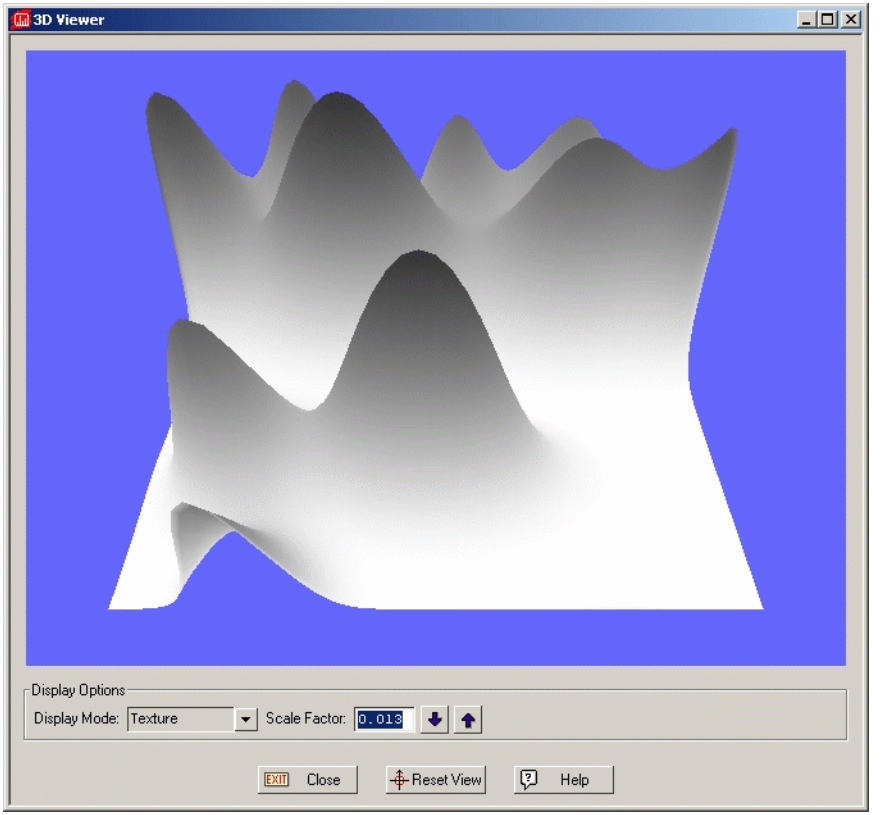


Fig. 3-7. 3D Viewer

3.5.a Positioning the Image

Use your mouse or keyboard to reposition and rotate the image.

Windows

- **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

- **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.

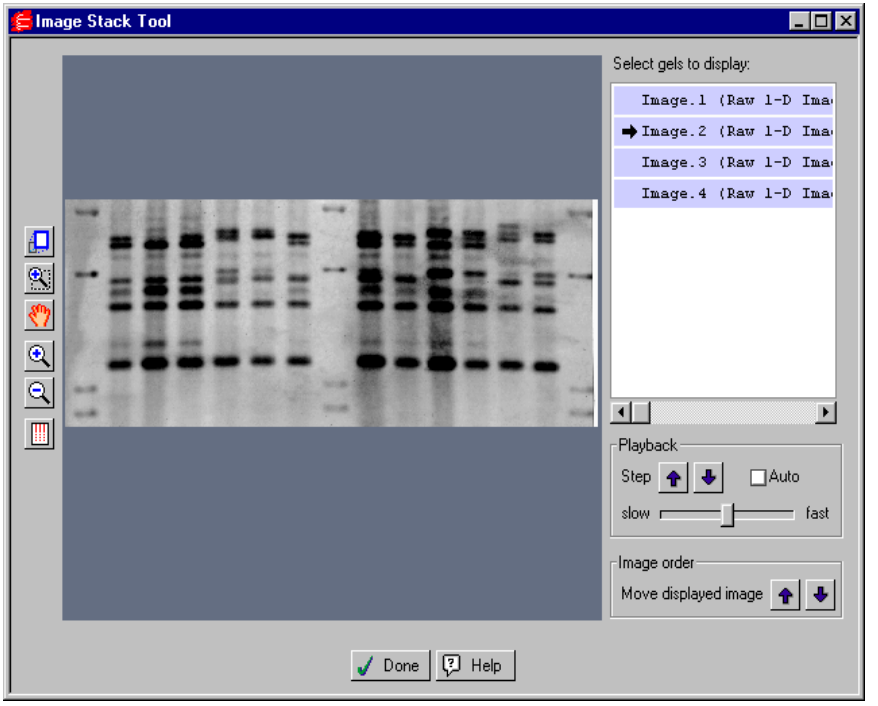


Fig. 3-8. Image Stack Tool.

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.

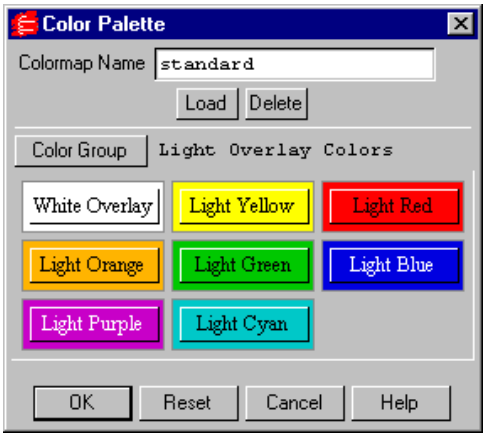


Fig. 3-9. Colors dialog.

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.).

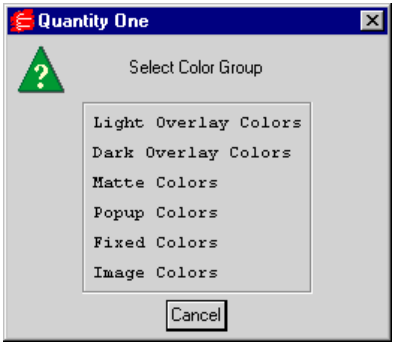


Fig. 3-10. List of Color Groups.

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.

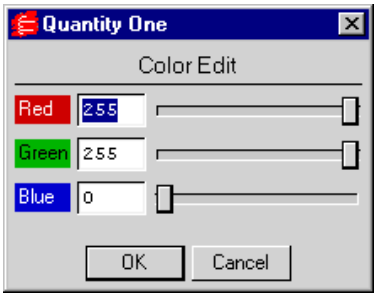


Fig. 3-11. Color Edit dialog.

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.

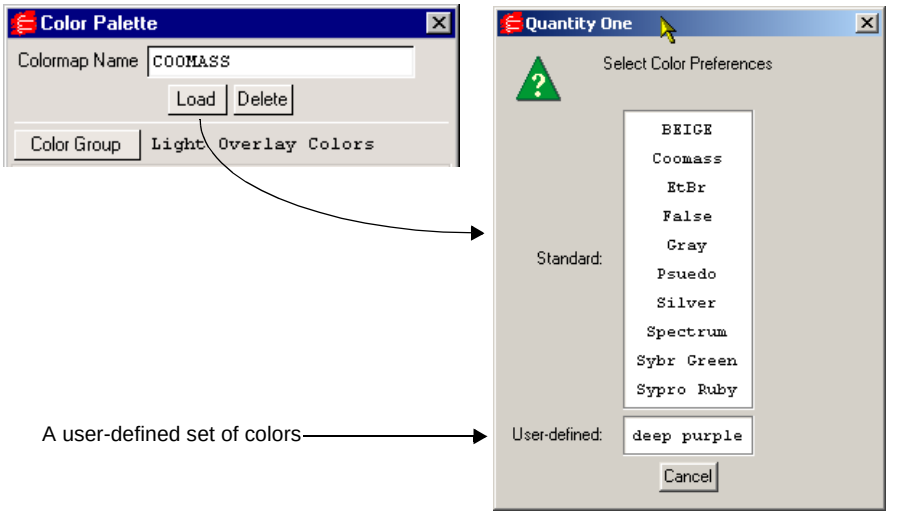


Fig. 3-12. Selecting a Colormap.

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.

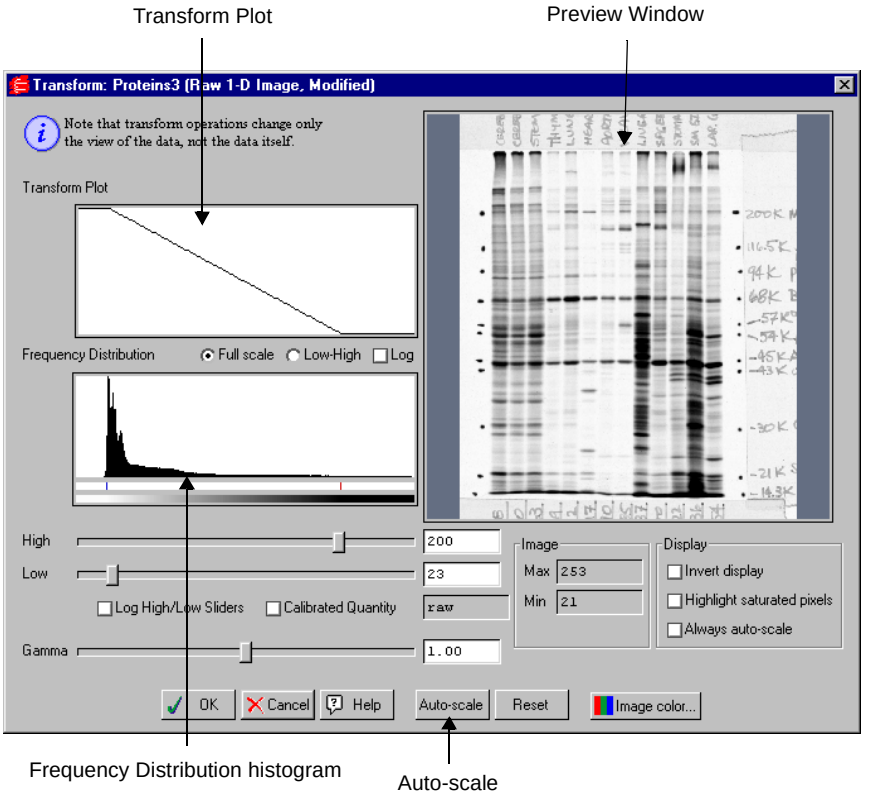


Fig. 3-14. Transform dialog.

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.

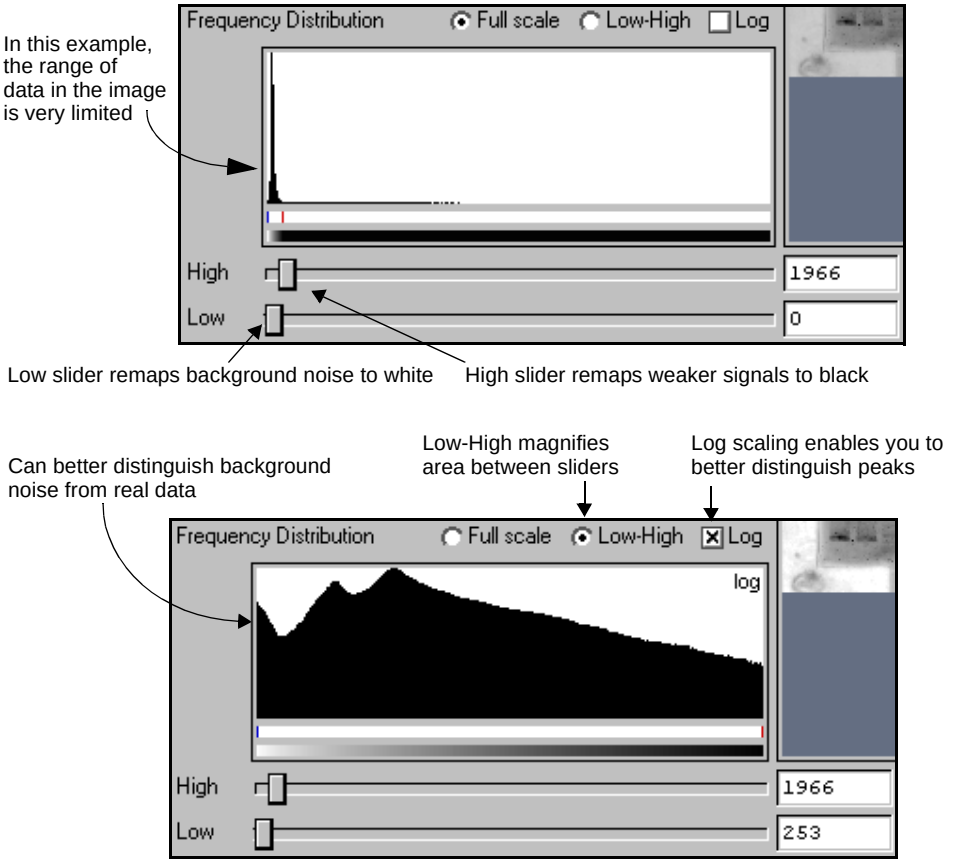


Fig. 3-15. Two views of the Frequency Distribution histogram.

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 unselected, the maximum and minimum numeric pixel values are displayed.

Image Color

Click on this button to open a list of colormaps, which you can define using the **Colors** command on the *Edit* menu (see section 3.7, Colors). Select a colormap 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.

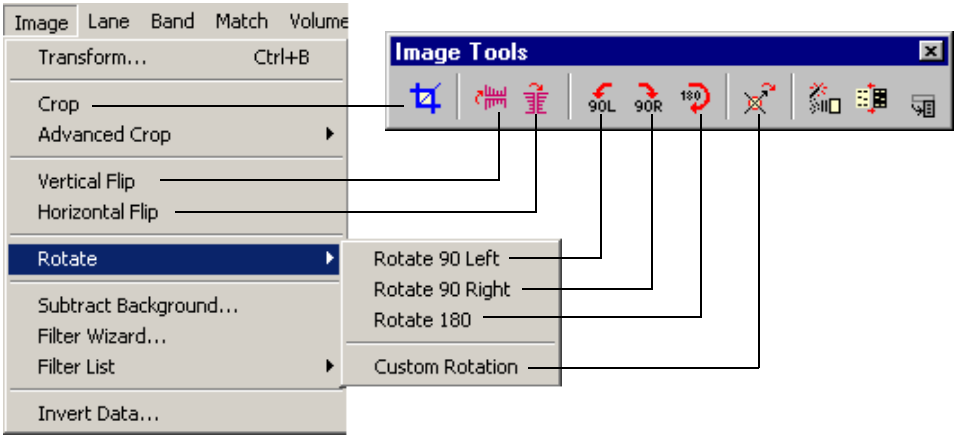


Fig. 3-16. Resizing and reorienting tools.

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.

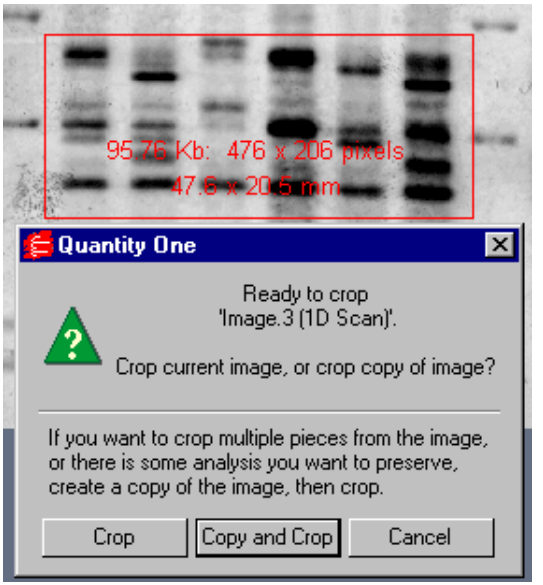


Fig. 3-17. Crop box and pop-up Crop dialog.

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.

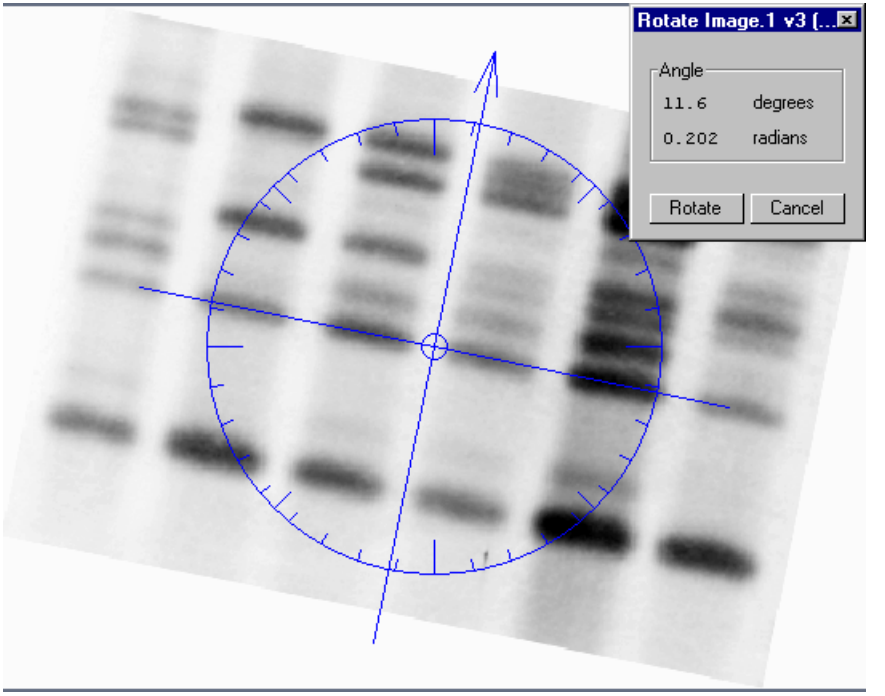


Fig. 3-18. Custom rotation; the arrow points in the direction of the new top of the image.

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 7.4, 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

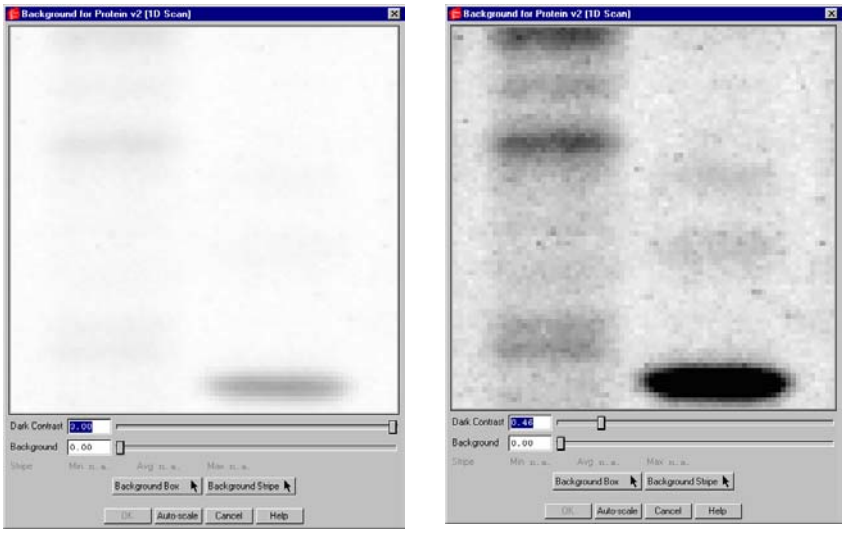


Fig. 3-20. Dark Contrast adjustment reveals true levels of background in the image.

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.

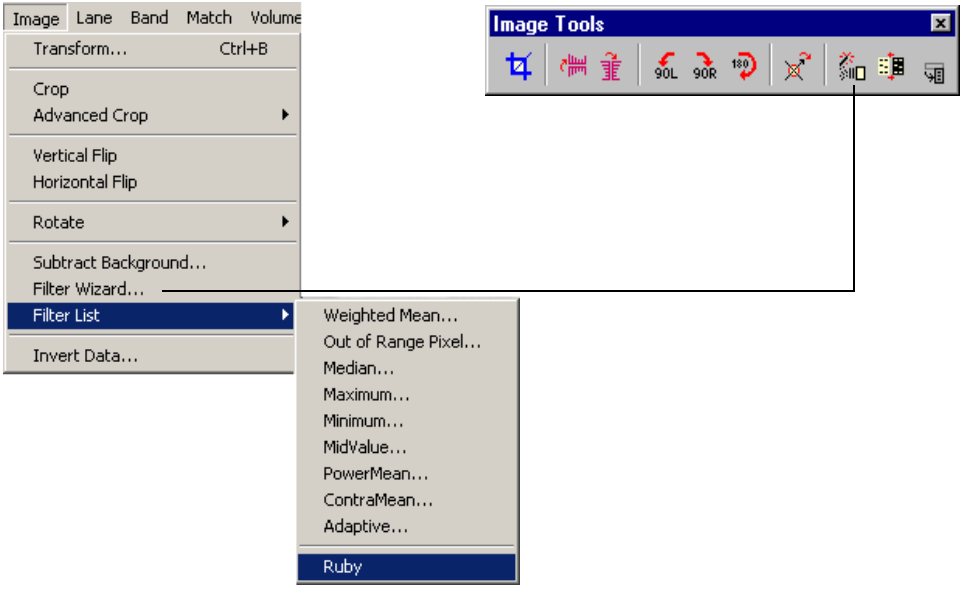


Fig. 3-21. Filtering commands.

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.

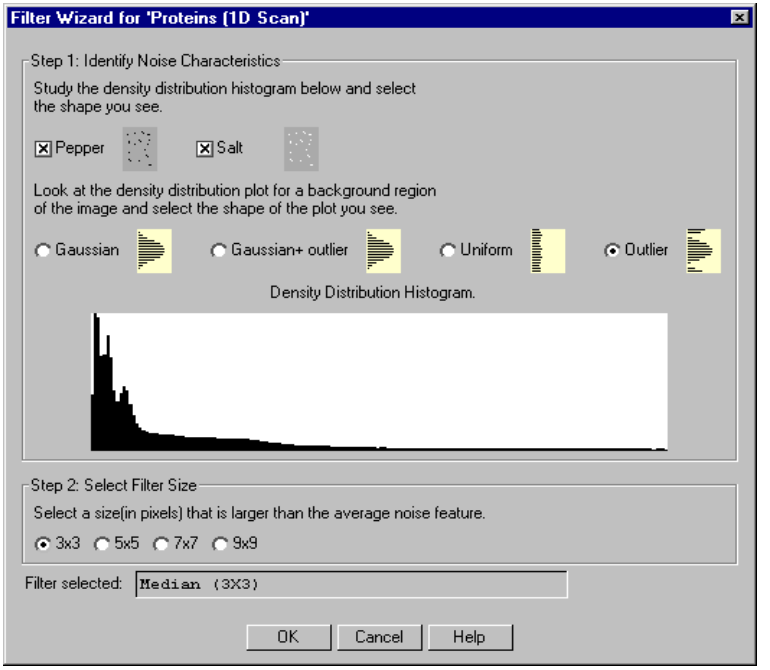


Fig. 3-22. Filter Wizard dialog.

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.

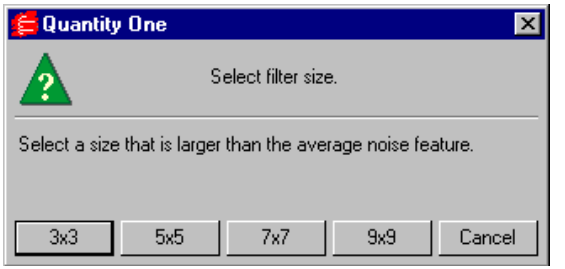


Fig. 3-23. Selecting a filter size.

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.

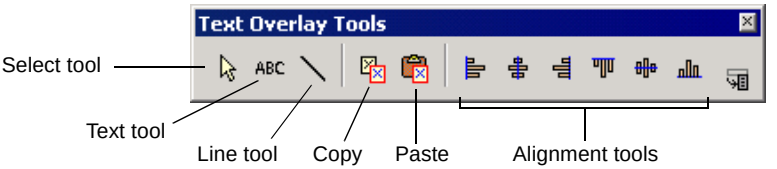


Fig. 3-24. Text Overlay Tools toolbar.

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.

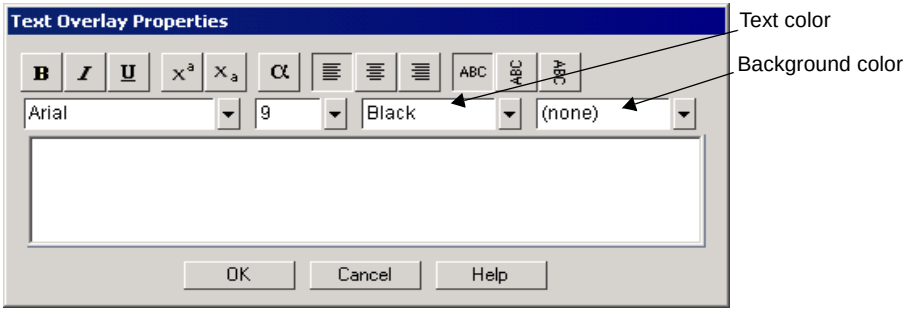


Fig. 3-25. Text Overlay Properties dialog.

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.

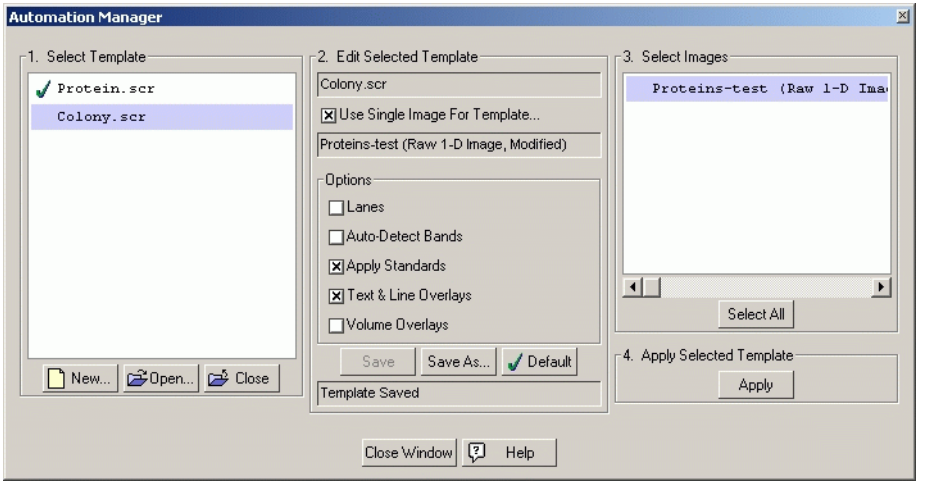


Fig. 3-26. Automation Manager dialog

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.

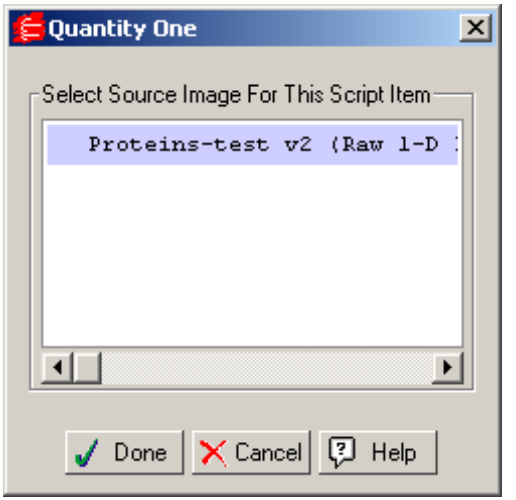


Fig. 3-27. Select an image

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.

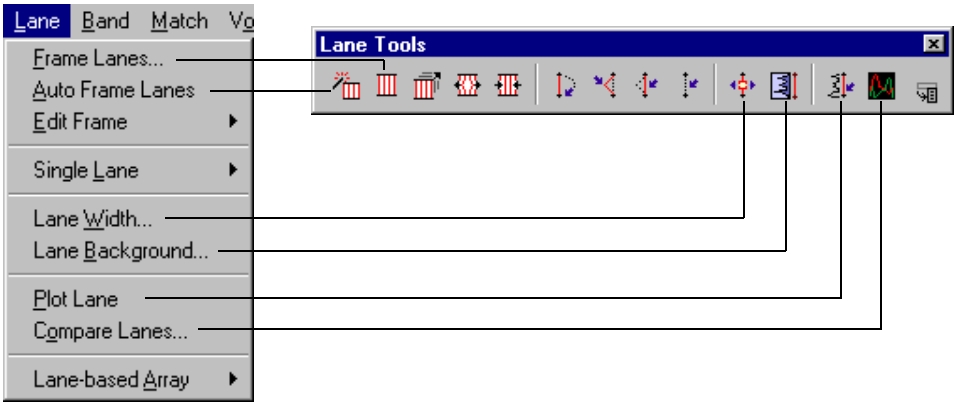


Fig. 4-1. Lane menu and toolbar.

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**.

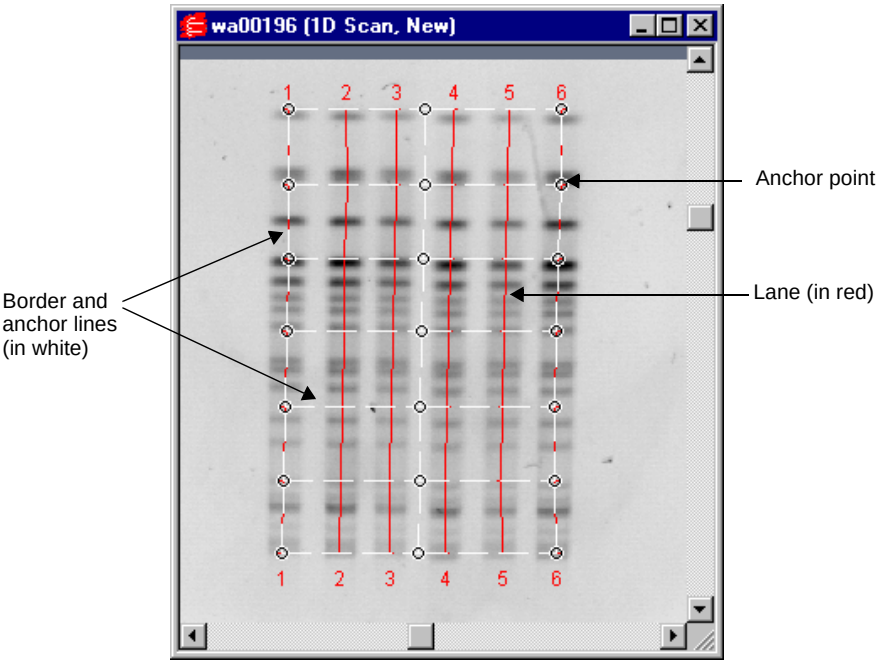


Fig. 4-2. Features of a lane frame.

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**.

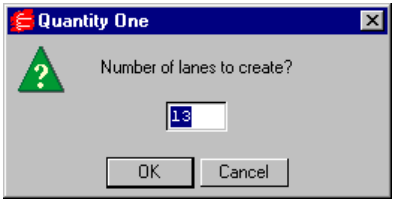


Fig. 4-3. Frame Lanes dialog.

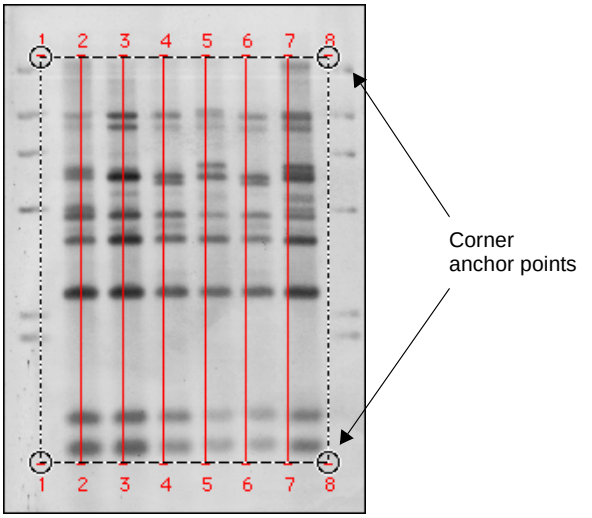


Fig. 4-4. Lane frame created using the Frame Lanes command.

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.

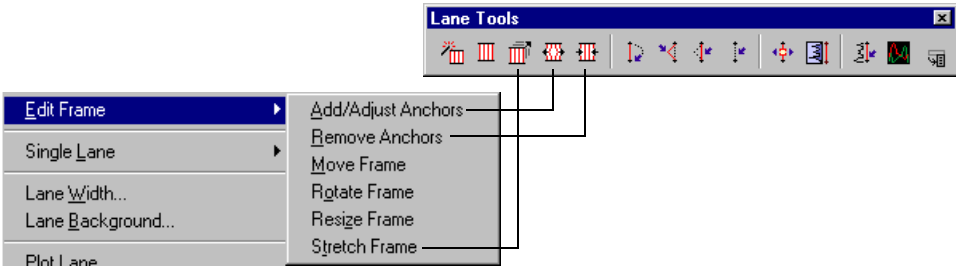


Fig. 4-5. Edit Frame tools.

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.

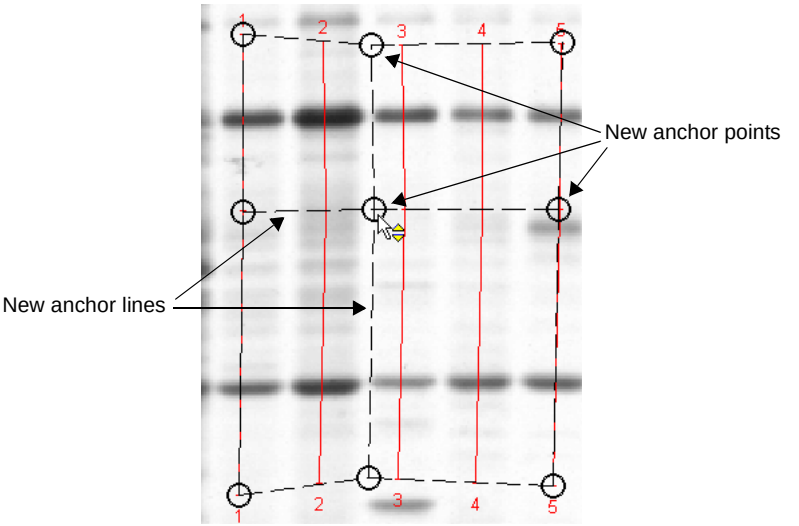


Fig. 4-6. Adjusting the anchor points of the lane frame.

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.

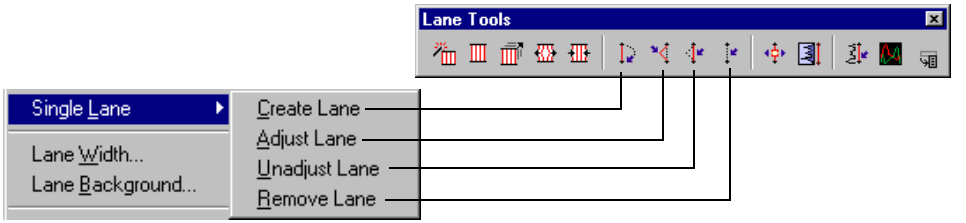


Fig. 4-7. Single Lane tools.

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.

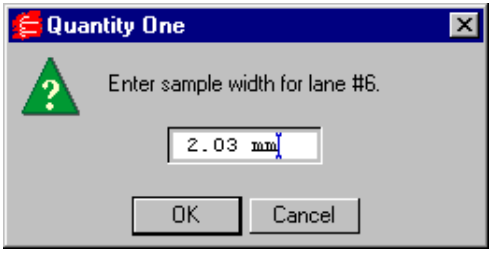


Fig. 4-8. Sample Width dialog.

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.

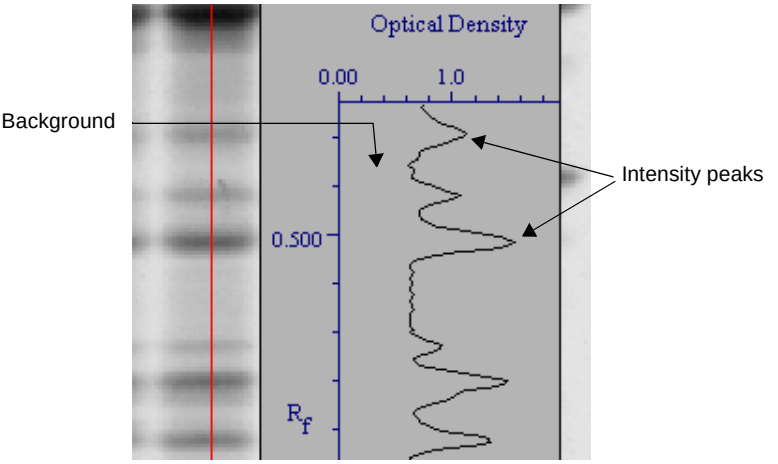


Fig. 4-9. Profile of a defined lane.

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

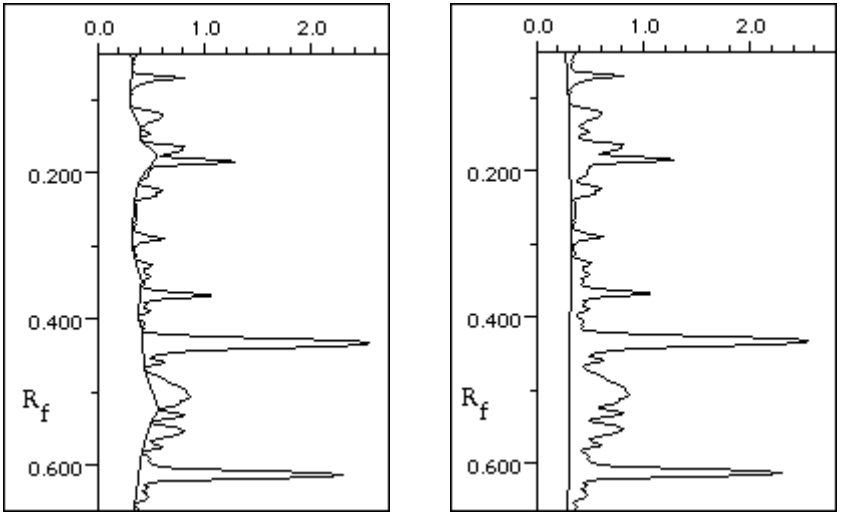
After defining lanes, we strongly recommend that you perform lane-based background subtraction. This is the best method for removing background intensity from lanes, and is required for Gaussian modeling of bands.

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.

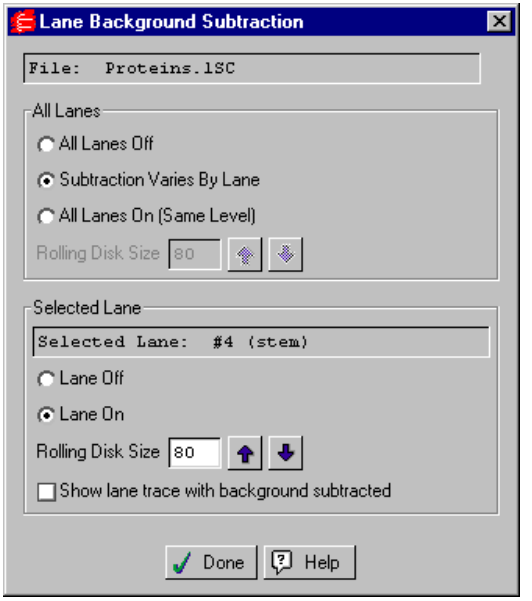


Fig. 4-11. Lane Background Subtraction dialog.

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.

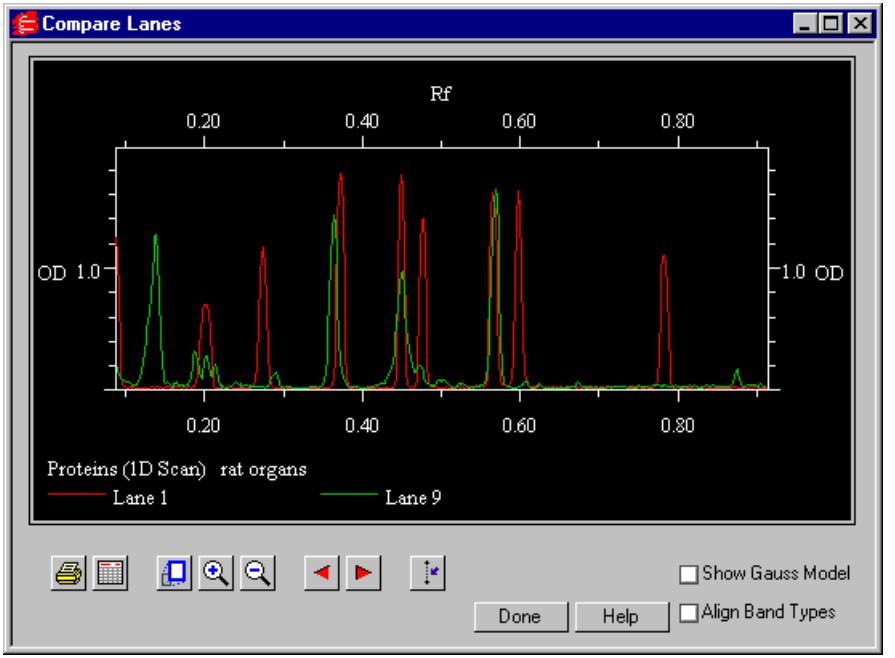


Fig. 4-12. The Compare Lanes dialog.

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

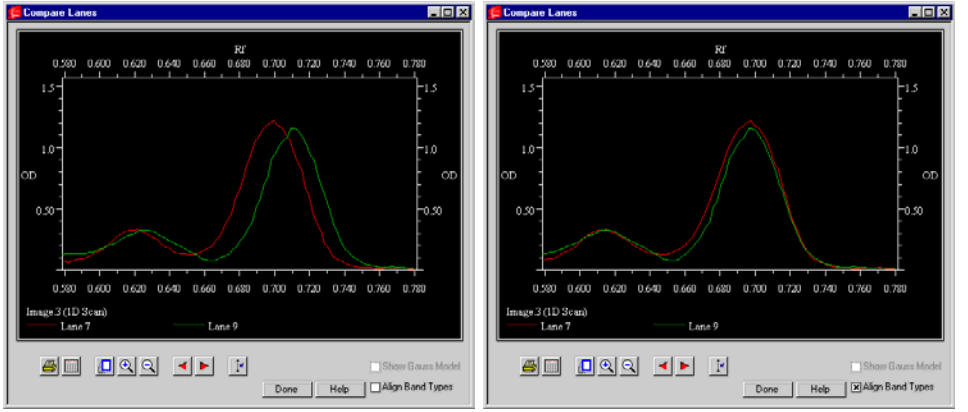
The **Show Gauss Model** checkbox is active if any of the selected lanes includes Gaussian modeling (see section 5.7, Gauss-Modeling Bands). If you select this checkbox, the Gaussian-fitted profiles will be superimposed on the regular lane profiles. The Gaussian profiles are displayed in white.

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.

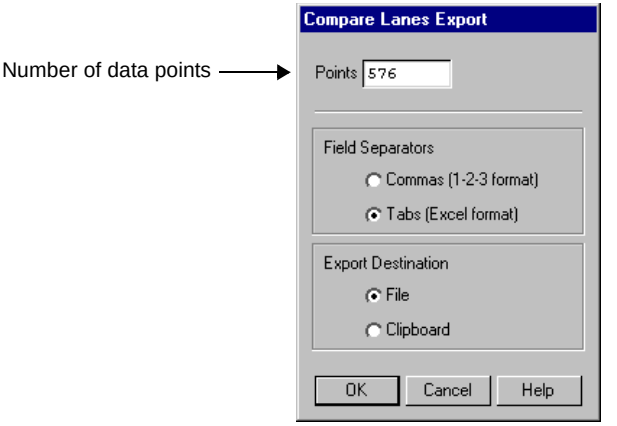


Fig. 4-14. Compare Lanes Export dialog.

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 7.5, 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**.

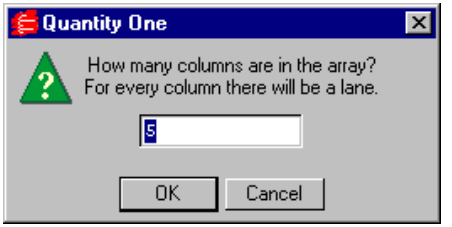


Fig. 4-16. Setting number of array columns.

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

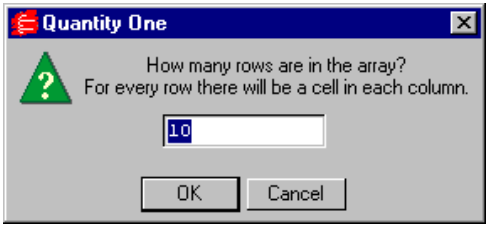


Fig. 4-17. Setting the number of array rows.

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.

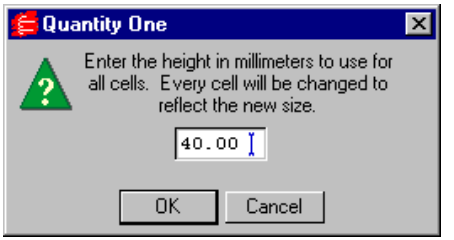


Fig. 4-18. Setting the array cell height.

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.

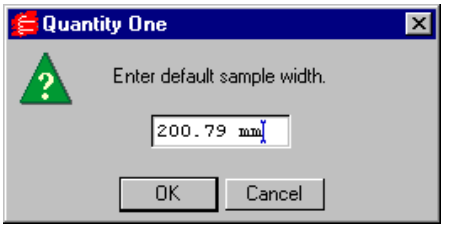


Fig. 4-19. Setting the array cell width.

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.

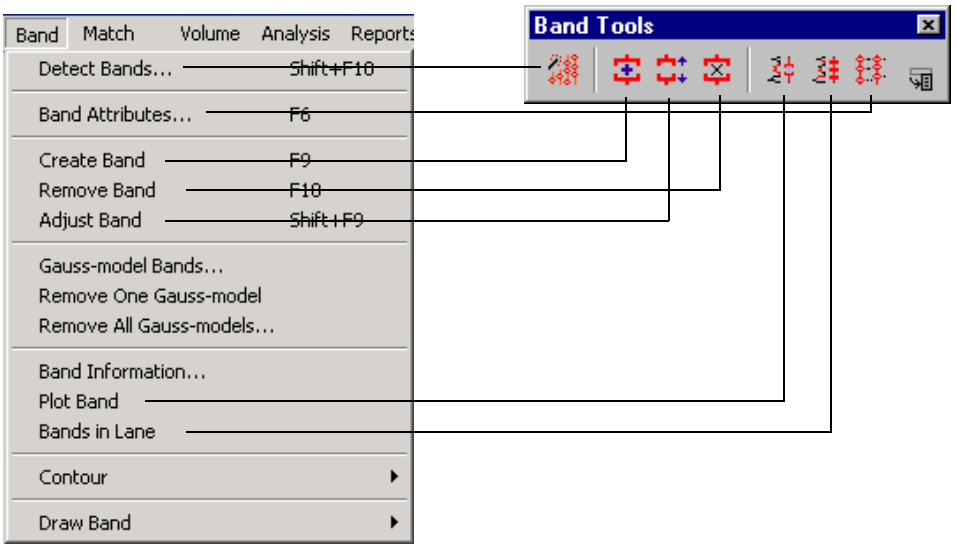


Fig. 5-1. Band menu and Band Tools toolbar.

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.

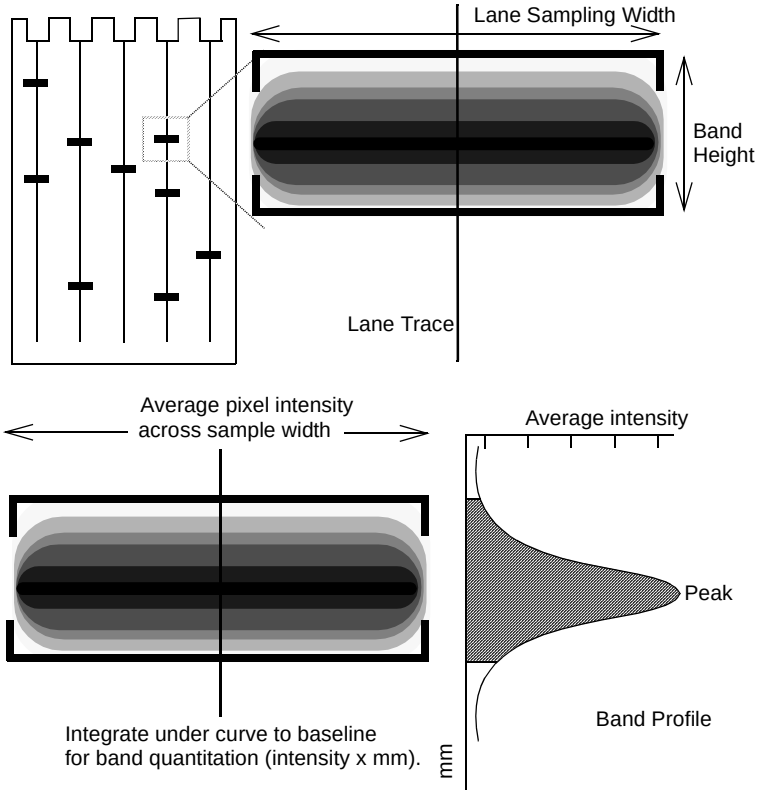


Fig. 5-2. Illustration of bracket quantitation.

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.

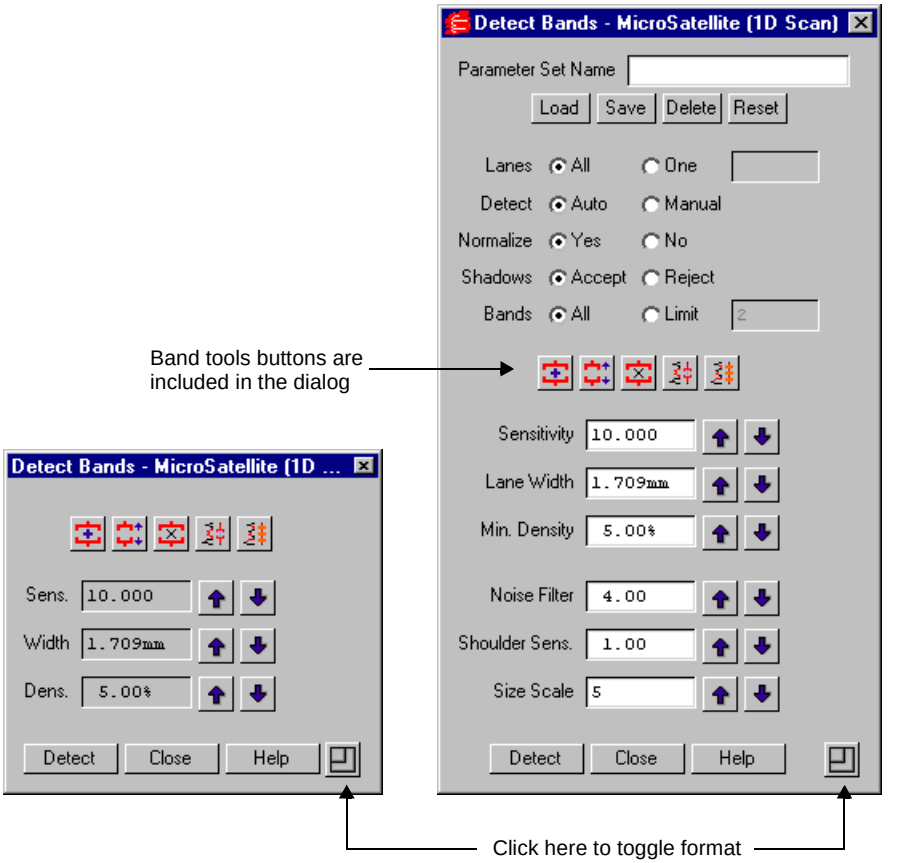


Fig. 5-3. Detect Bands dialog, short and expanded formats, with default values.

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.

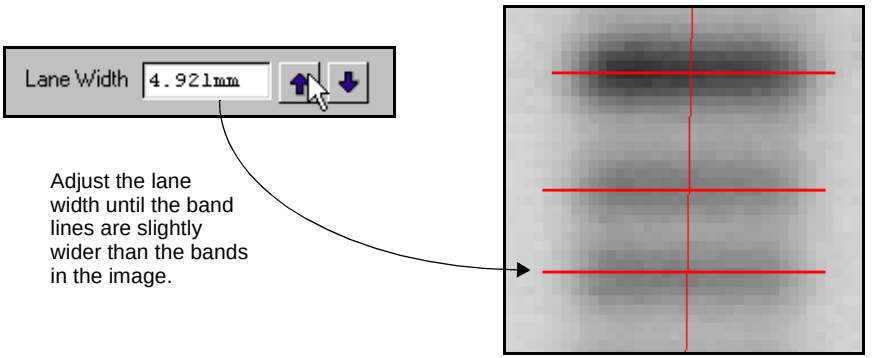


Fig. 5-4. Adjusting the lane width.

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.

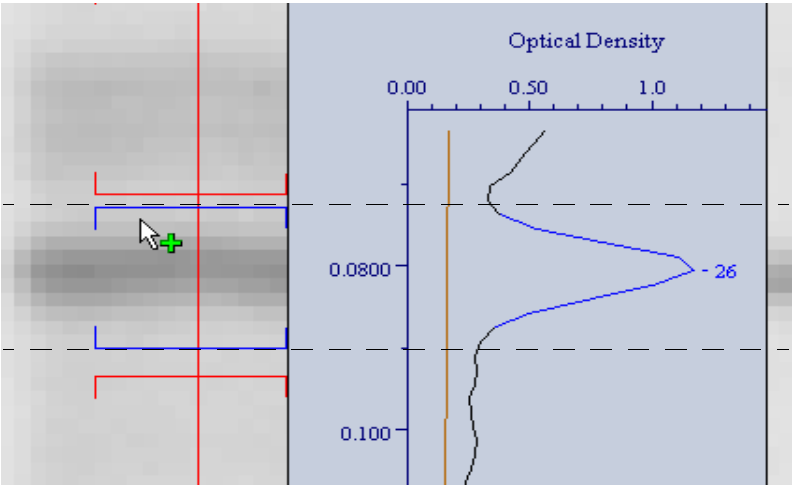


Fig. 5-7. Creating a band with brackets displayed.

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.

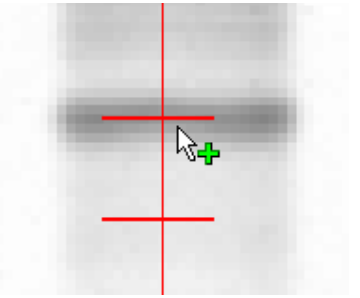


Fig. 5-8. Creating a band with lines displayed.

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.

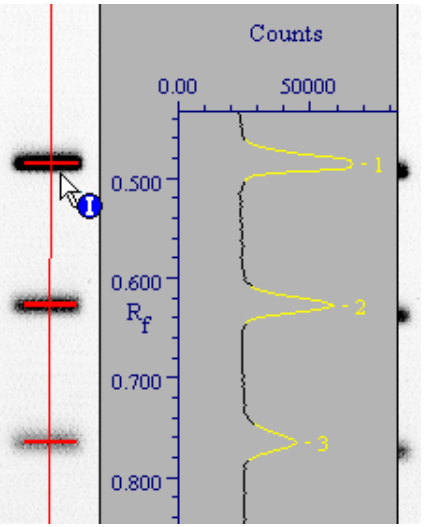


Fig. 5-10. Bands in Lane command.

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.

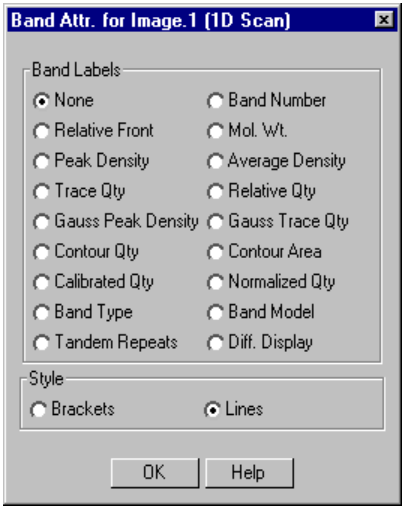


Fig. 5-11. Band Attributes dialog.

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 9.2, 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.

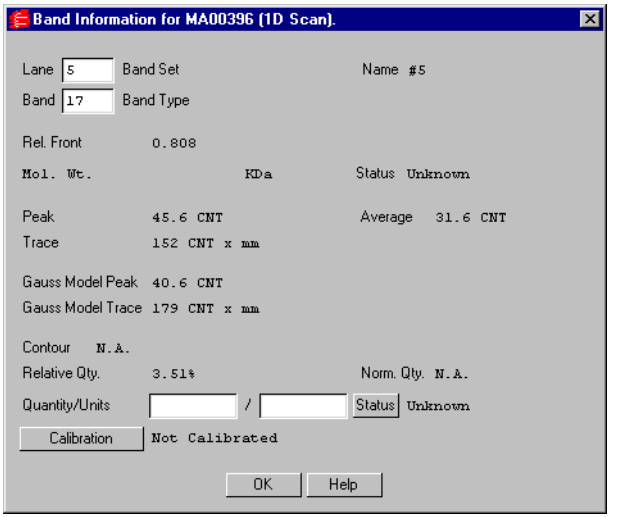


Fig. 5-12. Band Information dialog.

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.

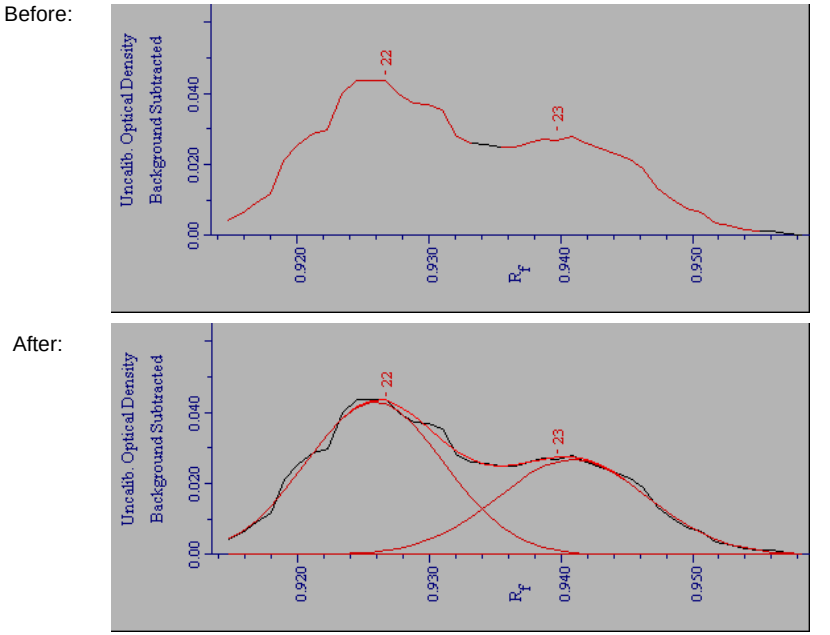


Fig. 5-13. Profiles of two overlapping bands, before (top) and after (bottom) Gaussian modeling. Modeling better resolves the band quantities.

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.

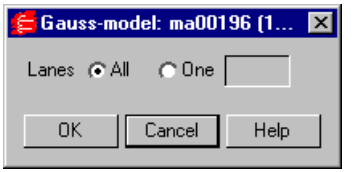


Fig. 5-14. Gauss-model Bands dialog.

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.

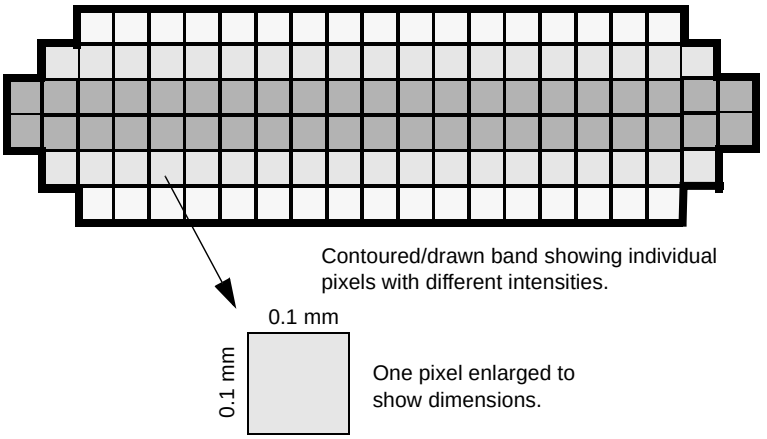


Fig. 5-15. A contoured band scanned at 100 x 100 micrometers.

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

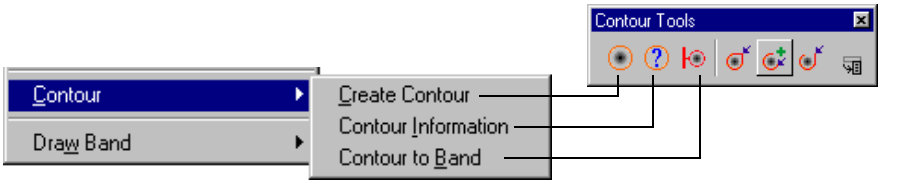


Fig. 5-16. Contour tools.

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.

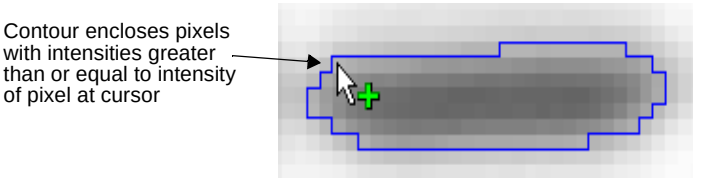


Fig. 5-17. Creating a Contour

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

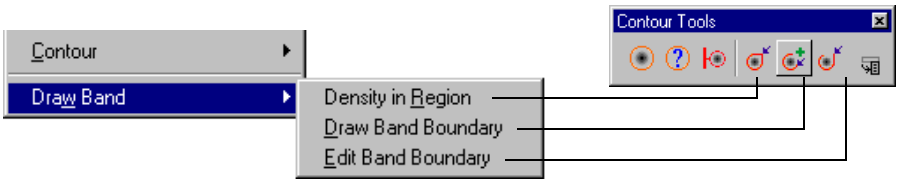


Fig. 5-18. Drawing tools.

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.

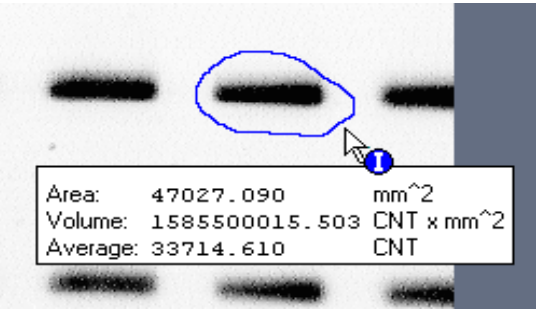


Fig. 5-19. Density in Region tool.

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.

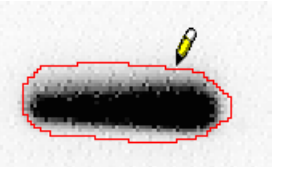


Fig. 5-20. Draw Band Boundary tool.

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.

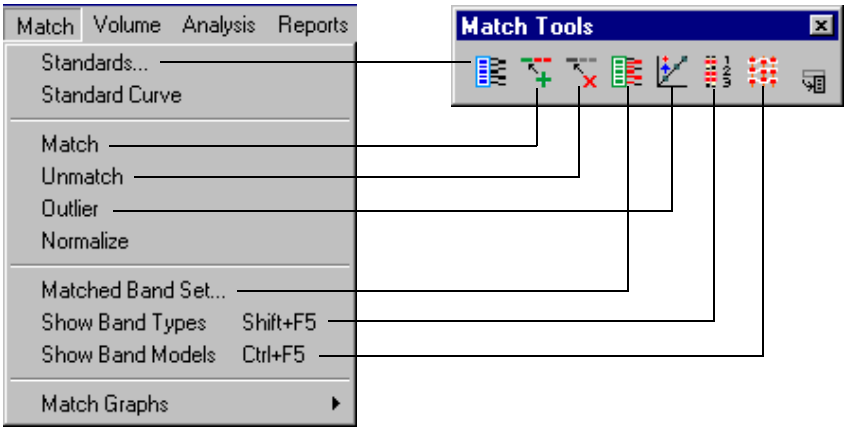


Fig. 6-1. Standards and matching tools.

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.

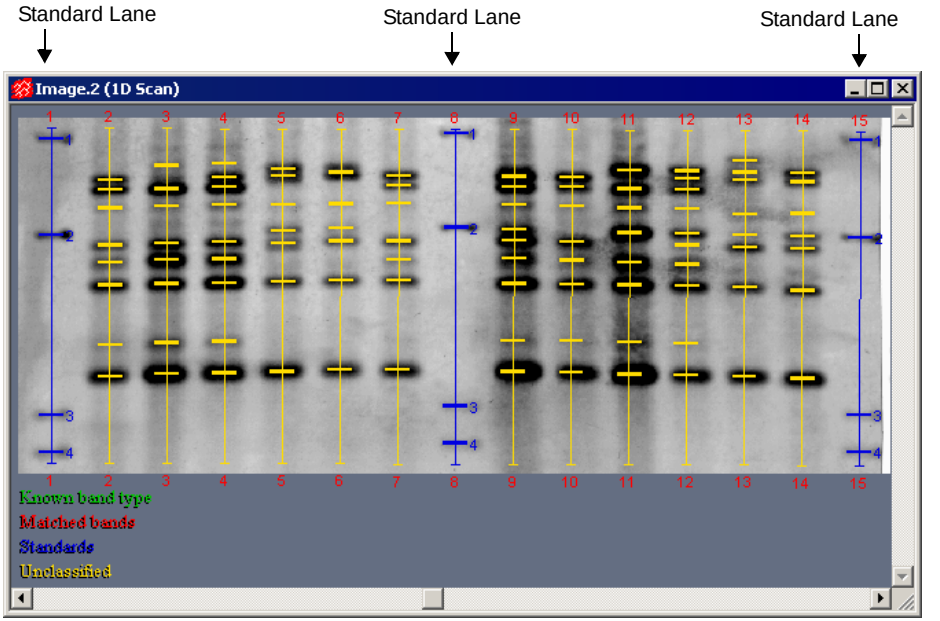


Fig. 6-2. Standard lanes as defined on a gel image.

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.

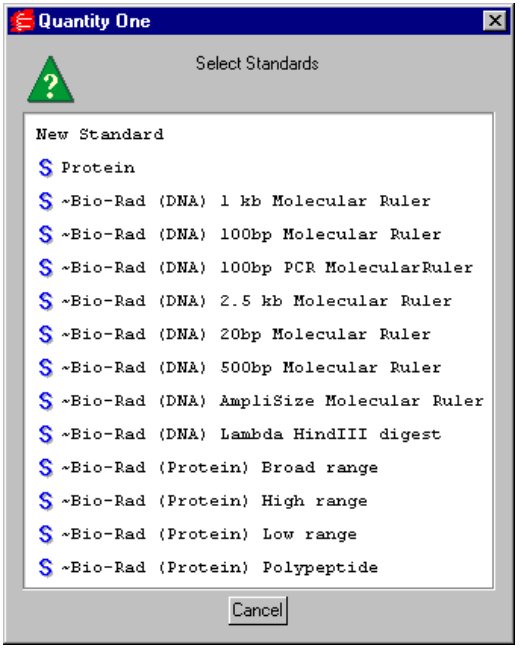


Fig. 6-3. Selecting a set of standards.

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.

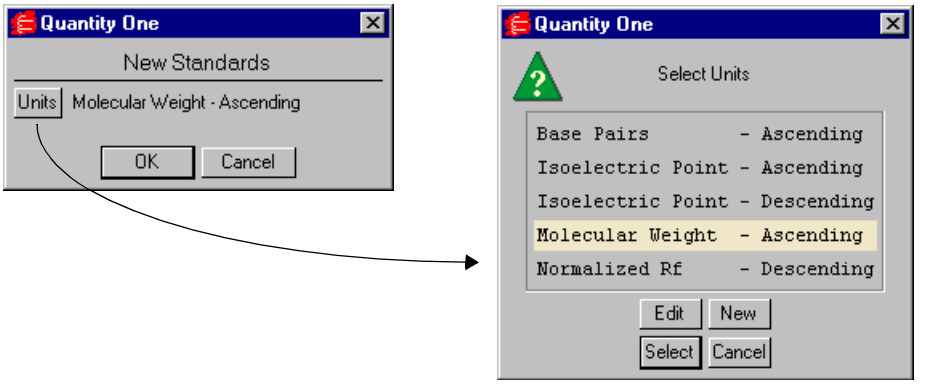


Fig. 6-4. Specifying units.

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**.

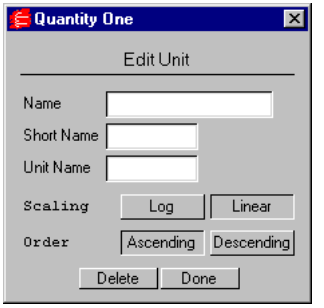


Fig. 6-5. Units dialog.

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.

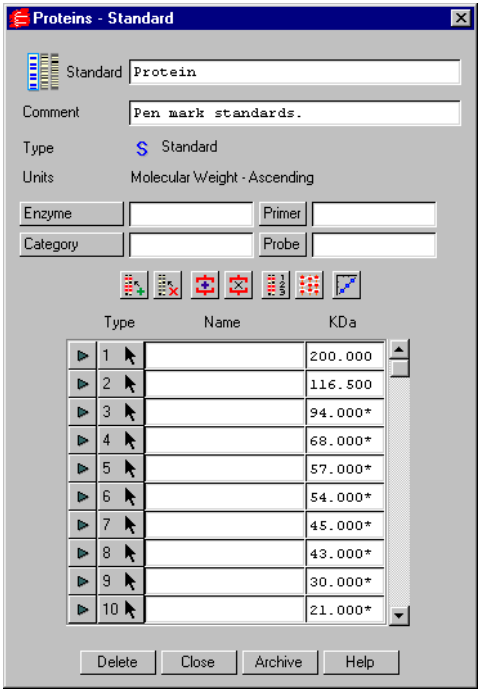


Fig. 6-6. Standards dialog.

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.

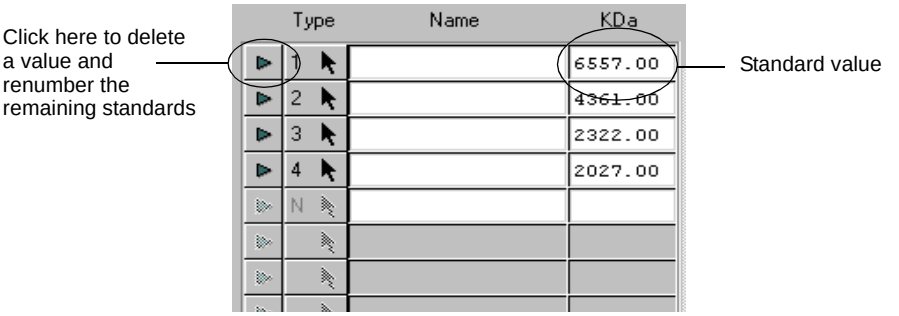


Fig. 6-7. Entering the values of the standards.

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.

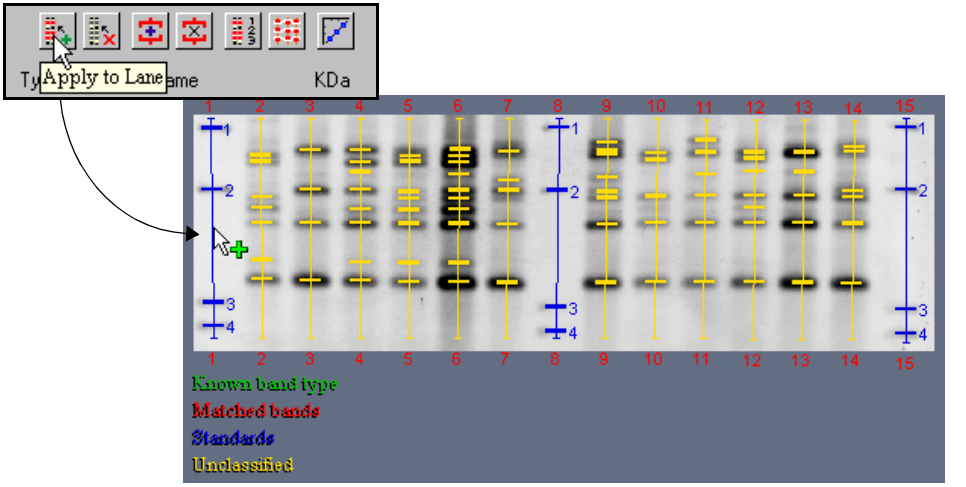


Fig. 6-8. Click on the Apply to Lane button and click on the lanes containing standards.

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.

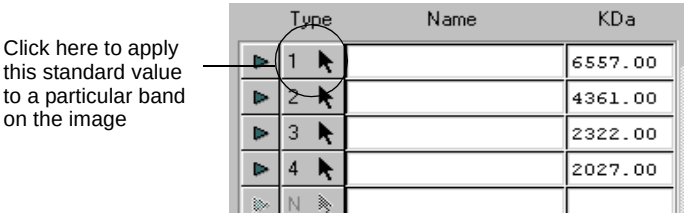


Fig. 6-9. Applying a single standard value.

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.

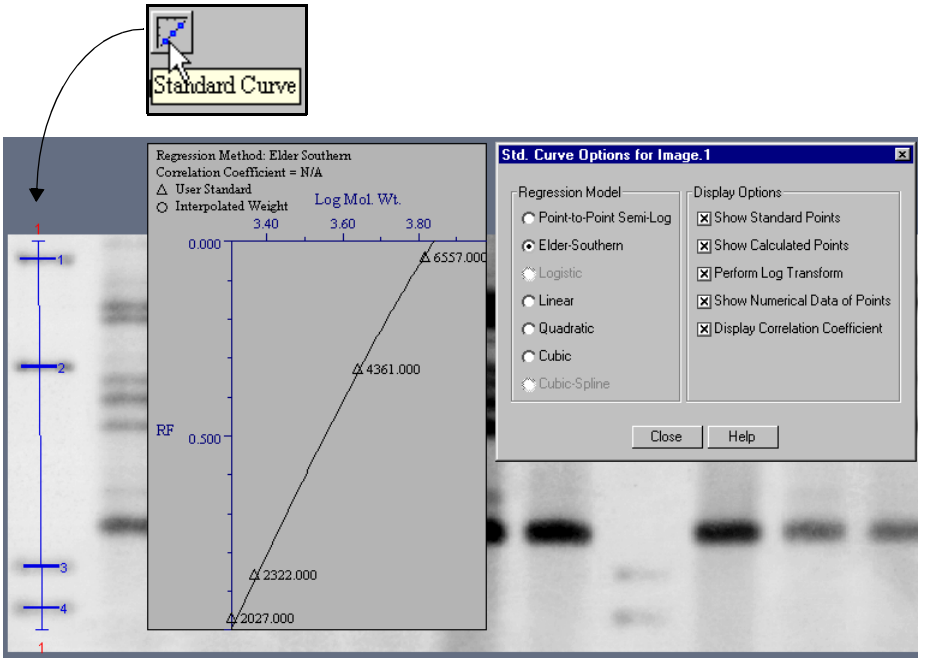


Fig. 6-10. Displaying the standard curve and options dialog.

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.

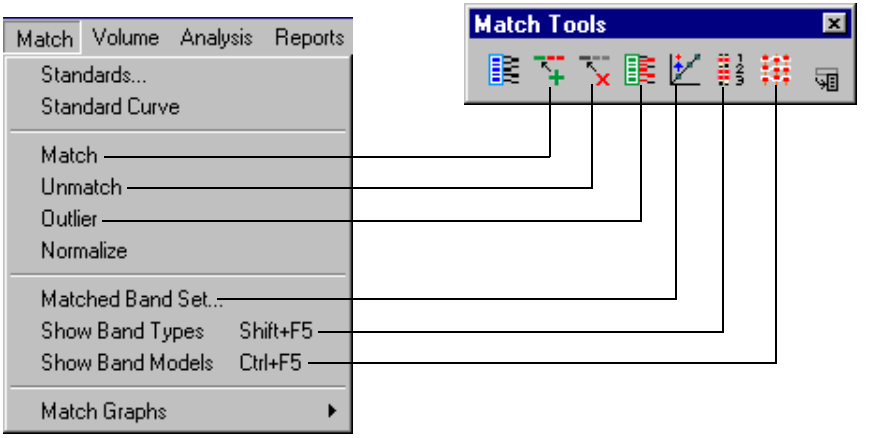


Fig. 6-11. Matching tools.

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.

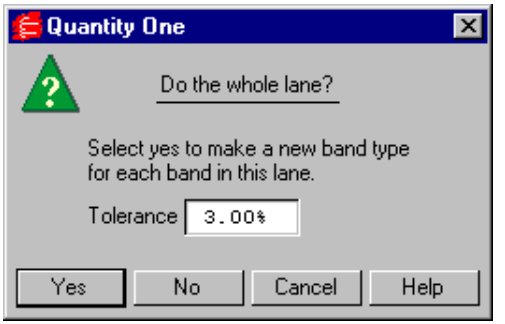


Fig. 6-12. Query box: Apply matching to the whole lane?

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.

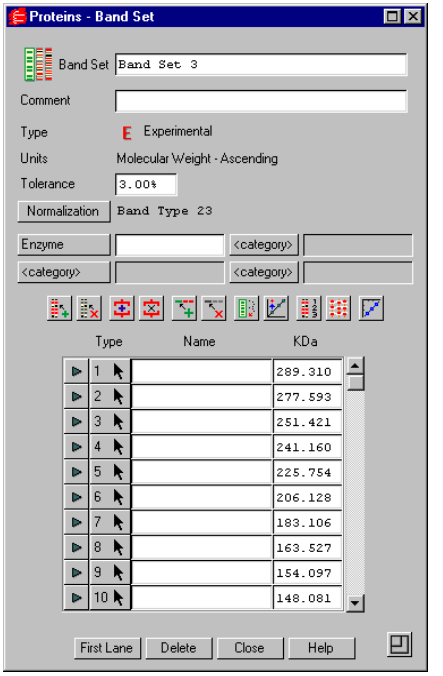


Fig. 6-13. Band Set dialog.

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.

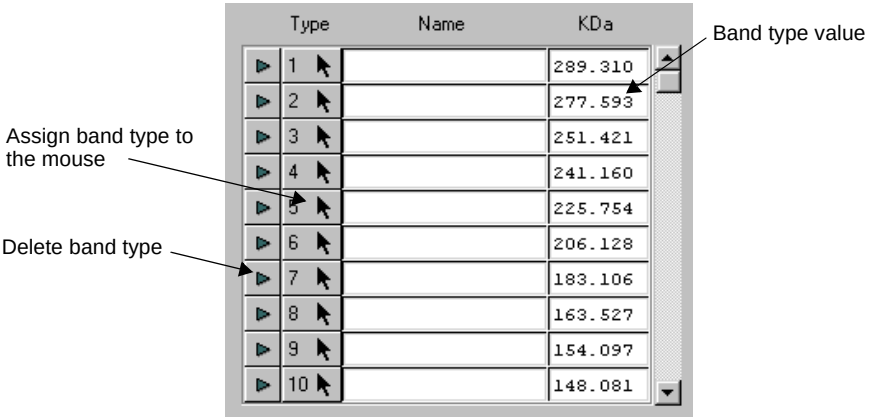


Fig. 6-14. Applying and editing band type values.

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.

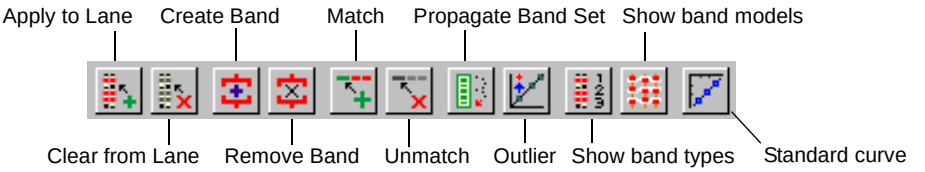


Fig. 6-15. Band set toolbar.

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 9.1, 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.

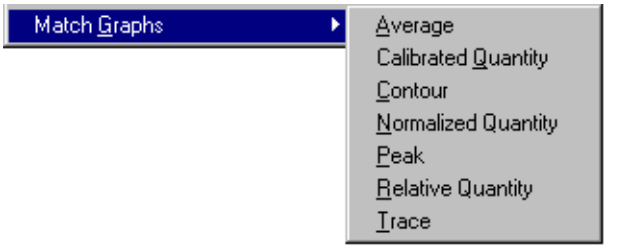


Fig. 6-16. Match Graphs submenu.

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.

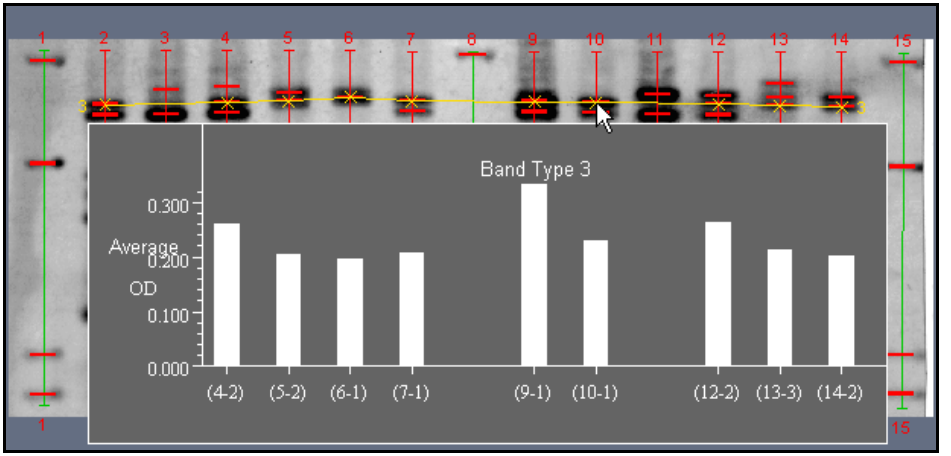


Fig. 6-17. Example of a match graph.

- **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.

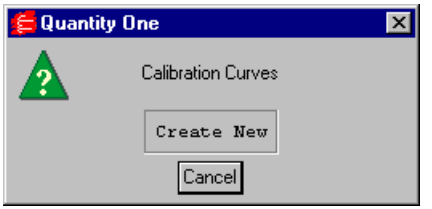


Fig. 6-18. Loading a quantity calibration curve.

Select **Create New** to open a blank *Quantity Standards* dialog.

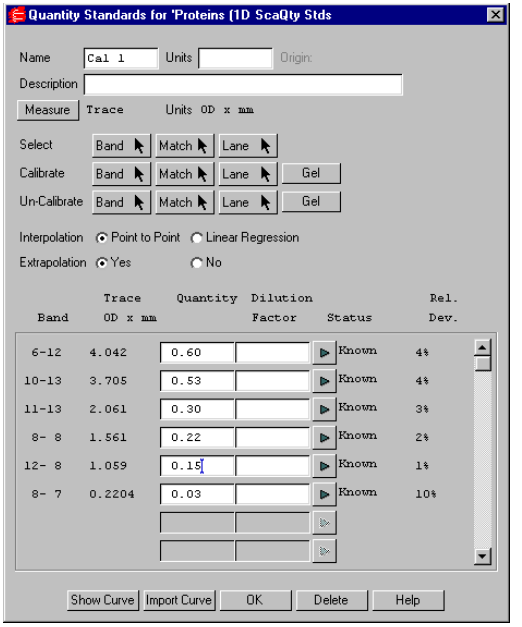


Fig. 6-19. Quantity Standards dialog.

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.

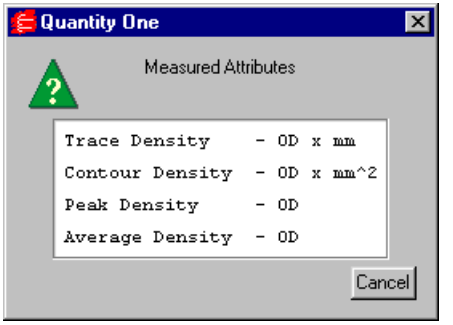


Fig. 6-20. Band attributes to measure for relative quantity.

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**.

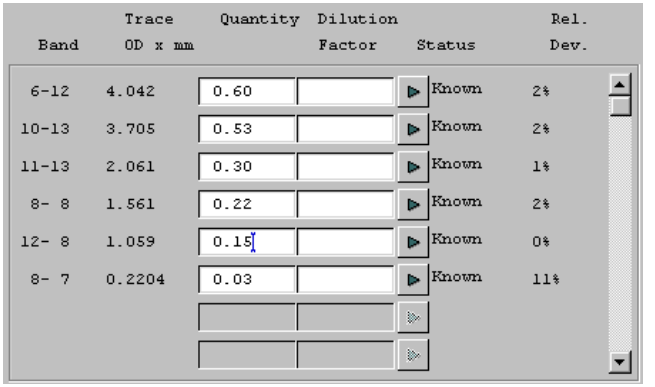


Fig. 6-22. Entering the known quantities.

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.

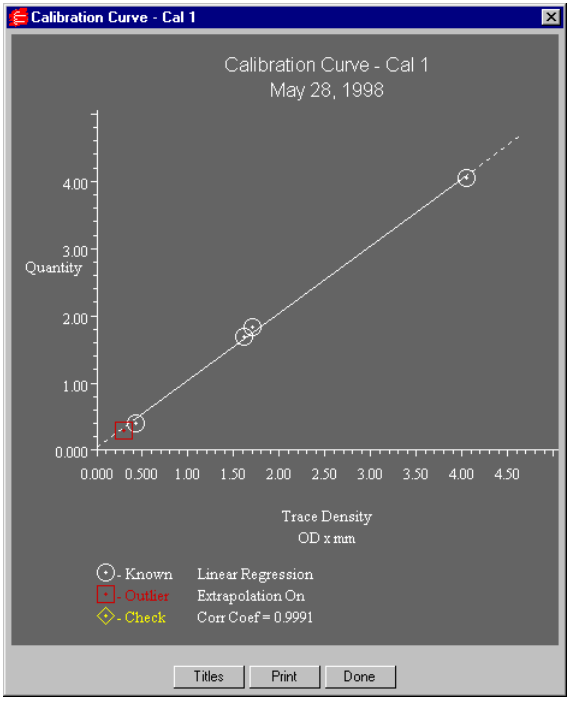


Fig. 6-23. Calibration curve with linear regression and extrapolation.

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.

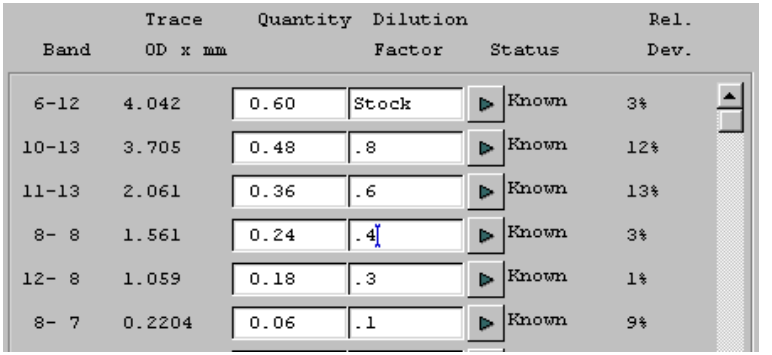


Fig. 6-25. Entering a dilution series.

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.

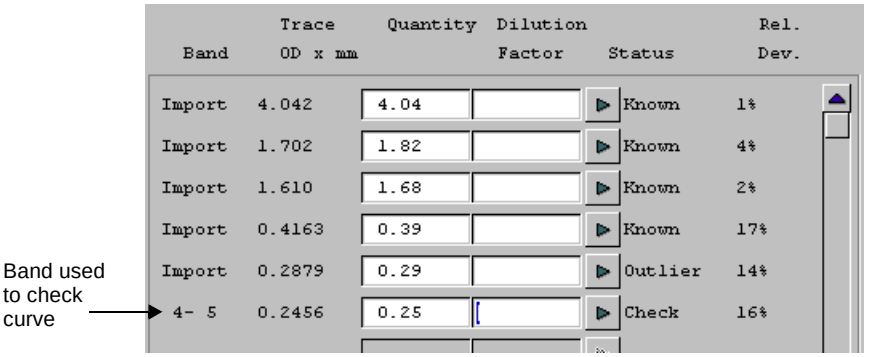


Fig. 6-26. Checking an imported calibration curve.

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 10.6, 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 Overlay Tools** from the main toolbar or the *Edit* menu. These commands are also located on the *Volume* menu.

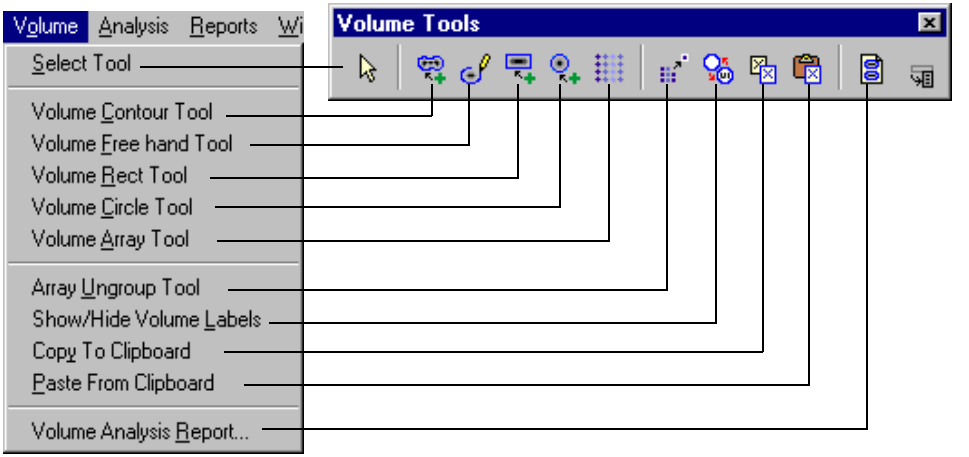


Fig. 7-1. Volume tools.

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 7.4, 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.

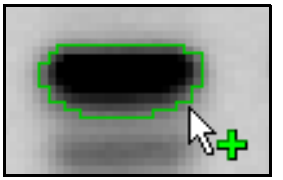


Fig. 7-2. Volume contour.

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.

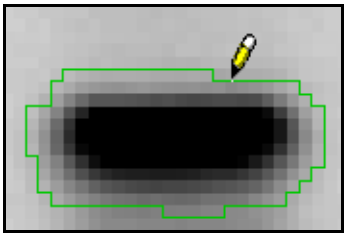


Fig. 7-3. Volume free hand.

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.

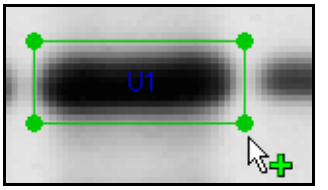


Fig. 7-4. Volume rectangle.

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.

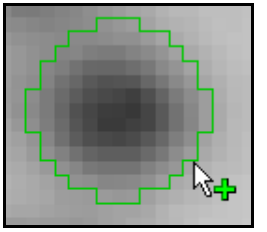


Fig. 7-5. Volume circle.

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.

Volume Labels

When you first create volumes, they will appear labeled. Click on **Show/Hide Volume Labels** on the *Volume* menu or toolbar to hide the labels.

The volumes will have default labels: U1, U2, U3, etc. The "U" stands for unknown, as distinguished from standard and background volumes. The number indicates the sequence in which the volume was created.

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.

Editing Volume Labels

The Edit Volume Labels tool allows you to change the text format of the label of the currently selected volume. In the Volume Properties dialog, click **Edit User Label** to open the Text Overlay Properties dialog. From this dialog you can change the size, color, orientation, and background of the selected text. Select the font style, font size, color of the text, and color of the background in the text box using the pull-down lists.

If you wish to change the text of the volume label, enter the new label in the text field. 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 of the edited volume label.

When you are satisfied with your edits, click OK. This returns you to the Volume Properties dialog.

Note: Changes made to a volume label will not change the default volume label formatting.

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 unselected—the border will change 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 Overlay 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.

7.2 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.

7.3 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.

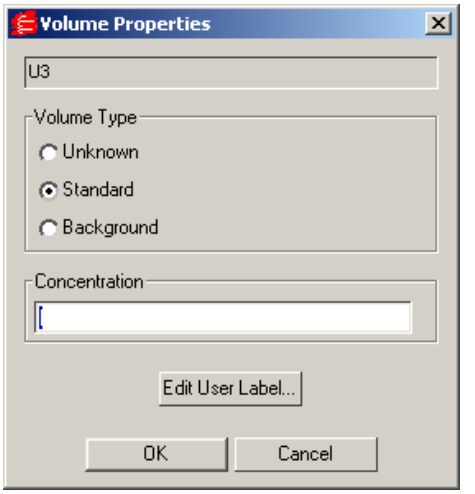


Fig. 7-6. Volume Properties dialog.

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 10.7, Volume Regression Curve) under the *Reports* menu to calculate the concentrations of the unknown volumes.

To change a standard back to an unknown, double-click on it, then select the **Unknown** button.

7.4 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 10.6.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.

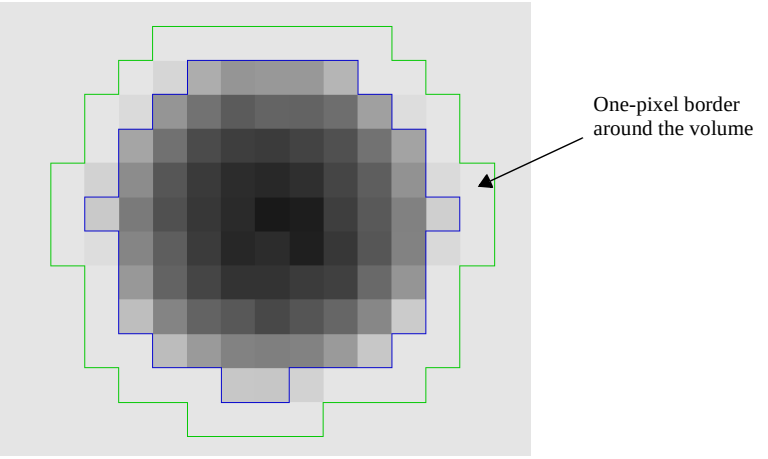


Fig. 7-7. Local background is calculated from a one-pixel border around the volume

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.

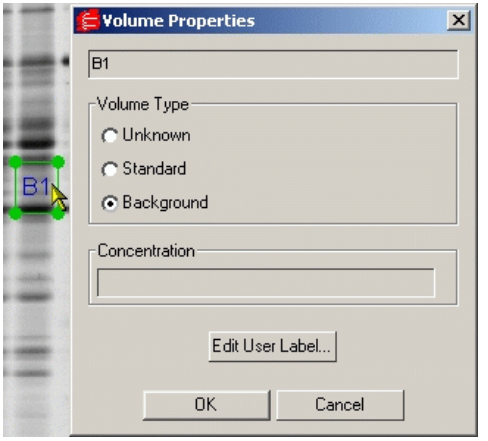


Fig. 7-8. Defining a background volume object.

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 10.6, 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.

7.5 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.

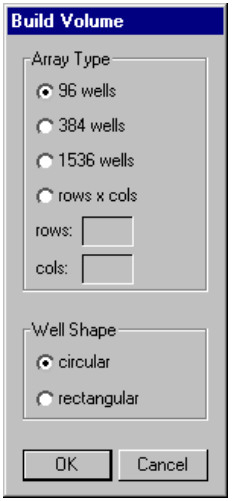


Fig. 7-9. Build Volume Array dialog.

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.

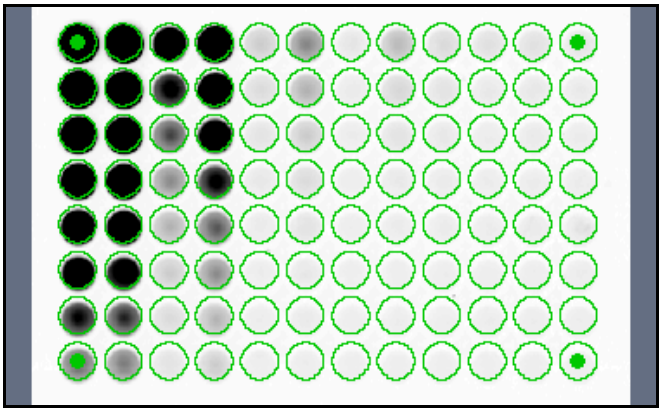


Fig. 7-10. Array overlay.

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.

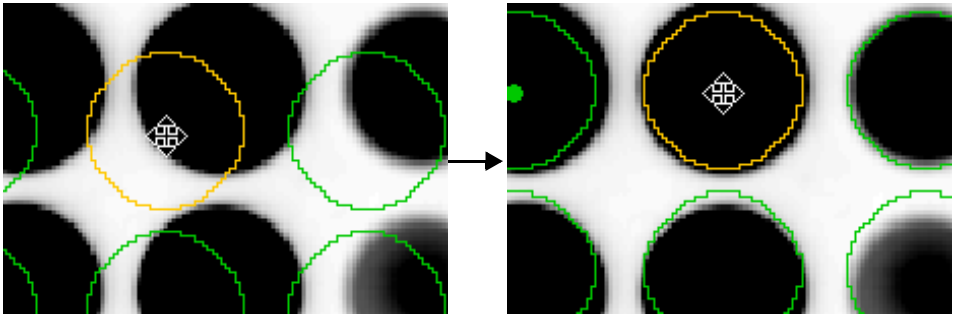


Fig. 7-11. Moving an array.

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.

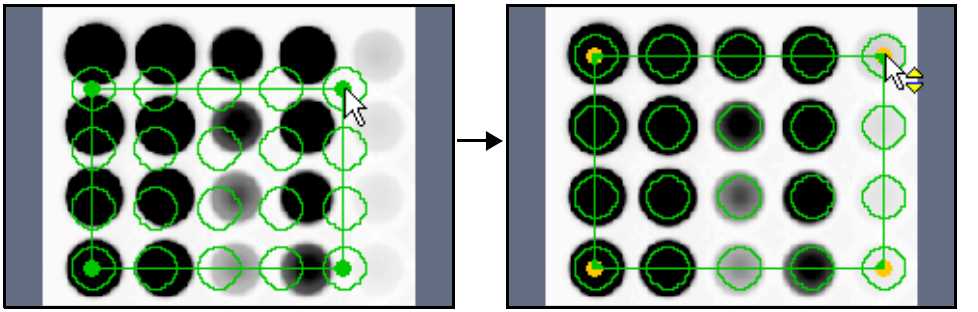


Fig. 7-12. Resizing an array.

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.

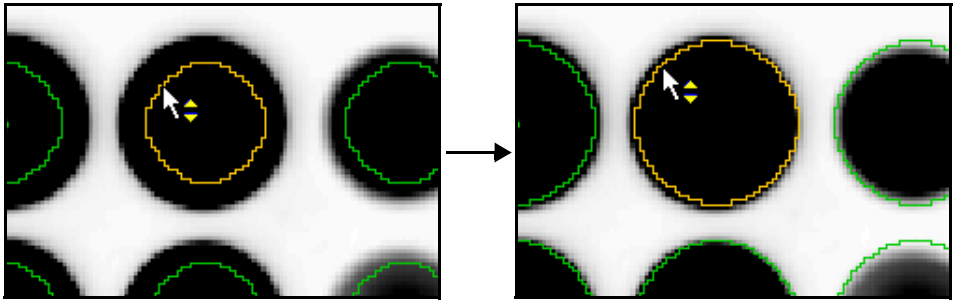


Fig. 7-13. Resizing an array cell.

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.

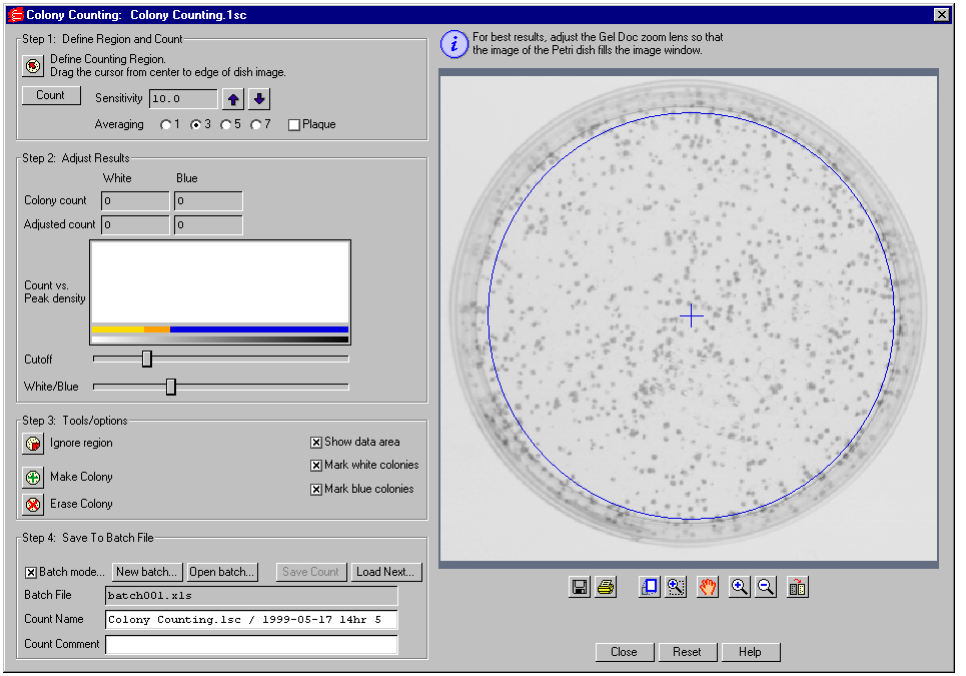


Fig. 8-1. Colony Counting dialog.

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.

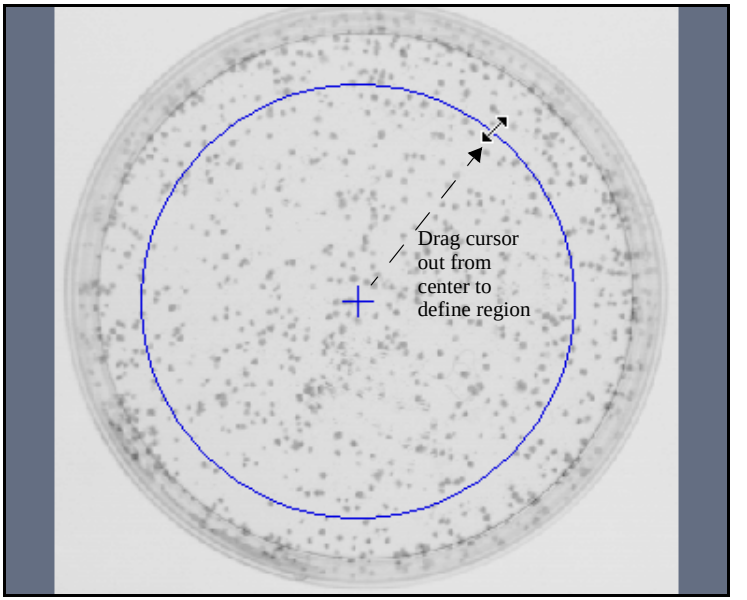


Fig. 8-2. Defining a counting region.

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.

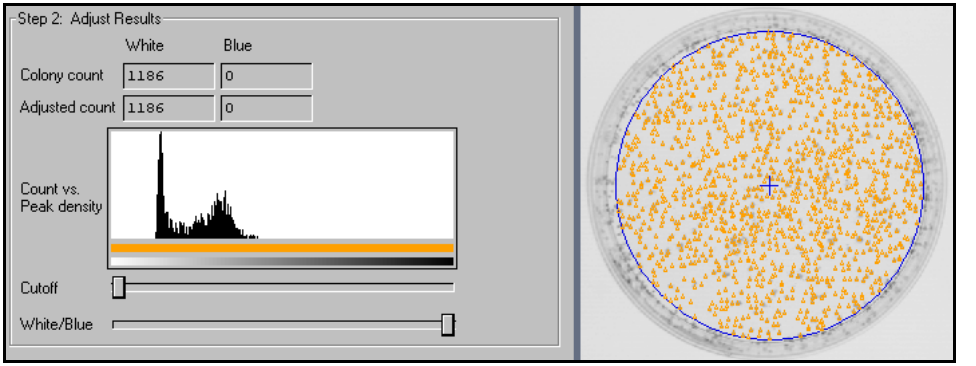


Fig. 8-3. Example of a dish with white colonies.

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.

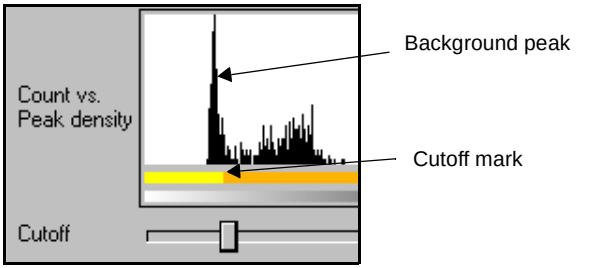


Fig. 8-5. Using the Cutoff slider.

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.

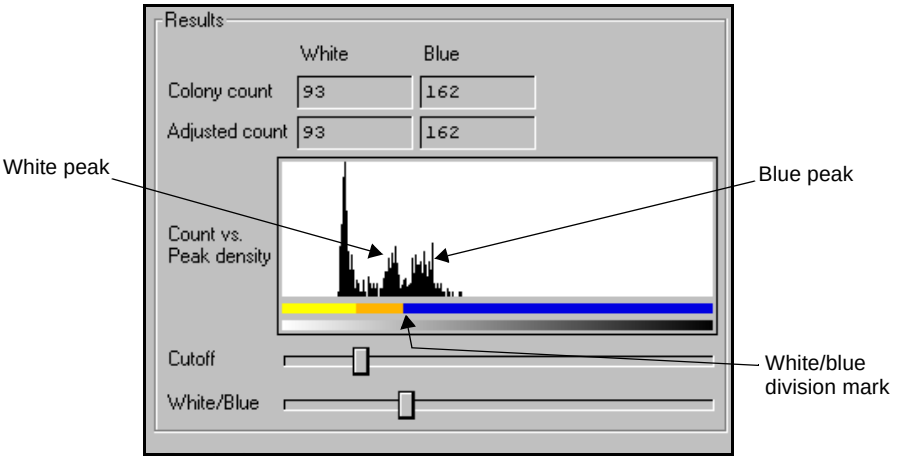


Fig. 8-6. Using the White/Blue slider.

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.

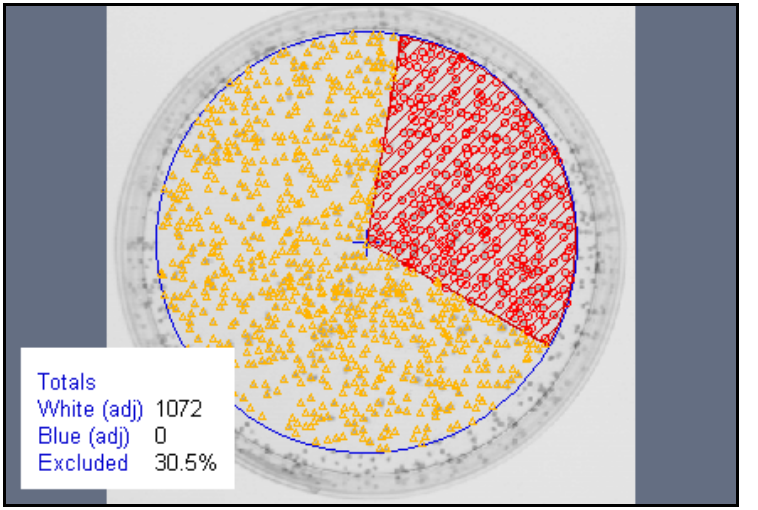


Fig. 8-7. Marking a region to ignore.

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.

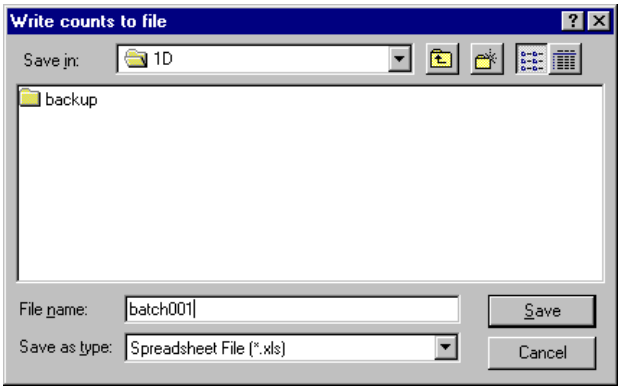


Fig. 8-9. Creating a batch file.

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. Differential Display and VNTRs

Quantity One has tools for studying gene expression using differential display analysis and counting VNTRs or other repeated elements in gels.

These functions are located on the *Analysis* menu.

9.1 Differential Display

Differential display is a popular technique using mRNA and PCR amplification to identify genes that are differentially expressed between and among cell types.[1] The mRNA in a cell is amplified using RT-PCR and resolved on a DNA sequencing gel, where it can be compared with mRNA from related cell types to determine differential gene expression.

The **Differential Display** tool in Quantity One facilitates the side-by-side comparison of the bands in a sequencing gel by highlighting the up or down regulation of bands across lanes.

Note: You must match the bands in the gel image before using this function. See Chapter 6 for more information.

9.1.a Normalization

Before using the **Differential Display** function, you must normalize the bands in the gel for quantity across all the lanes. With the gel open and matched, select **Normalize** from the *Match* menu and click on a distinct, well-resolved band that appears in *every lane* of the gel. The quantity of that band in each lane will be set to 100, and the quantities of the other bands in the lane will be normalized to that band. (If the band is not present in a lane, the normalized quantity for that lane is zero.)

---

1. Liang, P., and Pardee, A. (1992) *Science 257: 967-970.*

See section 6.2.d, Normalizing for Quantity, for more information on normalization.

9.1.b Differential Display Searches

After you have normalized the gel, select **Differential Display** from the *Analysis* menu. A dialog will open in which you can specify certain parameters.

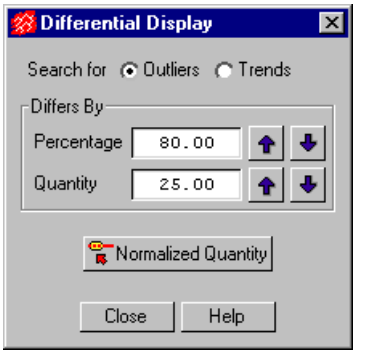


Fig. 9-1. The Differential Display dialog box.

You can perform two types of searches on bands across a differential display gel: an **Outliers** search or a **Trends** search. Both are determined by the **Percentage** and **Quantity** values that you specify in the **Differs By** field.

Outliers

Select the **Outliers** button to highlight bands with a normalized quantity that differs from the mean normalized quantity for that band type by the specified percentage and/or the specified quantity. An outlier is defined as a band with normalized quantity (n_q) that satisfies one of the following criteria:

1. n_q > ratio x mean and
   n_q > mean + quantity

2. n_q < (1/ratio) x mean and
   n_q < mean – difference

Where: ratio = 1.0 + (percentage/100)

Trends

Select the **Trend** button to search for increasing or decreasing levels of gene expression across samples, represented by trends in normalized quantity across lanes. With **Trend** selected, a linear regression of (n_q) versus lane number is computed for each band type. The leftmost and rightmost lanes containing that band type are determined. The normalized quantities for these lanes are calculated from the regression model. If:

abs(leftmost_n_q – rightmost_n_q) $\geq$ difference and
Max(leftmost_n_q, rightmost_n_q) / Min(leftmost_n_q, rightmost_n_q) $\geq$ ratio

then the band type is flagged as a trend.

Displaying Results

Bands identified as outliers or belonging to a trend are highlighted with a **white line** in the image.

If a lane is missing a band assigned to a band type that is flagged as an outlier or a trend, the expected location of the band will be highlighted by a **white box**.

Use the **Normalized Quantity** button in the *Differential Display* dialog to display a histogram of the normalized quantities and the mean quantity for a band type. Click on the button, then click on the band. A graph will display the normalized quantities of that band across the entire gel.

9.2 Variable Number Tandem Repeats

If your experiments involve the use of microsatellites, VNTRs, or other repeated elements, you can calculate the number of times a repeated element occurs in a band.

Note: You must define the **Standards** in the gel before you can use this function (see section 6.1, Standards).

To calculate the VNTRs in the bands in a gel, select **VNTR Calculations** from the *Analysis* menu. (You can also select the **VNTR Quick Guide** from the *Help* menu for guidance on analyzing gels with VNTRs.)

The *Tandem Repeat Calculations* dialog box will open.

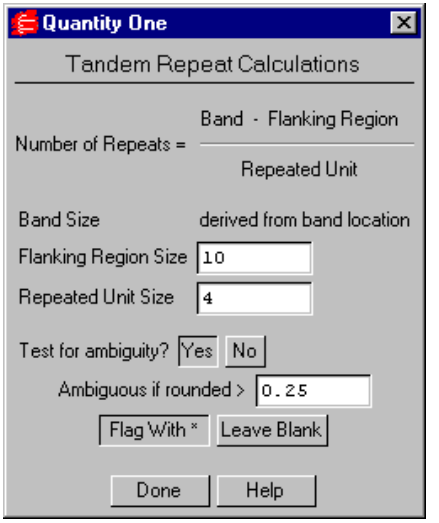


Fig. 9-2. Tandem Repeat Calculations dialog box.

At the top of the dialog box is the equation used to calculate the number of times an element is repeated in a band.

The band size is determined by the position of the band on the gel image.

In the **Flanking Region Size** field, enter the size (in base pairs or other repeated units) of the part of the fragment that does not include any repeated elements. This would include primer length and the length of any sequences that fall between the end of the primer region and the beginning of the stretch of repeats.

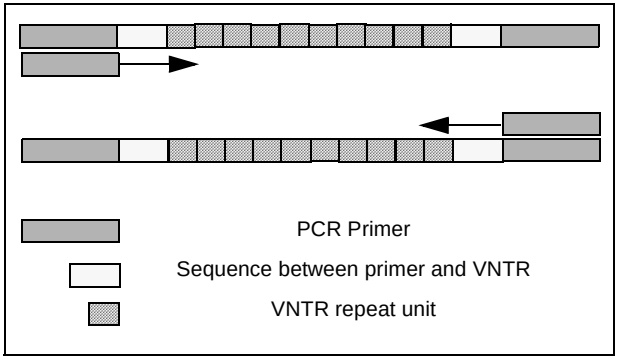


Fig. 9-3. Diagram of components of a DNA fragment.

In the **Repeated Unit Size** field, enter the size in base pairs of the repeated element (e.g., if you are working with (CA) repeats, enter “2”).

Testing for Ambiguity

The number of repeats calculated by the software may not be a whole number, due to the limitations of exact band size determination. Since the number of times an element is repeated must be a whole number, the calculated value is rounded to the nearest whole number.

In the dialog box, you have the option to “flag” those numbers

Select **Yes** after **Test for ambiguity?** to flag values that deviate from the nearest whole number by a certain fraction. These values may warrant further review. In the **Ambiguous if rounded >** field, specify how much the calculated value must deviate from the nearest whole number for it to be flagged.

Finally, specify how to identify the ambiguous values using the **Flag with *** button or the **Leave Blank** button.

When you have entered all the information in the dialog box, click on the **Done** button. The number of tandem repeats will be displayed next to the bands. (Note that if no standards have been defined on the gel, no numbers will be displayed.)

If the numbers have been concealed (e.g., by the **Hide Overlays** command), you can redisplay them by selecting **VNTR Display**.

10.Reports

Quantity One can display and print a variety of analysis reports. You can format the reports to include different kinds of data.

The available reports are listed under the *Reports* menu.

Fig. 10-1. Reports Menu.

10.1 Report Window

Many of the reports share the same basic report window.

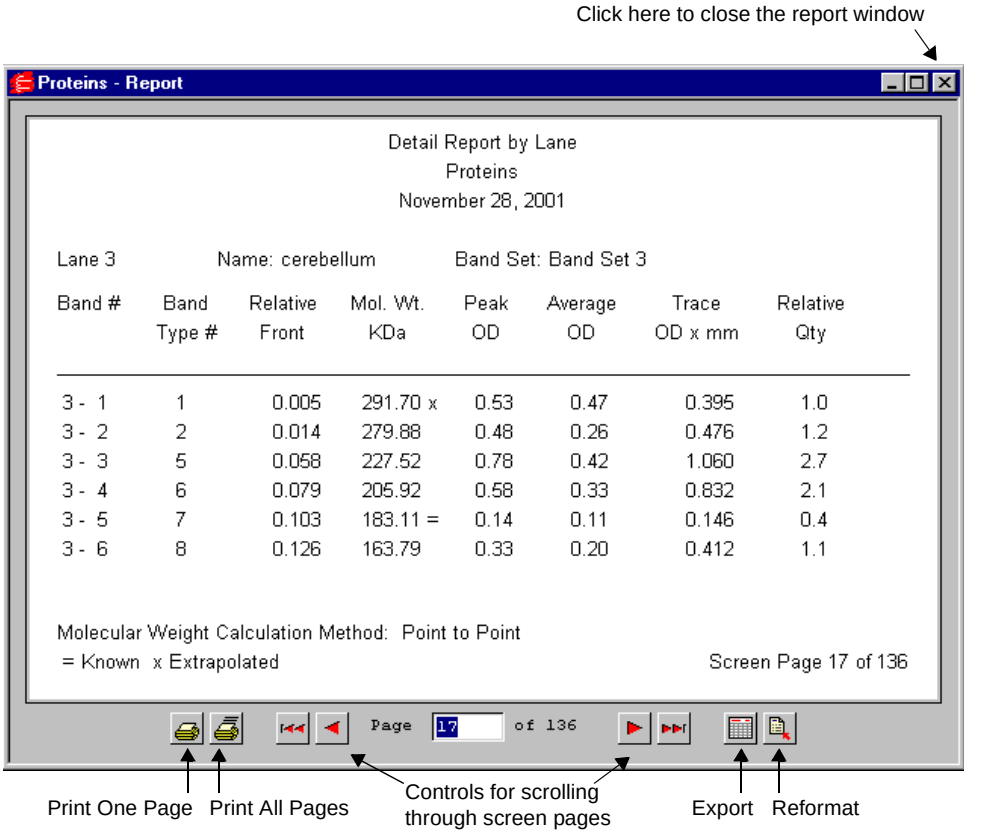


Fig. 10-2. Example of a report window.

The standard report window has buttons for printing the report, scrolling through the screen pages of the report, and exporting the report to a spreadsheet application. Some report windows also have a **Reformat** button for changing the data display.

To close a report window, click on the **Close** box in the upper right corner of the window.

Scrolling

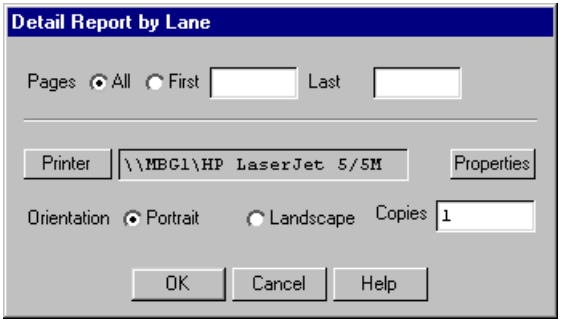
If a report has multiple pages, the scroll buttons in the report window become active. Use them to scroll to the next page, previous page, first page, or last page of the report. You can also enter a specific page number in the field to skip to that page.

Printing

Print the report using the **Print One Page** or **Print All Pages** commands in the report window.

Click on either of these buttons to open a smaller version of the standard print dialog (described in the next chapter).

Windows version:



Macintosh version:

Fig. 10-3. Print Report dialog.

Print One Page prints only the current screen page. **Print All Pages** prints all the pages in the report.

Note: If you select **Print All Pages**, the number of pages printed may be less than the number of screen pages listed in the report window. This is because the print command reformats the data to make maximum use of the paper size.

Exporting Report Data

You can export the report data to a spreadsheet application for further computation and analysis.

Click on the **Export** button to open the *Export Report* dialog.

Fig. 10-4. Export Report dialog.

Exported data can be separated by commas or tabs, depending on the requirements of your spreadsheet application.

You can save the data to a text file or to the clipboard by selecting the appropriate option button. If you select **File**, when you click on **OK** a dialog will open in which you can save the file.

Reformatting Report Data

Click on the **Reformat** button to open the options dialog for the report and select different display options. This button is not available in all reports.

10.2 Lane and Match Reports

There are four different lane and match reports: **Lane Report**, **All Lanes Report**, **Match Report**, and **All Matches Report**.

Lane Report generates a report on any you lane you select. First select the report from the *Reports* menu, then click on the particular lane.

All Lanes Report generates a report on all the lanes in the current gel image.

Match Report generates a report on a matched band that you select. First select the report from the menu, then click on a matched band. This report requires band matching (see section 6.2, Band Matching).

All Matches Report generates a report on all the matched bands in the gel . This report requires band matching (see section 6.2, Band Matching).

When you select any of these reports, the *Report Options* dialog will open, where you can specify the type and formatting of the data.

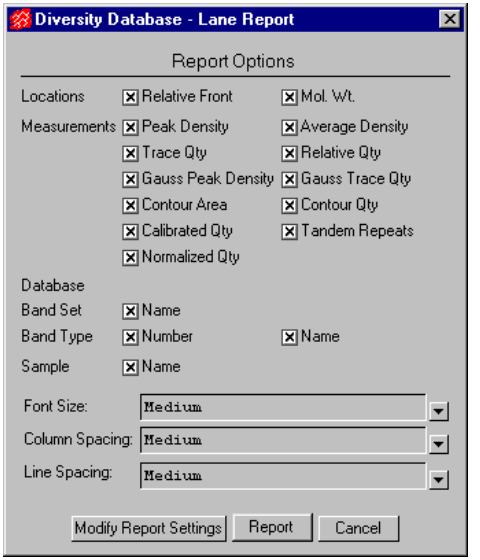


Fig. 10-5. Report Options dialog.

Select the data to display using the checkboxes.

Select the **Font Size, Column Spacing,** and **Line Spacing** settings to be used in the report by clicking on the button next to each field and selecting from the list of options.

To save the report options, click on the **Modify Report Settings** button and enter a name for the report settings in the field.

To load or delete previously saved settings, click on the button next to the **Settings to Load or Delete** field and select from the list of saved settings. Then click on **Load** or **Delete**.

To display the report, click on the **Report** button. The report will be displayed in a standard report window (see section 10.1, Report Window).

10.3 Band Types Report

The **Band Types Report** shows the presence or absence of specific bands in specific lanes of a gel. The report window displays a schematic representation of all the bands and lanes in the gel, with the presence or absence of bands shown as specified in the options dialog.

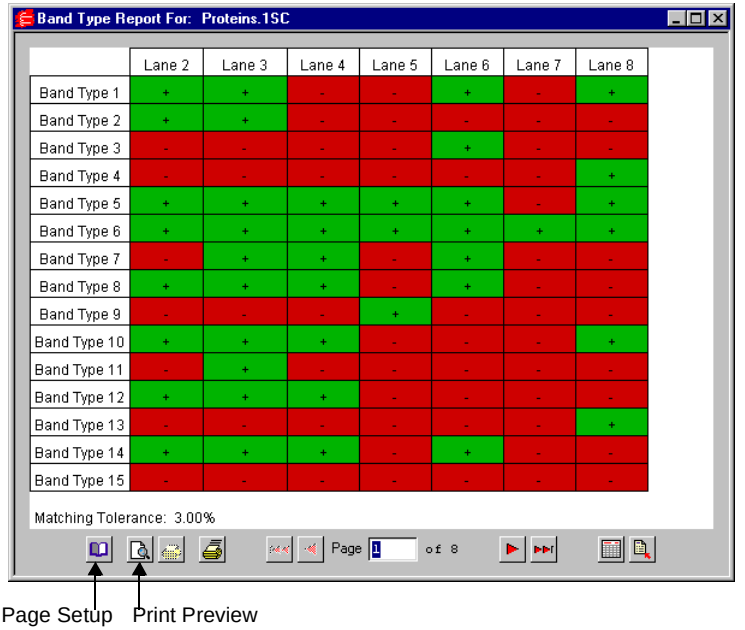


Fig. 10-6. Band Type Report.

Note: This report requires band matching (see section 6.2, Band Matching).

Select the report from the *Reports* menu, and select the display options in the *Band Types Report Options* dialog.

To save the report options, click on the **Settings** button in the options dialog and enter a name for the settings in the field. To load or delete previously saved settings, click on the button next to the **Settings to Load or Delete** field and select from the list of saved settings. Then click on **Load** or **Delete**.

To display the report, click on the **Report** button.

This report window includes two additional options: **Page Setup** and **Print Preview**.

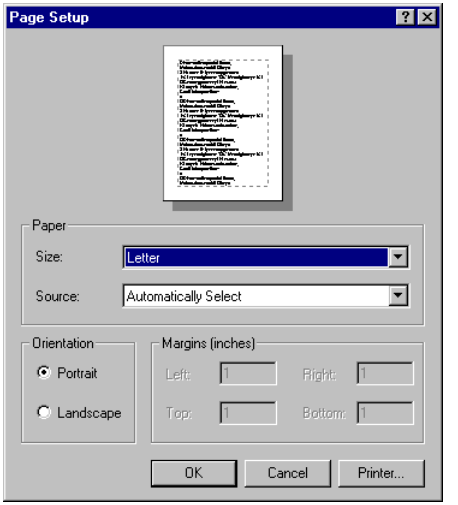


Fig. 10-7. Page Setup dialog.

The **Page Setup** dialog includes controls for selecting the paper size and orientation, paper tray, page margins, and printer. The **Print Preview** dialog displays the report as it will be printed.

10.4 1-D Analysis Report

The **1-D Analysis Report** displays all the advanced analysis data (including band types, normalized quantities, amount of sample loaded, etc.) for all the lanes in the gel. The lanes will also be ranked in similarity to the lane you initially select to generate the report.

Note: This report requires band matching (see section 6.2, Band Matching).

Select **1-D Analysis Report** from the *Reports* menu, then click on any experimental lane in the gel. A dialog will pop up, in which you can select the report data to display.

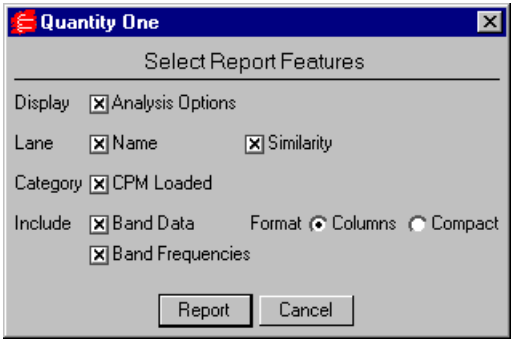


Fig. 10-8. 1-D Analysis Report options.

To display the selected data, click on the **Report** button. The report will be displayed in a standard report window (see section 10.1, Report Window).

10.5 Similarity Comparison Reports

Quantity One has three reports for comparing the similarity of lane-based samples in a gel: **Compare Lane Images**, **Phylogenetic Tree**, and **Similarity Matrix**.

Note: These reports require band matching (see section 6.2, Band Matching).

Comparison Options

Before opening any of these reports, select **Comparison Options** from the *Reports* menu to specify some similarity settings.

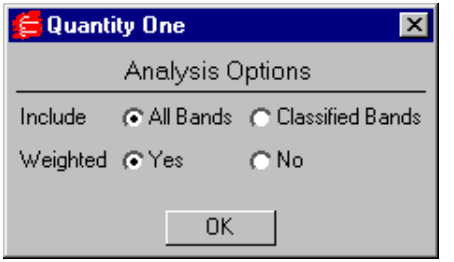


Fig. 10-9. Comparison Options dialog.

In the *Comparison Options* dialog, select **All Bands** to include every band in the sample similarity comparison, or selected **Classified Bands** to include only matched bands.

Next to **Weighted**, select **Yes** to use both band position *and* intensity when comparing sample similarity. Select **No** to use only band position when comparing samples. The next section describes how weighting is used in the similarity comparion equation.

Comparison Method

The method for computing similarity in Quantity One is the Dice Coefficient. The formula for the Dice Coefficient is:

$$sim = 200 \times \frac{\sum_{i=1}^{B} Min(s_i, t_i)}{\sum_{i=1}^{B} (s_i + t_i)}$$

$$dist = 100 - sim$$

where S and T are vectors representing two lanes in the same band set that are being compared.

To compute similarity, a vector is constructed that represents the bands identified in the lane. The vector depends on the comparison options (see above) selected. If the

search was done on classified (matched) bands only, then the vector S contains B elements (S = (s1, s2, s3 ... sB)), where B is the number of band types in the lane's band set. The values for s1, s2, s3 ... sB have the following values:

Weighting Off Search

s_i = 1 if the i'th band type is found in the lane.

s_i = 0 if it is not found.

Weighting On Search

If the band set has a normalizing band type, then:

s_i = The normalized density of the band assigned to the i'th band type.

s_i = 0 if the lane does not have a band assigned to the i'th type.

Otherwise:

si = The quantity of the band assigned to the i'th band type.

s_i = 0 if the lane does not have a band assigned to the i'th type.

10.5.a Compare Lane Images

The **Compare Lane Images** report displays the lanes in decreasing order of similarity to a lane that you select.

Select **Compare Lane Images** from the *Reports* menu, then click on a reference lane. Select the report features in the pop-up box.

:

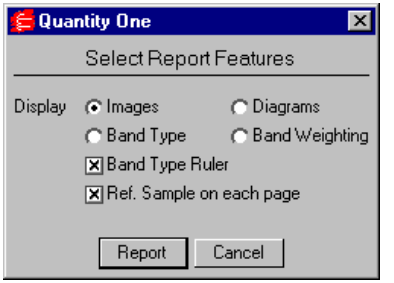


Fig. 10-10. Compare Lane Images options.

If you select the **Images** option, actual images of the lanes will be displayed in the report. If you select **Diagrams**, schematic representations of the lanes will be displayed.

To display the selected data, click on the **Report** button.

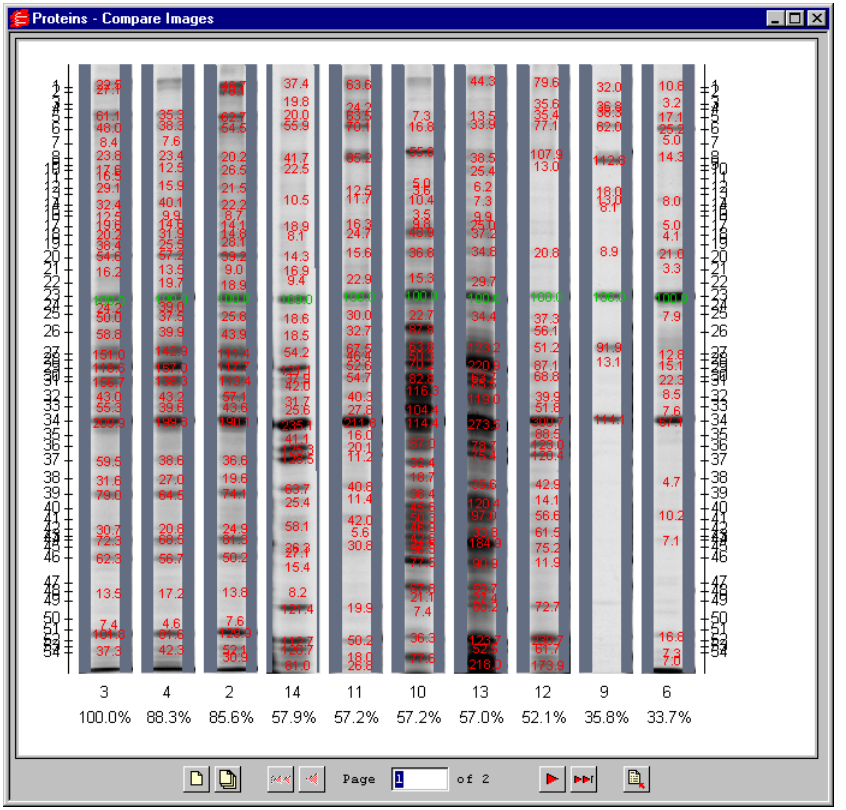


Fig. 10-11. Compare Lane Images report.

The *Compare Lane Images* report is displayed in a standard report window (see section 10.1, Report Window). Change the report options by clicking on the **Reformat** button.

The **Print Report** dialog for this report contains special fields for entering a report title.

10.5.b Phylogenetic Tree

Phylogenetic trees are schematic representations of sample similarity. To compare the similarity of samples in a phylogenetic tree format, select **Phylogenetic Tree** from the *Reports* menu. (You can also select **Phylogenetic Tree Quick Guide** under the *Help* menu to guide you through the process of generating a phylogenetic tree.)

In the pop-up box, select a clustering method for creating the tree. See the following pages for information about the different methods.

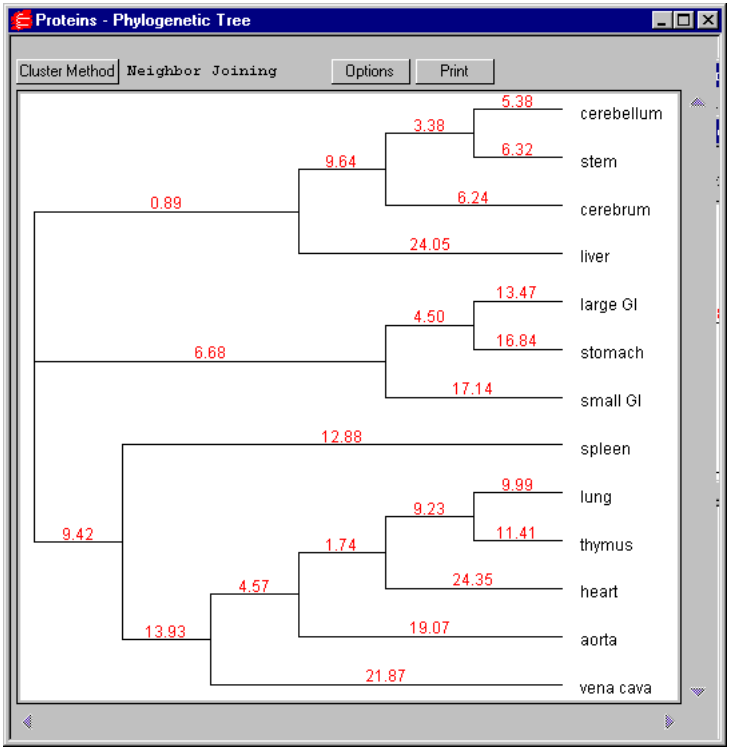


Fig. 10-12. Phylogenetic Tree.

Note: We have found that Ward's method, UPGAMA, and WPGAMA give the most plausible clusters and are affected the least by samples that are outliers.

The report window for a phylogenetic tree is slightly different than the standard report window. The tree appears in a window with scroll bars for moving up and down and left and right.

To print the tree, click on the **Print** button.

Click on the **Cluster Method** button to select a different clustering method for displaying the tree. This will automatically recalculate and redisplay the tree.

Neighbor Joining

This type of phylogenetic tree is computed is based on minimizing the total branch length at each stage of clustering. The method also finds branch lengths between nodes. The approximate distance between any two samples in this tree can be found by adding the branch lengths that connect the samples.

Other Methods[1],[2]

The following methods are based on the algorithm below:

1. Begin with n clusters—one cluster for each sample.
2. Compute the similarity matrix for the samples.
3. Convert the similarity matrix into a distance matrix d using the appropriate distance formula.
4. Join the two clusters with the minimum distance into one cluster. Compute the similarity value for this cluster.
5. Recompute the distance matrix d using the cluster that was formed in Step 4.

Steps 4 and 5 are repeated until there is only one cluster. The difference between the methods below is based on the definition of minimum distance in Step 4, and on the

---

1. For a complete explanation of the calculations and assumptions used to generate these dendrograms, please refer to Sneath and Sokal. *Numerical Taxonomy*, San Francisco: W. H. Freeman & Company, 1973.
2. Vogt and Nagel, *Clinical Chemistry* **38** (2): 182-198, (1992).

method of computing the new distance matrix in step 5. In the discussion that follows, let:

p, q be indices indicating two clusters that are to be joined into a single cluster.

k be the index of the cluster formed by joining clusters p and q.

i be the index of any remaining clusters other than cluster p, q, or k.

n_p the number of samples in the p'th cluster.

n_q the number of clusters in the q'th cluster.

n the number of clusters in the k'th cluster formed by joining the p'th and q'th cluster. $n = n_p + n_q$

d_{pq} the distance between cluster p and cluster q.

Single Linkage

This is also called Nearest Neighbor or Minimum Method.

$$d_{ki} = min(d_{pi}, d_{qi})$$

Complete Linkage

This is also called the Furthest Neighbor or Maximum Method.

$$d_{ki} = max(d_{pi}, d_{qi})$$

Single and Complete linkage are good algorithms for indicating outlier clusters.

UPGAMA

Unweighted pair group method using arithmetic averages. This is also called Weighted Average Linkage.

$$d_{ki} = \left(\frac{n_p}{n}\right) \cdot d_{pi} + \left(\frac{n_q}{n}\right) \cdot d_{qi}$$

WPGAMA

Weighted pair group method using arithmetic averages. This is also called Average Linkage.

$$d_{ki} = 0.5 \cdot d_{pi} + 0.5 \cdot d_{qi}$$

WPGAMA is a special case of UPGAMA that favors the most recent member clusters in forming new clusters.

Centroid

$$d_{ki} = \left(\frac{n_p}{n}\right) \cdot d_{pi} + \left(\frac{n_q}{n}\right) \cdot d_{qi} - \frac{(n_p \cdot n_q)}{n^2 d_{pq}}$$

Median

$$d_{ki} = 0.5 \cdot d_{pi} + 0.5 \cdot d_{qi} - 0.25 \cdot d_{pq}$$

Centroid and Median are similar to UPGAMA and WPGAMA, respectively, but the distance formula contains an additional third term. Centroid and Median methods are not monatomic hierarchical clustering algorithms. In other words, the similarity value between cluster k and any other cluster may be greater than the similarity between cluster p and cluster q. This condition occurs if the centroids (or medians) of the different clusters have approximately the same distance as the distances between the samples that make up the cluster.

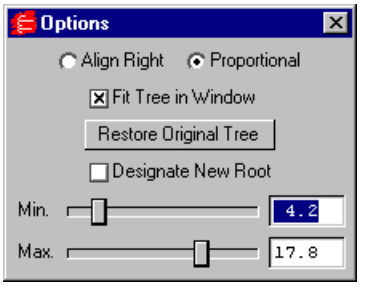
Ward's

This method attempts to minimize the information by describing a set of N samples using a fewer number of clusters.

$$d_{ki} = \frac{n_p + n_i}{n + n_i} d_{pi} + \frac{n_q + n_i}{n + n_i} d_{qi} - \frac{n_i}{n + n_i} d_{pq}$$

Options

Click on the **Options** button in the *Phylogenetic Tree* window to open the *Options* dialog.



Neighbor Joining Only

All Other Methods

Fig. 10-13. Two forms of the Options dialog.

The box on the left has controls that are specific for the Neighbor Joining method. The box on the right is applicable to the other tree methods. Both boxes share certain features.

Each allows you to choose between aligning the tree to the right or proportionally aligning it. Right alignment uses a fixed branch length for displaying the distance between nodes; proportional alignment differs for Neighbor Joining and other tree modes.

With the Neighbor Joining method, proportional alignment shows branch length sizes proportional to the min/max values set in the *Options* dialog. Other methods plot the nodes at locations determined by their similarity values.

The **Fit Tree in Window** option scales the tree so that the entire tree will fit onto a single printed page. With this turned off, the entire tree will appear with the correct distances between nodes preserved, and the printed tree can be tiled across multiple sheets of paper.

The Neighbor Joining *Options* dialog, click on the **Designate New Root** button to designate a new root node (the node at the very top of the tree), enabling you to look at the relationships between lanes from a different perspective. This will highlight all the nodes in the tree. Click on the node that you want to serve as the new root and the

dendrogram will be recalculated. To return to the original tree, click on **Restore Original Tree**.

The **Min.** and **Max.** sliders are active only for proportionally aligned Neighbor Joining trees. Use them to adjust the minimum and maximum distances between nodes on branches to highlight specific regions of data. Distances below the **Min.** or above the **Max.** values will be collapsed to fit in the window.

For non-Neighbor Joining trees, use the **Clusters** option to define the number of clusters (0 to 18) in the tree. The separate clusters will be identified in the tree by letters.

10.5.c Similarity Matrix

The **Similarity Matrix** report compares the similarity of all the lanes to one another. If there are N lanes in the gel, then the similarity matrix is an N by N matrix that is computed using the Dice Coefficient as described in section 10.4, 1-D Analysis Report.

The matrix has the following properties:

- The diagonal elements always have values of 100. This is because a lane is always 100 percent similar to itself.
- The matrix is symmetrical ($M_{ij} = M_{ji}$).

Select **Similarity Matrix** from the *Reports* menu to open this report.

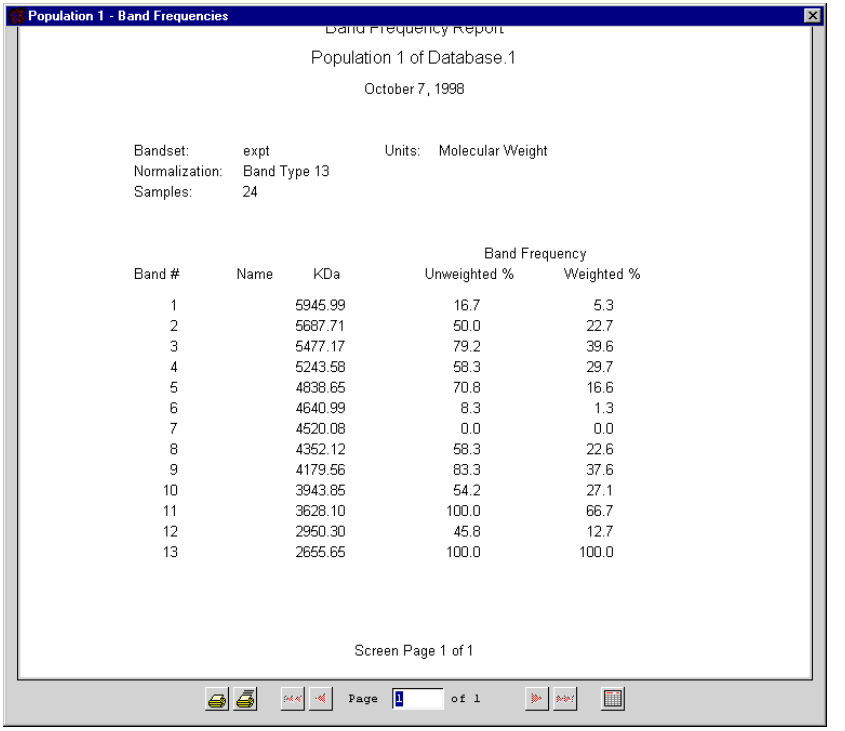


Fig. 10-14. Similarity Matrix report.

The *Similarity Matrix* report is displayed in a standard report window (see section 10.1, Report Window).

10.6 Volume Analysis Report

The **Volume Analysis Report** displays volume data.

Select **Volume Analysis Report** from the *Reports* menu or *Volume* toolbar. The *Volume Report Options* dialog will open, in which you can specify the information that will appear in the report.

When you click on **OK**, the report will be displayed in a standard report window (see section 10.1, Report Window).

10.6.a Volume Report Options

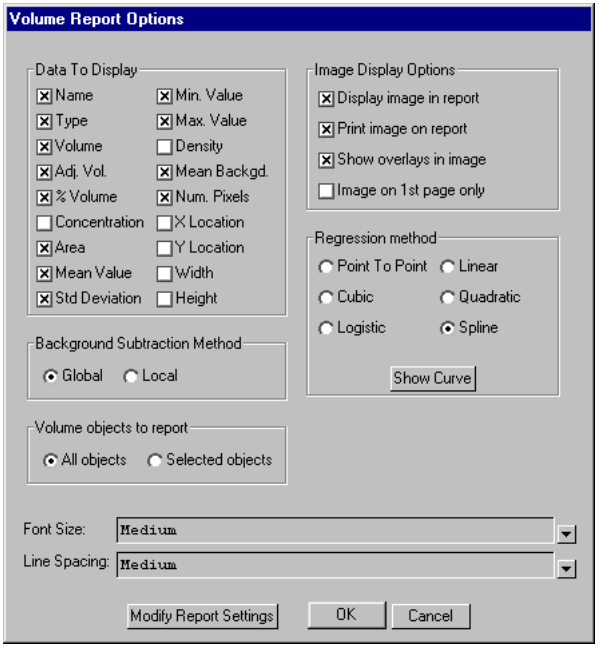


Fig. 10-15. Volume Report Options dialog.

The volume report options are described below:

- **Name**—The name that is automatically assigned to the volume based on its type (U=Unknown, Std=Standard, B=Background) and order in which it was created.
- **Type**—Unknown, standard, or background.
- **Volume**—Sum of the intensities of the pixels inside the volume boundary x area of a single pixel (in mm^2).
- **Adj. Vol.**—Volume minus the background volume; if there is no background volume, this is simply the volume.

- **% Volume**—The volume expressed as a percentage of all the volumes in the image.
- **Concentration**—The quantity as calculated from the standards and the regression method. If you have not defined standards, this is not calculated.
- **Area**—The total area of the volume box you have drawn in mm^2.
- **Mean Value**—The mean intensity of the pixels inside the volume boundary.
- **Std. Deviation**—The standard deviation from the mean intensity.
- **Min. Value**—The value of the lowest intensity pixel in the volume.
- **Max. Value**—The value of the highest intensity pixel in the volume.
- **Density**—The total intensity of all the pixels in the volume divided by the area of the volume.
- **Mean Background**—The mean intensity of the pixels in the background volume.
- **Num. Pixels**—The number of pixels inside the volume.
- **X location**—The distance in mm from the left edge of the image to the center of the volume.
- **Y location**—The distance in mm from the top edge of the image to the center of the volume.
- **Width**—The width of the volume in mm.
- **Height**—The height of the volume in mm.

Specify the preferred **Background Subtraction Method** (**Global** or **Local**).

Note: If you select **Global** and have not defined a background volume, no background subtraction will be performed on the image.

The **Image Display Options** affect how the image is displayed and/or printed on the report.

You can choose whether to report on all volume objects (**All objects**) or only those objects you have selected (**Selected objects**)

Select the regression method for calculating the **Volume Regression Curve**. To display the curve, click on the **Show Curve** button.

Select the **Font Size** and **Line Spacing** settings to be used in the report by clicking on the button next to each field and selecting from the list of options.

Saving the Report Options

To save the report options, click on the **Modify Report Settings** button at the bottom of the dialog. In the pop-up dialog, enter a name for the report settings in the field.

To load or delete previously saved settings, click on the button next to the **Settings to Load or Delete** field and select from the list of saved settings. Then click on **Load** or **Delete**.

10.7 Volume Regression Curve

If you have defined at least two standard volumes on the image, you can display a regression curve for calculating the quantities of the unknowns.

Select **Volume Regression Curve** from the *Reports* menu.

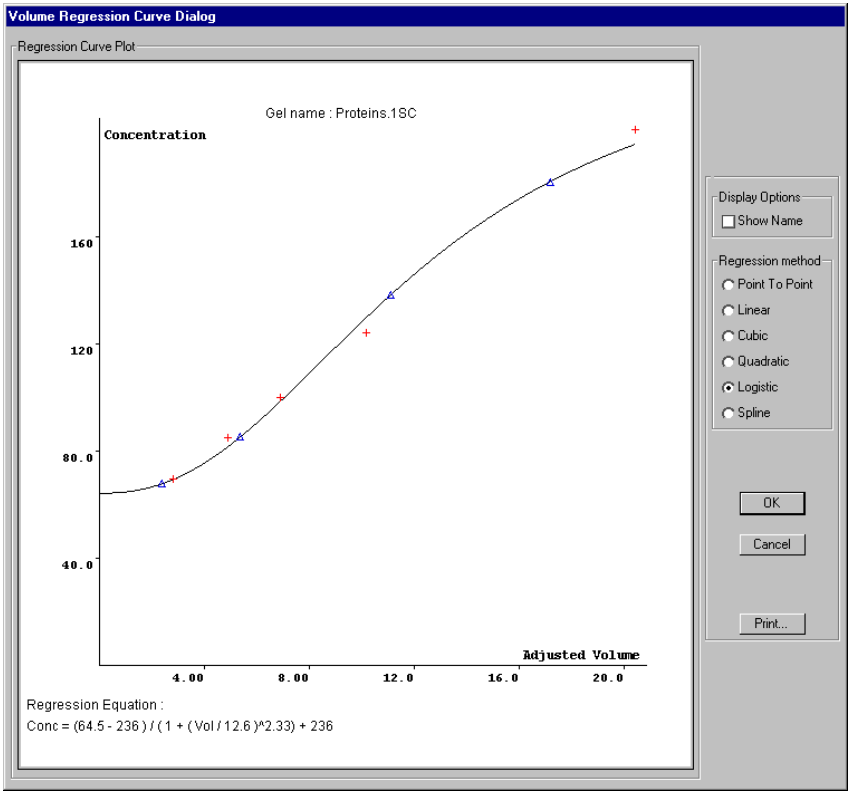


Fig. 10-16. Volume Regression Curve window.

On the curve, each standard volume is marked by a red X and each unknown is marked by a blue triangle.

The X axis is the adjusted volume and the Y axis is the concentration, based on the standards you have marked on the image.

To display the numbers and names of the volumes, click on the **Show Name** checkbox.

Select the preferred regression method from the list of option buttons. The regression equation for the selected method is displayed in the lower left of the window.

To print the curve, click on the **Print** button. To close the window, click on the **OK** button.

10.8 VNTR Report

The **VNTR Report** displays the calculated VNTR data (see section 9.2, Variable Number Tandem Repeats).

Select **VNTR Report** from the *Reports* menu. A pop-up box will ask if you want to display the gel image in the report.

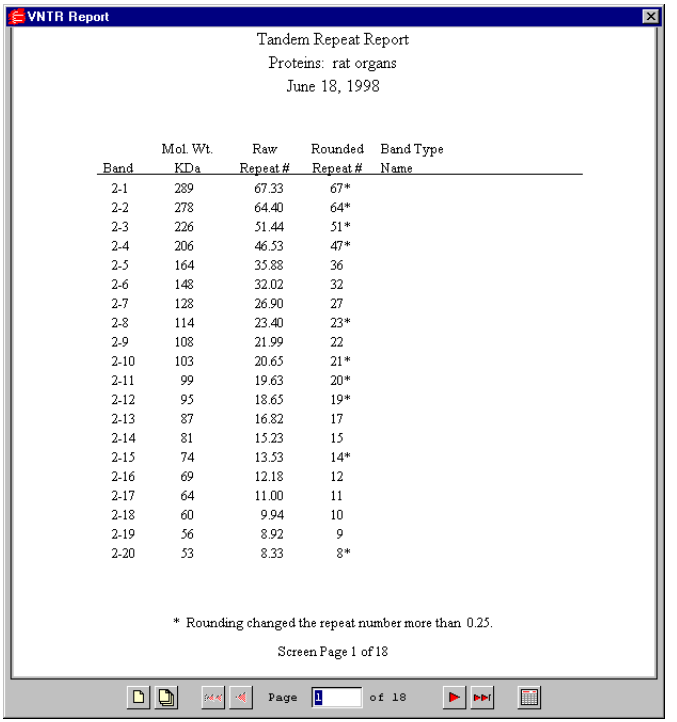


Fig. 10-17. Example of a VNTR Report.

The report is displayed in a standard report window (see section 10.1, Report Window).

The report displays the molecular weight, the raw repeat number, and the rounded repeat number for each band in the gel image.

The raw repeat number is the number of repeats calculated using the information that you provided in the *Tandem Repeat Calculation* dialog, and is likely to include fractional values. The rounded repeat number is the raw repeat number rounded to the nearest whole number.

An asterisk (*) will appear next to some of the rounded repeat numbers if you selected **Test for Ambiguity** and **Flag with *** in the *Tandem Repeat Calculations* dialog. The asterisk will appear next to numbers that vary from the raw repeat number by more than the selected ambiguity value.

11.Printing and Exporting

The commands for printing and exporting images are located on the *File* menu.

Reports are printed from within the individual report windows.

11.1 Print Image and Print Actual Size

The **Print Image** and **Print Actual Size** commands on the *File* > *Print* submenu open a standard Windows or Macintosh printer dialog box, in which you can select the printer, paper size, paper source, page layout, etc.

You can use these print commands to print the plain image, or the image plus overlays (volumes, text overlays, defined lanes and bands, band data, etc.). To include image overlays in the printout, first display the overlays on the image and then select the appropriate print command.

Select **Print Image** to fit the selected image to the printed page.

Select **Print Actual Size** to print an actual-size copy of the selected image. If you are using the Gel Doc, ChemiDoc, ChemiDoc XRS, Fluor-S, Fluor-S MAX, Fluor-S MAX2, or VersaDoc, you must specify the correct image area size when capturing images to ensure accurate 1:1 printing. Specify the image area size in the acquisition window for the instrument. See the chapter on each imaging device for more information.

11.2 Page Setup

Specify the printer settings using the **Page Setup** command on the *File* > *Print* submenu. This will open the standard *Page Setup* dialog box for Windows or Macintosh, in which you can select the paper size, page orientation, printer, etc.

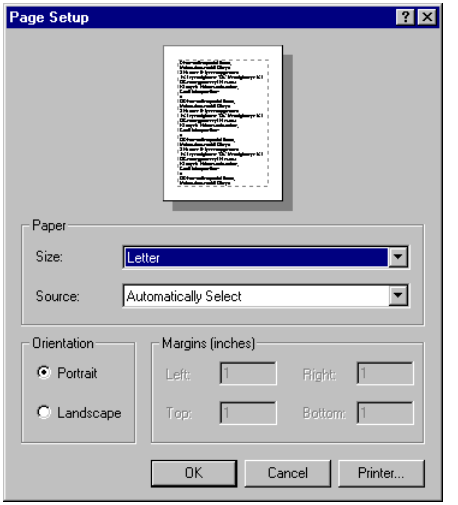


Fig. 11-1. Page Setup dialog box.

11.3 Image Report

The **Image Report** displays the image and basic image information, including the image dimensions, pixel size, date of scan, type of imager, etc.

To print an image report for a particular gel, select **Image Report** from the *File* > *Print* submenu. This will open a smaller version of the standard printer dialog box.

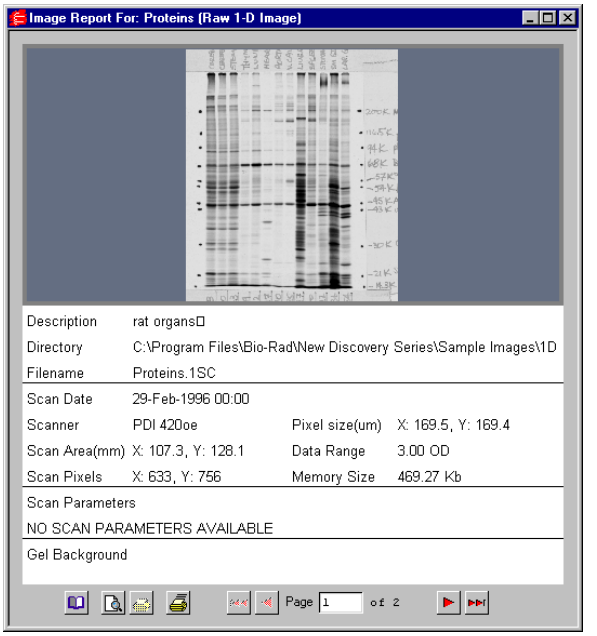


Fig. 11-2. Image Report.

Note: TIFF images do not contain all the tagged information that would normally be included in an image file (for example, imaging device, scan date, image color, etc.). For this reason, the **Image Report** may list this information as “Unknown” for imported TIFF files.

11.4 Video Print

Select **Video Print** from the *File* > *Print* submenu to print images and reports to a video printer.

Note: Video printing requires installation of the video board and cable that come with the Gel Doc and ChemiDoc systems. The video board and cable can also be ordered separately.

Settings for the Mitsubishi P90W/P91W Video Printer

There are three settings for the Mitsubishi P90W/P91W video printer. Set **Contrast** to zero, **Brightness** to zero, and **Gamma** to 5.

The dip switches should stay in the orientation in which they are shipped: Pin 1 is up (on), and Pins 2–10 are down (off).

11.5 Export to TIFF Image

You can export images in TIFF format for analysis and publishing using other applications. Select **Export to TIFF Image** from the *File* menu to open a dialog box in which you can specify the export parameters.

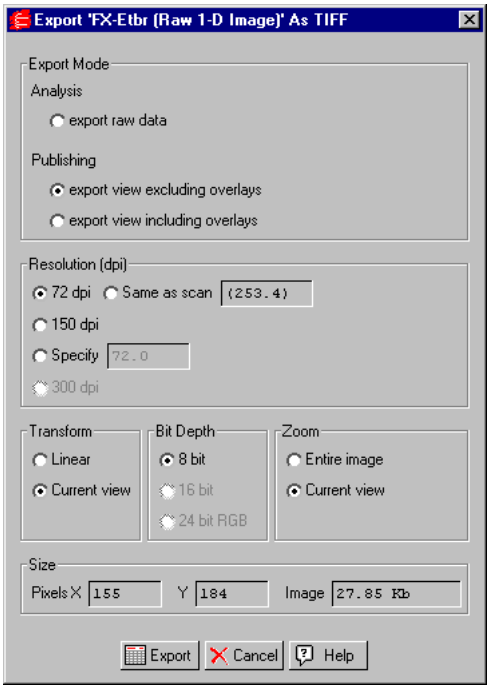


Fig. 11-3. Export to TIFF Image dialog box.

There are two basic export options:

- Export the **raw image data** for further analysis
- Export the **displayed view** of the image for publishing.

If you choose to export the displayed view, you can export with or without the image overlays.

11.5.a Analysis Export Mode

To export a TIFF image for further analysis, select the **Export raw data** button. The publishing controls in the dialog box will become inactive.

11.5.b Publishing Export Mode

To export a TIFF image that looks like the image as it is currently displayed on the screen, select one of the options under **Publishing**.

Export View Including Overlays

Select this option to include overlays with the exported image. The other controls in the dialog box will become inactive.

Export View Excluding Overlays

Select this option to *not* include overlays with the exported image. This is the only mode available if you are exporting images from the *Multi-channel Viewer* (see section 3.4, Multi-Channel Viewer).

With this option selected, specify a resolution for the TIFF image by selecting **72 dpi** (typical computer screen resolution), **150 dpi**, **300 dpi** (standard printing resolution), **Same As Scan**, or any resolution that you enter in the **Specify** field (up to the resolution of the scan).

Under **Transform**, you can specify a **Linear** transform or preserve the **Current View** (which is automatically log transformed).

Images with a higher **Bit Depth** (e.g., **16 bit** or **24 bit RGB**) can be compressed to a lower bit depth (e.g., **8 bit**) during export.

Note: TIFF images are automatically exported from the *Multi-channel Viewer* in **24 bit RGB** mode to preserve the colors displayed in the viewer.

Finally, if you are only displaying part of the image due to magnification or repositioning, you can preserve the **Current View** or export the **Entire Image**.

11.5.c Exporting the Image

The size of the pixels in the image and the file size of the image are listed at the bottom of the dialog. When you are ready to export, click on the **Export** button.

In the **Save As** dialog box, the default file name will have a .tif extension, and the file type will indicate that this is a TIFF image.

Appendix A
Gel Doc 2000

Fig. A-1. Gel Doc.

Before you can begin acquiring images, the Gel Doc system must be properly installed and connected with the host computer. See the Gel Doc hardware manual for installation, startup, and operating instructions.

To use the Gel Doc, you will need to have the Bio-Rad-supplied acquisition board installed in your PC or Macintosh. The drivers for this board will be installed when you install the main software application.

Make sure that your Gel Doc camera is turned on. If the camera is not turned on, the Gel Doc acquisition window will open and an image capture error will be displayed.

Simulation Mode

Any of the imaging device acquisition windows can be opened in "simulation mode." In this mode, an acquisition window will open and the controls will appear active, but instead of capturing real images, the window will create "dummy" images of manufactured data.

You do not need to be connected to an imaging device to open a simulated acquisition window. This is useful for demonstration purposes or practice scans.

To enter simulation mode, hold down the CTRL key and select the name of the device from the File menu. The title of the acquisition window will indicate that it is simulated.

A.1 Gel Doc Acquisition Window

To acquire images using the Gel Doc, go to the File menu and select Gel Doc.... The acquisition window for the instrument will open, displaying a control panel and a video display window.

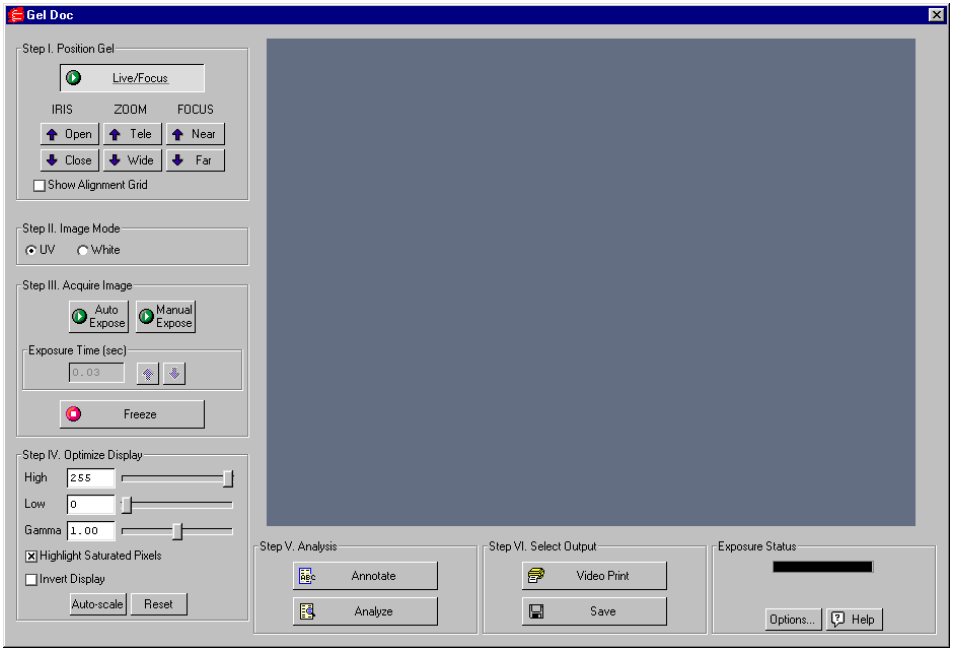


Fig. A-2. Gel Doc acquisition window

The Gel Doc video display window will open in “live” mode, giving you a live video display of your sample. If no image is visible, make sure the camera is on, check the cable connections, make sure the iris on the camera is not closed, and make sure that the protective cap is off the camera lens. Also check to see that the transilluminator is on and working.

The control panel has been arranged from top to bottom to guide you through the acquisition procedure. There are six basic steps to acquiring an image using the Gel Doc:

1. Position and focus the gel or other object to be imaged.
2. Select Mode.
3. Acquire the image.

4. Optimize the display.
5. Analysis.
6. Select the output.

A.2 Step I. Position Gel

The Gel Doc window will open in “live” mode, giving you a live video display of your sample. In this mode, the Live/Focus button will appear selected, and frames will be captured and displayed at about 10 frames per second, depending on the speed of your computer.

You can use live mode to zoom, focus, and adjust the aperture on the camera, while positioning the sample within the area.

Note: Newer versions of the Gel Doc feature a motorized zoom lens that can be controlled directly from the acquisition window using the Iris, Zoom, and Focus arrow buttons. Click on the Up/Down buttons while viewing your sample in the window to adjust the lens. These buttons will not be visible if you are connected to older versions of the Gel Doc without the motorized zoom lens.

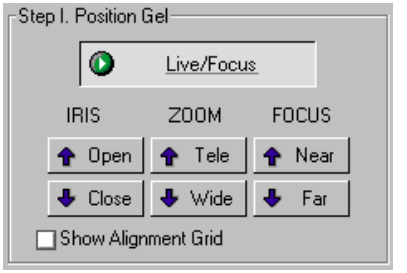


Fig. A-3. Newer Gel Docs feature camera control buttons in the acquisition window.

You can also select the Show Alignment Grid checkbox to facilitate positioning.

Note: After positioning your sample, you should check the Imaging Area dimensions under Options (see section A.9, Options) to make sure that they conform to the size of the area you are focusing on. To determine the size of the area you are

focusing on, you can place a ruler in the Gel Doc cabinet so that it is visible in the image.

A.3 Step II. Select Image Mode

The Image Mode option buttons allow you to set the type and scale of your data.

UV

Select this mode for fluorescent samples. With this mode selected, the data will be measured in linear intensity units.

White Light

Select this mode for reflective and transmissive samples. With this mode selected, the data will be measured in uncalibrated optical density (uOD) units.

A.4 Step III. Acquire Image

For many white light applications, you can skip this step and save and print images directly from Live/Focus mode.

For UV light or faint samples, you can take an automatic exposure based on the number of saturated pixels in the image or you can enter a specific exposure time.

Note: "Exposure" refers to the integration of the image on the camera CCD over a period of time. The effect is analogous to exposing photographic film to light over a period of time.

Auto Expose

Auto Expose will take an exposure whose time length is determined by the number of saturated pixels in the image. This is useful if you are uncertain of the optimal exposure length.

Note: If you know the approximate exposure time you want (± 3 seconds), you can skip this step and go directly to Manual Expose.

Click on the Auto Expose button to cancel Live/Focus mode and begin an automatic exposure. The Auto Expose button will appear selected throughout the exposure.

During the auto exposure, the image is continuously integrated on the camera CCD until it reaches a certain percentage of saturated pixels. This percentage is set in the Options dialog box. (Default = 0.15 percent. See section A.9, Options.)

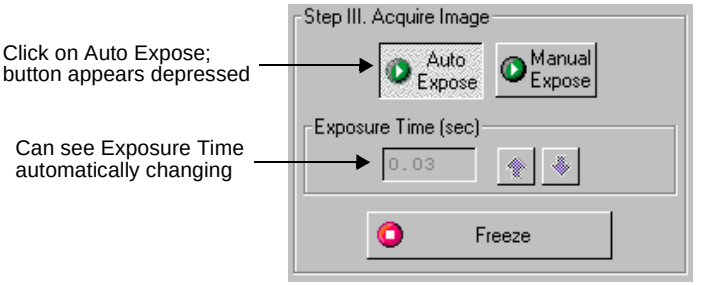


Fig. A-4. Auto Expose.

Once an image has reached the specified percent of saturated pixels, it is captured and displayed in the video display window, Auto Expose is automatically deactivated, the exposure time appears active in the Exposure Time field, and Manual Expose is activated.

Note: If you are having difficulty auto-exposing your sample, you can use Manual Expose to adjust your exposure time directly. Most applications only require an exposure time of a few seconds, which can be quickly adjusted using Manual Expose.

Manual Expose

If you know the approximate exposure time you want, you can click on the Manual Expose button. Manual Expose is automatically activated after Auto Expose has deactivated.

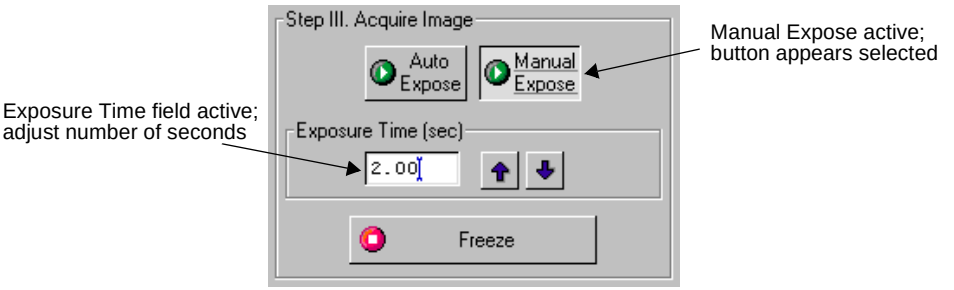


Fig. A-5. Manual Expose.

With Manual Expose activated, you can adjust the exposure time directly by changing the number of seconds in the Exposure Time field. Type in a number or use the arrow buttons next to the field.

When the specified exposure time is reached, the last captured image will be displayed in the Gel Doc image window. The camera continues to integrate the image on the CCD, updating the display whenever the specified number of seconds is reached.

Once you are satisfied with the quality of the displayed image, click on the Freeze button to stop the exposure process. The last full exposure will be displayed in the image window.

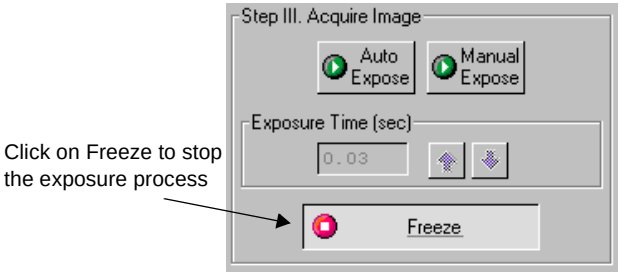


Fig. A-6. Freezing the exposure.

Note: Freeze is automatically activated if you adjust any of the subsequent controls (e.g., Video Print, Image Mode, Display controls, etc.).

A.5 Step IV. Optimize Display

The Display controls are useful for quickly adjusting the appearance of your image for output to a video printer. Adjusting these controls will automatically freeze the video display and allow you to alter the image within the Gel Doc window.

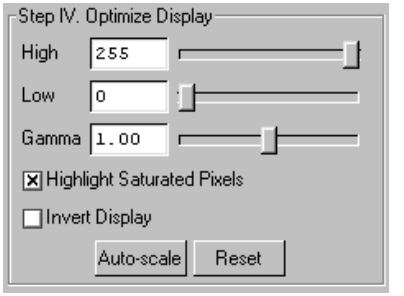


Fig. A-7. Display controls.

These controls are similar to those in the Transform dialog box.

Note: The Display controls will only change the appearance of the image. They will not change the underlying data.

High/Low Sliders

If Auto-scale doesn't give you the appearance you want, you can use the High and Low sliders to redraw the image yourself. In white light mode, dragging the High slider handle to the left will make weak signals appear darker. In UV mode, dragging the High slider handle to the left will make weak signals appear brighter. Dragging the Low slider handle to the right will reduce background noise.

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

Gamma Slider

Some images may be more effectively visualized if their data are mapped to the computer screen in a nonlinear fashion. Adjusting the Gamma slider handle changes the light and dark contrast nonlinearly.

Highlight Saturated Pixels

When this box is checked, any saturated pixels in the image will appear highlighted in red in the scan window and in the pop-up image window. To view/hide saturated pixels in the pop-up image window, use the Image > Transform command.

Invert Display

This checkbox will switch light spots on a dark background to dark spots on a light background, and visa versa. This will only affect how the image is displayed on the screen, not the actual image data.

Auto-scale

Clicking on Auto-scale will adjust your displayed 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). You can then “fine tune” the display using the High, Low, and Gamma sliders described below.

Reset

Reset will return the image to its original, unmodified appearance.

A.6 Step V. Analysis

The Analysis step of the Gel Doc acquisition window allows you to add annotations and analyze the newly acquired image.

Annotate

Clicking on Annotate will open a separate image window displaying the captured image. The default name for the image will include the date, time, and user (if known).

The Text Overlay toolbar will also pop up to allow you to annotate your image.

The image will not be saved until you select Save or Save As from the File menu.

Analyze

Clicking on Analyze will open a separate image window displaying the captured image. The default name for the image will include the date, time, and user (if known).

You can then analyze the image using the other features in the main application.

The image will not be saved until you select Save or Save As from the File menu.

A.7 Step VI. Select Output

In Select Output you can select Video Print and Save as your output options.

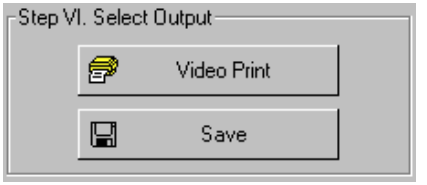


Fig. A-8. Output options.

Video Print

Clicking on Video Print will automatically send the currently displayed frame (either live or integrated) to a video printer. You can add information about your image to the

bottom of the printout by selecting the appropriate checkboxes in the Options dialog box. (See section A.9, Options.)

Save

Clicking on Save will open a separate image window displaying the captured image. A Save As dialog box will automatically open displaying the default file name for the image, which will include the date, time, and user (if known). You can then change the file name and storage directory.

You can also export your image as a TIFF image for viewing with other applications.

A.8 Exposure Status

The Exposure Status bar shows the progress of your exposure. If your exposure time is greater than 1 second, the status bar display will give you a graphical representation of the remaining time before exposure is complete.

If the exposure time is less than 1 second, the status bar will not refresh itself for each exposure; it will remain at 100 percent.

A.9 Options

Click on the Options button to open the Options dialog box. Here you can specify certain settings for your Gel Doc system.

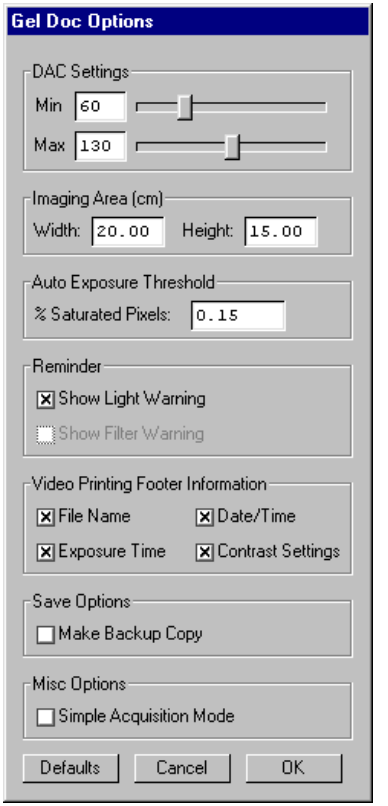


Fig. A-9. Available options in the Gel Doc acquisition window.

Click on OK to implement any changes you make in this box. Clicking on Defaults restores the settings to the factory defaults.

DAC Settings

Note: The default DAC settings are highly recommended and should be changed with caution.

These sliders may be used to adjust the minimum and maximum voltage settings of your video capture board. The minimum slider defines the pixel value that will appear

as white in the image, while the maximum slider defines the pixel value that will appear as black. The slider scale is 0–255, with the defaults set to 60 minimum and 130 maximum.

Imaging Area

These fields are used to specify the size of your imaging area in centimeters, which in turns determines the size of the pixels in your image (i.e., resolution). When you adjust one imaging area dimension, the other dimension will change to maintain the aspect ratio of the camera lens.

Note: Your imaging area settings must be correct if you want to do 1:1 printing. These are also important if you are comparing the size of objects (e.g., using the Volume Tools) between images.

Auto Exposure Threshold

When you click on Auto Expose, the exposure time is determined by the percentage of saturated pixels you want in your image. This field allows you to specify that percentage.

Typically, you will want less than 1 percent of the pixels in your image saturated. Consequently, the default value for this field is 0.15 percent.

Reminder

When this checkbox is selected, the software will warn you to turn off your transilluminator light when you exit the Gel Doc acquisition window or when your system is “idle” for more than 5 minutes.

Video Printing Footer Information

The checkboxes in this group allow you to specify the information that will appear at the bottom of your video printer printouts.

Save Options

To automatically create a backup copy of any scan you create, select the Make Backup Copy checkbox.

With this checkbox selected, when you save a scan, a backup copy will be placed in the same directory as the scanned image. Windows backup files will have an ".sbk" extension. Macintosh backup files will have the word "backup" after the file name.

Simple Acquisition Mode

The Simple Acquisition mode option allows you to reconfigure the Gel Doc acquisition window to simple mode. In simple mode, the acquisition window contains the same steps as the advanced acquisition window with the exception of Step IV, Optimize display, and Step V, Analysis. Manual exposure is also disabled in simple mode.

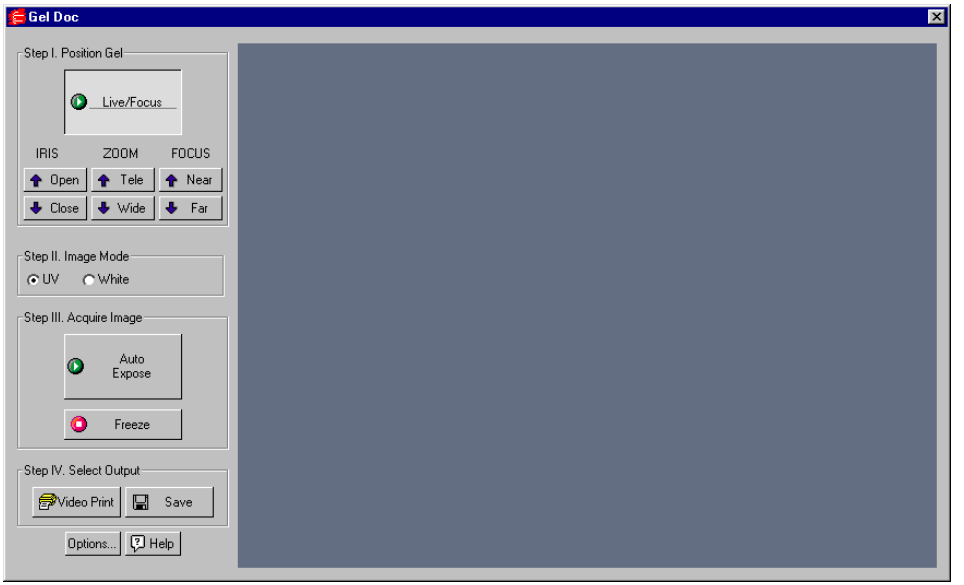


Fig.A-10. Simple Acquisition Window

To change the acquisition window to Simple Acquisition Mode, check the box marked Simple Acquisition Mode. The change will take effect the next time you open the acquistion window.

Appendix B
ChemiDoc

Fig.B-1. ChemiDoc.

Before you can begin acquiring images, the ChemiDoc system must be properly installed and connected with the host computer. See the ChemiDoc hardware manual for installation, startup, and operating instructions.

To use the ChemiDoc, you will need to have the Bio-Rad-supplied acquisition board installed in your PC or Macintosh. The drivers for this board will be installed when you install the main software application.

Make sure that your ChemiDoc camera is turned on. If the camera is not turned on, the ChemiDoc acquisition window will open and an image capture error will be displayed.

Simulation Mode

Any of the imaging device acquisition windows can be opened in "simulation mode." In this mode, an acquisition window will open and the controls will appear active, but instead of capturing real images, the window will create "dummy" images of manufactured data.

You do not need to be connected to an imaging device to open a simulated acquisition window. This is useful for demonstration purposes or practice scans.

To enter simulation mode, hold down the CTRL key and select the name of the device from the File menu. The title of the acquisition window will indicate that it is simulated.

B.1 ChemiDoc Acquisition Window

To acquire images using the ChemiDoc, go to the File menu and select ChemiDoc.... The acquisition window for the instrument will open, displaying a control panel and a video display window.

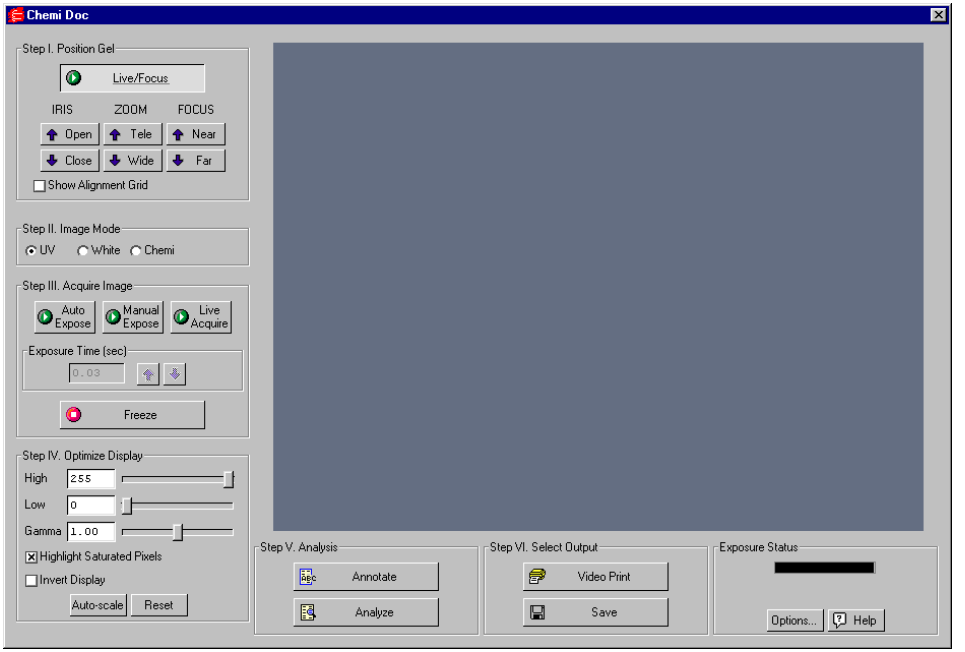


Fig.B-2. ChemiDoc acquisition window

The ChemiDoc video display window will open in “live” mode, giving you a live video display of your sample. If no image is visible, make sure the camera is on, check the cable connections, make sure the iris on the camera is not closed, and make sure that the protective cap is off the camera lens. Also check to see that the transilluminator is on and working.

The control panel has been arranged from top to bottom to guide you through the acquisition procedure. There are six basic steps to acquiring an image using the ChemiDoc:

1. Position and focus the gel or other object to be imaged.
2. Select Mode.
3. Acquire the image.

4. Optimize the display.
5. Analysis.
6. Select the output.

B.2 Step I. Position Gel

The ChemiDoc window will open in “live” mode, giving you a live video display of your sample. In this mode, the Live/Focus button will appear selected, and frames will be captured and displayed at about 10 frames per second, depending on the speed of your computer.

You can use live mode to zoom, focus, and adjust the aperture on the camera, while positioning the sample within the area.

Note: Newer versions of the ChemiDoc feature a motorized zoom lens that can be controlled directly from the acquisition window using the Iris, Zoom, and Focus arrow buttons. Click on the Up/Down buttons while viewing your sample in the window to adjust the lens. These buttons will not be visible if you are connected to older versions of the ChemiDoc without the motorized zoom lens.

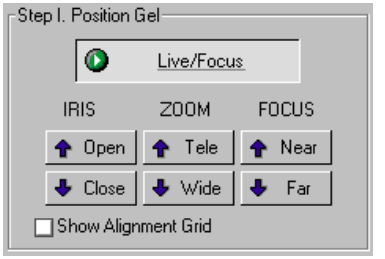


Fig.B-3. Live/Focus button and camera control buttons.

You can also select the Show Alignment Grid checkbox to facilitate positioning.

Note: After positioning your sample, you should check the Imaging Area dimensions under Options (see section B.9, Options) to make sure that they conform to the size of the area you are focusing on. To determine the size of the area you are

focusing on, you can place a ruler in the ChemiDoc cabinet so that it is visible in the image.

B.3 Step II. Image Mode

The Image Mode option buttons change the type and scale of the data, as well as the behavior of the ChemiDoc when acquiring an image.

UV

Select this mode for fluorescent samples. With this mode selected, the data will be measured in linear intensity units.

White Light

Select this mode for reflective and transmissive samples. With this mode selected, the data will be measured in uncalibrated optical density (uOD) units.

Chemi

This mode is designed for chemiluminescent samples. With this mode selected, the data is measured in linear intensity units; however, the data is inverted, so that samples will appear dark on a light background.

Also, Chemi mode changes the behavior of the Auto and Manual Expose functions, as described above.

B.4 Step III. Acquire Image

For many white light applications, you can skip this step and save and print images directly from Live/Focus mode.

For UV light, chemiluminescent applications, or faint samples, the ChemiDoc control panel has several features for creating image exposures. You can take an automatic

exposure based on the number of saturated pixels in the image, you can enter a specific exposure time, or you can take a series of exposures and select the best one.

Note: "Exposure" refers to the integration of the image on the camera CCD over a period of time. The effect is analogous to exposing photographic film to light over a period of time.

Auto Expose

Use Auto Expose if you want to take a single exposure but are uncertain of the optimal exposure time.

Note: If you know the approximate exposure time you want (± 3 seconds), you can skip this step and go directly to Manual Expose.

Click on the Auto Expose button to cancel Live/Focus mode and begin an automatic exposure. The Auto Expose button will appear selected throughout the exposure.

During the auto exposure, the image is continuously integrated on the camera CCD until it reaches a certain percentage of saturated pixels. This percentage is set in the Options dialog box. (Default = 0.15 percent. See section B.9, Options.)

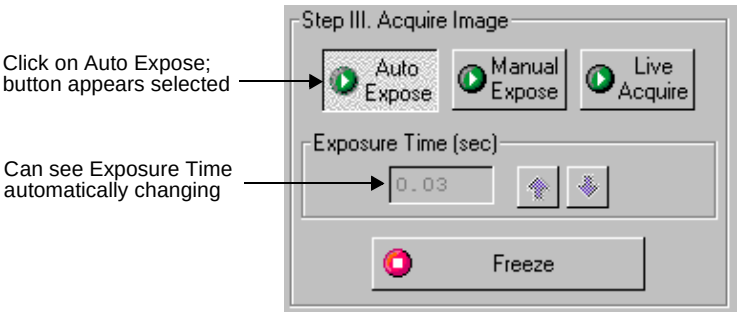


Fig.B-4. Selecting Auto Expose.

Once an image has reached the specified percentage of saturated pixels, it is captured and displayed in the video display window, Auto Expose is automatically deactivated, and the exposure time appears active in the Exposure Time field.

At this point, if you are in UV or White image mode, Manual Expose will be automatically activated. If you are in Chemi mode, the Freeze button will be automatically activated.

Note: If you are having difficulty auto-exposing your sample, you can use Manual Expose to adjust your exposure time directly. Most non-chemi applications only require an exposure time of a few seconds, which can be quickly adjusted using Manual Expose.

Manual Expose

If you know the approximate exposure time you want, you can click on the Manual Expose button. In UV or White image mode, Manual Expose is automatically activated after Auto Expose is complete.

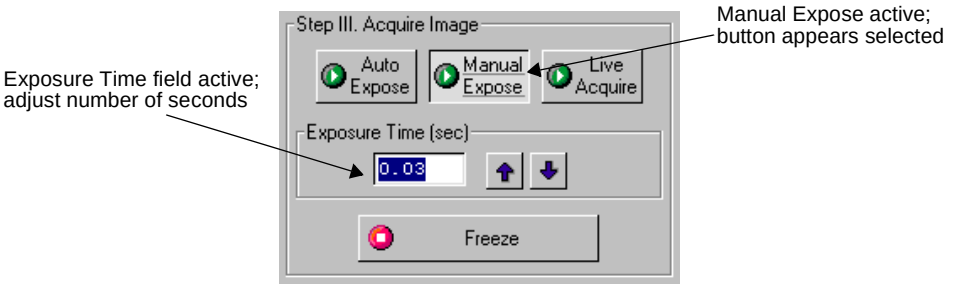


Fig.B-5. Setting a manual exposure.

With Manual Expose activated, you can adjust the exposure time directly by changing the number of seconds in the Exposure Time field. Type in a number or use the arrow buttons next to the field.

In UV or White image mode, when the specified exposure time is reached, the last captured image will be displayed in the ChemiDoc image window. The camera will continue to integrate the image on the CCD, updating the display whenever the specified number of seconds is reached.

Once you are satisfied with the quality of the displayed image, click on the Freeze button to stop the exposure process. The last full exposure will be displayed in the image window.

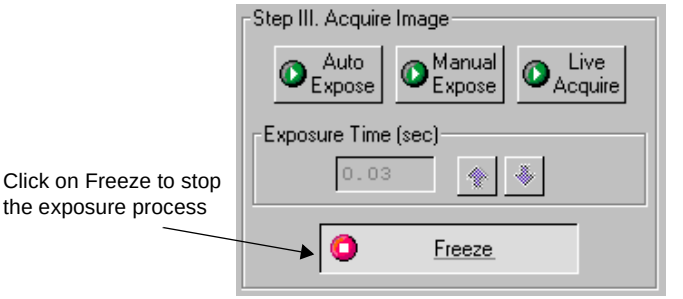


Fig.B-6. Freezing the manual exposure.

In Chemi mode, Manual Expose will expose an image over the specified exposure time and then stop automatically.

Note: Freeze is automatically activated if you adjust any of the subsequent controls (e.g., Video Print, Image Mode, Display controls, etc.).

Live Acquire

Live Acquire mode allows you to specify an interval over which a series of progressively longer exposures are taken. All exposures are then displayed on the screen, and you can choose the one with the best image.

Click on the Live Acquire button. A settings dialog box will open in which you can specify the total exposure time, starting exposure time, and number of exposures.

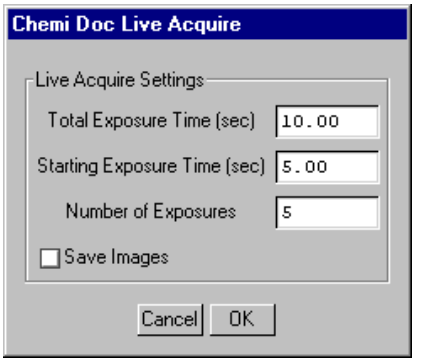


Fig.B-7. Live Acquire settings.

Note: You should specify no more than 10 exposures in the Live Acquire Settings dialog, to avoid excessive build-up of image background in later exposures. The fewer the exposures, the less background will be added to the image. See the Release Notes for additional instructions on reducing background in images captured using Live Acquire.

Select the Save Images checkbox if you want to automatically save each exposure as it is taken.

Click on OK in the settings dialog to begin taking exposures. If you selected Save Images, a Save dialog box will open in which you can specify the base file name and location of the exposure files. When you click on Save, the exposures will be taken.

The specified number of exposures will be taken at equal intervals between the starting exposure time and total exposure time. When each exposure is complete, an image window containing that exposure will open behind the ChemiDoc window. When the full exposure time has lapsed, all the image windows will move in front of the ChemiDoc window.

Note that the first exposure will have the base file name (the default base file name is the computer user name and a time stamp). Each subsequent exposure will have a version number (v2, v3, v4, etc.) appended to the base file name. The highest version number will be the final exposure. If you did not elect to auto-save the exposures as they were created, then each image will be unsaved.

To stop the Live Acquire, click on the Freeze button or adjust any of the subsequent controls (e.g., Video Print, Image Mode, Display controls, etc.).

Note: Exposures captured before freezing will be displayed in image windows.

Study the different images and select the best exposure(s) to keep. You can then proceed to the next step.

B.5 Step IV. Optimize Display

The Display controls are useful for quickly adjusting the appearance of your image for output to a video printer. Adjusting these controls will automatically freeze the video display and allow you to alter the image within the ChemiDoc window.

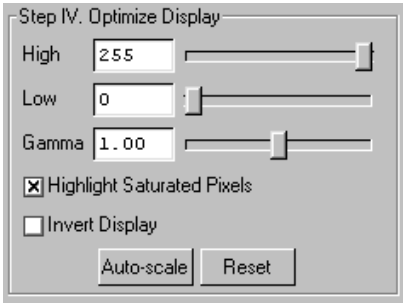


Fig.B-8. Display controls.

These controls are similar to those in the Transform dialog box.

Note: The Display controls will only change the appearance of the image. They will not change the underlying data.

High/Low Sliders

If Auto-scale doesn't give you the appearance you want, you can use the High and Low sliders to redraw the image yourself. In white light mode and chemi mode, dragging the High slider handle to the left will make weak signals appear darker. In

UV mode, dragging the High slider handle to the left will make weak signals appear brighter. Dragging the Low slider handle to the right will reduce background noise.

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

Gamma Slider

Some images may be more effectively visualized if their data are mapped to the computer screen in a nonlinear fashion. Adjusting the Gamma slider handle changes the light and dark contrast nonlinearly.

Highlight Saturated Pixels

When this box is checked, any saturated pixels in the image will appear highlighted in red in the scan window and in the pop-up image window. To view/hide saturated pixels in the pop-up image window, use the Image > Transform command.

Invert Display

This checkbox will switch light spots on a dark background to dark spots on a light background, and visa versa. This will only affect how the image is displayed on the screen, not the actual image data.

Auto-scale

Clicking on Auto-scale will adjust your displayed 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). You can then “fine tune” the display using the High, Low, and Gamma sliders described below.

Reset

Reset will return the image to its original, unmodified appearance.

B.6 Step V. Analysis

The Analysis step of the ChemiDoc acquisition window allows you to add annotations and analyze the newly acquired image.

Annotate

Clicking on Annotate will open a separate image window displaying the captured image. The default name for the image will include the date, time, and user (if known).

The Text Overlay toolbar will also pop up to allow you to annotate your image.

The image will not be saved until you select Save or Save As from the File menu.

Analyze

Clicking on Analyze will open a separate image window displaying the captured image. The default name for the image will include the date, time, and user (if known).

You can then analyze the image using the other features in the main application.

The image will not be saved until you select Save or Save As from the File menu.

B.7 Step VI. Select Output

The ChemiDoc window has several output options.

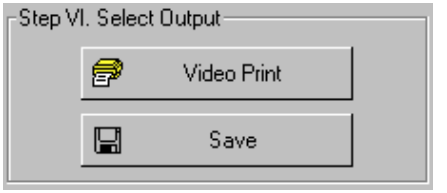


Fig.B-9. Output options.

Video Print

Clicking on Video Print will automatically send the currently displayed frame (either live or integrated) to a video printer. You can add information about your image to the bottom of the printout by selecting the appropriate checkboxes in the Options dialog box. (See section B.9, Options.)

Save

Clicking on Save will open a separate image window displaying the captured image. A Save As dialog box will automatically open displaying the default file name for the image, which will include the date, time, and user (if known). You can then change the file name and storage directory.

You can also export your image as a TIFF image for viewing with other applications.

B.8 Exposure Status

The Exposure Status bar shows the progress of your exposure. If your exposure time is greater than 1 second, the status bar display will give you a graphical representation of the remaining time before exposure is complete.

If the exposure time is less than 1 second, the status bar will not refresh itself for each exposure; it will remain at 100 percent.

B.9 Options

Click on the Options button to open the Options dialog box. Here you can specify certain settings for your ChemiDoc system.

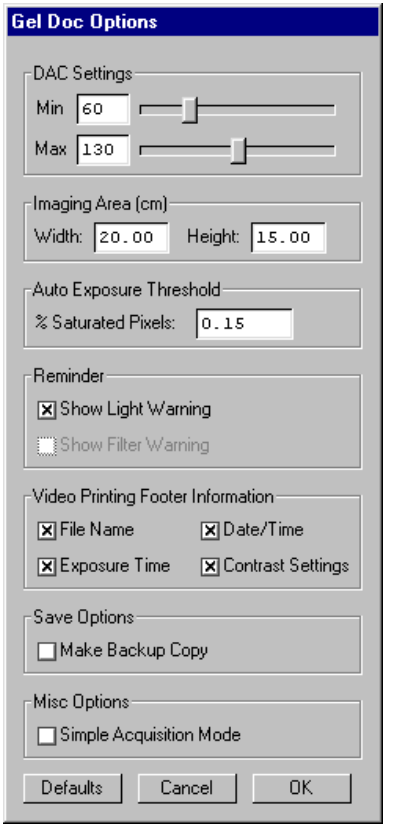


Fig.B-10. Available options in the ChemiDoc acquisition window.

Click on OK to implement any changes you make in this box. Clicking on Defaults restores the settings to the factory defaults.

DAC Settings

Note: The default DAC settings are highly recommended and should be changed with caution.

These sliders may be used to adjust the minimum and maximum voltage settings of your video capture board. The minimum slider defines the pixel value that will appear as white in the image, while the maximum slider defines the pixel value that will appear as black. The slider scale is 0–255, with the defaults set to 60 minimum and 130 maximum.

Imaging Area

These fields are used to specify the size of your imaging area in centimeters, which in turns determines the size of the pixels in your image (i.e., resolution). When you adjust one imaging area dimension, the other dimension will change to maintain the aspect ratio of the camera lens.

Note: Your imaging area settings must be correct if you want to do 1:1 printing. These are also important if you are comparing the size of objects (e.g., using the Volume Tools) between images.

Auto Exposure Threshold

When you click on Auto Expose, the exposure time is determined by the percentage of saturated pixels you want in your image. This field allows you to specify that percentage.

Typically, you will want less than 1 percent of the pixels in your image saturated. Consequently, the default value for this field is 0.15 percent.

Reminder

When this checkbox is selected, the software will warn you to turn off your transilluminator light when you exit the ChemiDoc acquisition window or when your system is “idle” for more than 5 minutes.

Note: If you are performing experiments that are longer than 5 minutes (e.g., chemiluminescence), this should be deselected.

Video Printing Footer Information

The checkboxes in this group allow you to specify the information that will appear at the bottom of your video printer printouts.

Save Options

To automatically create a backup copy of any scan you create, select the Make Backup Copy checkbox.

With this checkbox selected, when you save a scan, a backup copy will be placed in the same directory as the scanned image. Windows backup files will have an ".sbk" extension. Macintosh backup files will have the word "backup" after the file name.

Simple Acquisition Mode

The Simple Acquisition mode option allows you to reconfigure the ChemiDoc acquisition window to simple mode. In simple mode, the acquisition window contains the same steps as the advanced acquisition window with the exception of Step IV, Optimize display, and Step V, Analysis. Manual exposure is also disabled in simple mode.

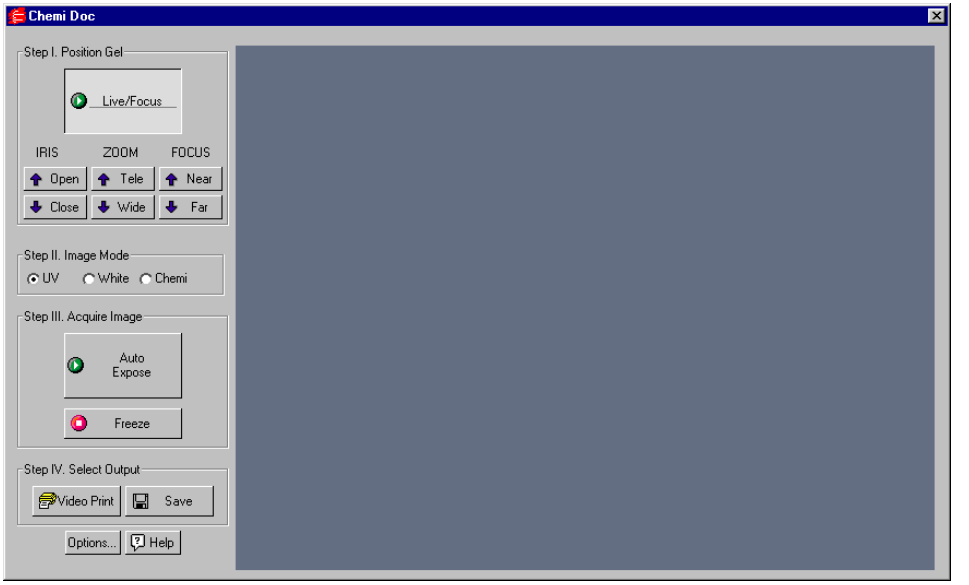


Fig.B-11. Simple Acquisition Window

To change the acquisition window to Simple Acquisition Mode, check the box marked Simple Acquisition Mode. The change will take effect the next time you open the acquistion window.

Appendix C
ChemiDoc XRS

Fig.C-1. ChemiDoc XRS.

Before you can begin acquiring images, the ChemiDoc XRS system must be properly installed and connected with the host computer. See the ChemiDoc XRS hardware manual for installation, startup, and operating instructions.

To use the ChemiDoc XRS, you will need to have the Bio-Rad-supplied acquisition board installed in your PC or Macintosh. The drivers for this board will be installed when you install the main software application.

Make sure that your ChemiDoc XRS camera is turned on. If the camera is not turned on, the ChemiDoc XRS acquisition window will not open.

Simulation Mode

Any of the imaging device acquisition windows can be opened in "simulation mode." In this mode, an acquisition window will open and the controls will appear active, but instead of capturing real images, the window will create "dummy" images of manufactured data.

You do not need to be connected to an imaging device to open a simulated acquisition window. This is useful for demonstration purposes or practice scans.

To enter simulation mode, hold down the CTRL key and select the name of the device from the File menu. The title of the acquisition window will indicate that it is simulated.

C.1 ChemiDoc XRS Acquisition Window

To acquire images using the ChemiDoc XRS, go to the File menu and select ChemiDoc XRS.... The acquisition window for the instrument will open, displaying a control panel and a video display window.

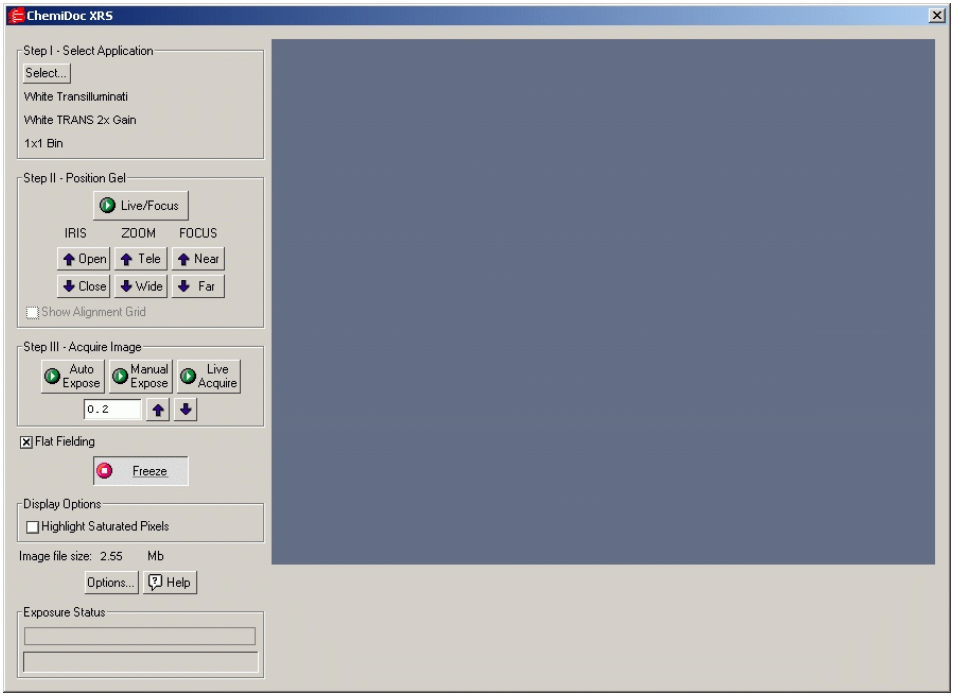


Fig.C-2. ChemiDoc XRS acquisition window

When the ChemiDoc XRS window first opens, no image will be displayed.

The control panel has been arranged from top to bottom to guide you through the acquisition procedure. There are three basic steps to acquiring an image using the ChemiDoc XRS:

1. Select the application.
2. Position and focus the gel or other object to be imaged.
3. Acquire the image.

C.2 Step I. Select Application

To set the appropriate parameters for the type of object you are imaging, click on the Select button under Select Application. You have the option of selecting a UV, White, or Chemiluminescence application. You can also select a custom setting. Once you select an application, the name of the application and its settings appear in the Select application step.

C.2.a. Custom Applications

The Bio-Rad installed applications have pre-set gain and bin settings. If the settings for the available applications do not meet your needs, you have the option of creating a custom application, which allows you to set your own gain and binning settings.

To use a custom application, click Select and choose custom. Next, select a custom application from the list, or select Create to create a new application. This opens the custom dialog.

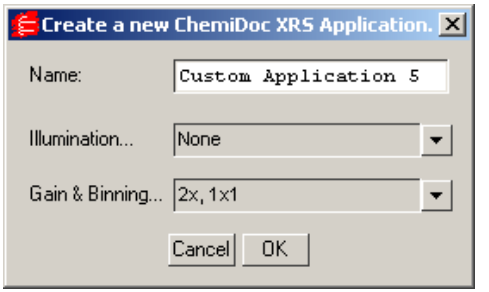


Fig.3-3. Creating a custom ChemiDoc XRS application.

Enter a name for the application. From the Illumination drop down list, select an illumination mode. You can select UV, white or no illumination. Next select gain and binning settings from the drop down list.

A higher Binning setting (2x2, 3x3) provides optimal sensitivity for low-light applications such as chemiluminescence. In this mode, the pixels in the camera are “binned” (e.g., four pixels are combined into one) to increase the amount of signal per pixel without increasing noise. Note that combining the pixels results in a reduction in the resolution of the image.

Selecting a higher Gain setting (2x) provides higher sensitivity without reduced resolution; however, noise will also increase. This is useful for faint signals (bright spots will saturate).

Click OK to save and add the new custom application to the custom list. The new application is automatically selected as your application.

C.3 Step II. Position Gel

Click the Live/Focus button, and frames will be captured and displayed at about 10 frames per second, depending on the speed of your computer.

You can use live mode to zoom, focus, and adjust the aperture on the camera, while positioning the sample within the area.

Note: ChemiDoc XRS features a motorized zoom lens that can be controlled directly from the acquisition window using the Iris, Zoom, and Focus arrow buttons. Click on the Up/Down buttons while viewing your sample in the window to adjust the lens.

Fig.C-4. Live/Focus button and camera control buttons.

You can also select the Show Alignment Grid checkbox to facilitate positioning.

Note: After positioning your sample, you should check the Imaging Area dimensions under Options (see C.5, Options) to make sure that they conform to the size of the area you are focusing on. To determine the size of the area you are focusing on, you can place a ruler in the ChemiDoc XRS hood so that it is visible in the image.

C.4 Step III. Acquire Image

The ChemiDoc XRS control panel has several features for creating image exposures. You can take an automatic exposure based on the number of saturated pixels in the image, you can enter a specific exposure time, or you can take a series of exposures and select the best one.

Note: "Exposure" refers to the integration of the image on the camera CCD over a period of time. The effect is analogous to exposing photographic film to light over a period of time.

Auto Expose

Use Auto Expose if you want to take a single exposure but are uncertain of the optimal exposure time.

Note: If you know the approximate exposure time you want (± 3 seconds), you can skip this step and go directly to Manual Expose.

Click on the Auto Expose button to cancel Live/Focus mode and begin an automatic exposure. The Auto Expose button will appear selected throughout the exposure.

During the auto exposure, the image is continuously integrated on the camera CCD until it reaches a certain percentage of saturated pixels. This percentage is set in the Options dialog box. (Default = 0.75 percent. See C.5, Options.)

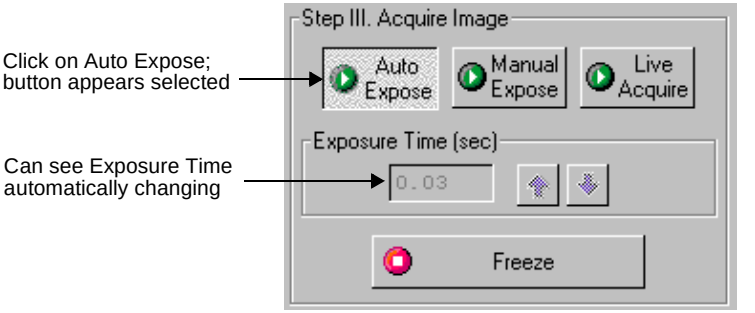


Fig.C-5. Selecting Auto Expose.

Once an image has reached the specified percentage of saturated pixels, it is captured and displayed in the video display window, Auto Expose is automatically deactivated, and the exposure time appears active in the Exposure Time field.

Note: If you are having difficulty auto-exposing your sample, you can use Manual Expose to adjust your exposure time directly. Most non-chemiluminescent applications only require an exposure time of a few seconds, which can be quickly adjusted using Manual Expose.

Manual Expose

If you know the approximate exposure time you want, you can click on the Manual Expose button. Manual Expose is automatically activated after Auto Expose is complete.

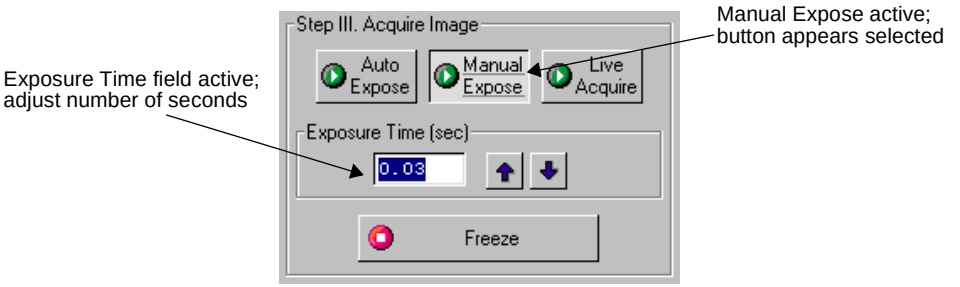


Fig.C-6. Setting a manual exposure.

With Manual Expose activated, you can adjust the exposure time directly by changing the number of seconds in the Exposure Time field. Type in a number or use the arrow buttons next to the field.

When the specified exposure time is reached, the last captured image will be displayed in the ChemiDoc XRS image window.

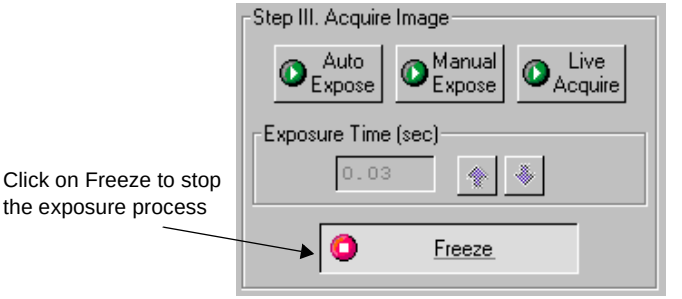


Fig.C-7. Freezing the manual exposure.

Live Acquire

Live Acquire mode allows you to specify an interval over which a series of progressively longer exposures are taken. All exposures are then displayed on the screen, and you can choose the one with the best image.

Click on the Live Acquire button. A settings dialog box will open in which you can specify the total exposure time, starting exposure time, and number of exposures.

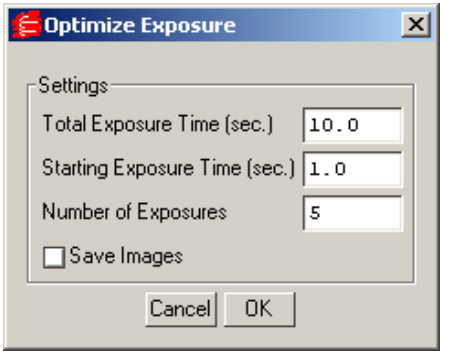


Fig.C-8. Live Acquire settings.

Note: You should specify no more than 10 exposures in the Live Acquire Settings dialog, to avoid excessive build-up of image background in later exposures. The

fewer the exposures, the less background will be added to the image. See the Release Notes for additional instructions on reducing background in images captured using Live Acquire.

Select the Save Images checkbox if you want to automatically save each exposure as it is taken.

Click on OK in the settings dialog to begin taking exposures. If you selected Save Images, a Save dialog box will open in which you can specify the base file name and location of the exposure files. When you click on Save, the exposures will be taken.

The specified number of exposures will be taken at equal intervals between the starting exposure time and total exposure time. When each exposure is complete, an image window containing that exposure will open in front of the ChemiDoc XRS window.

Note that the first exposure will have the base file name (the default base file name is the computer user name and a time stamp). Each subsequent exposure will have a version number (v2, v3, v4, etc.) appended to the base file name. The highest version number will be the final exposure. If you did not elect to auto-save the exposures as they were created, then each image will be unsaved.

To stop the Live Acquire, click on the Freeze button.

Note: Exposures captured before freezing will be displayed in image windows.

Study the different images and select the best exposure(s) to keep.

C.4.a. Exposure Status

The Exposure Status bar shows the progress of your exposure. If your exposure time is greater than 1 second, the status bar display will give you a graphical representation of the remaining time before exposure is complete.

Illumination Flat Fielding

For UV or white light transilluminatoin applications, you should use the appropriate reference plate to ensure a uniform intensity in the image. This will compensate for normal variations in image pixel intensity that occur with a transilluminating light source.

To enable this feature, select the Flat Fielding checkbox.

UV Illumination Flat Fielding: When you first select the Flat fielding checkbox, and then acquire an image using the UV transilluminator, you will be prompted to remove your sample and place the fluorescent reference plate on the ChemiDoc XRS sample stage and turn on the UV transilluminator (see the ChemiDoc XRS User Manual).

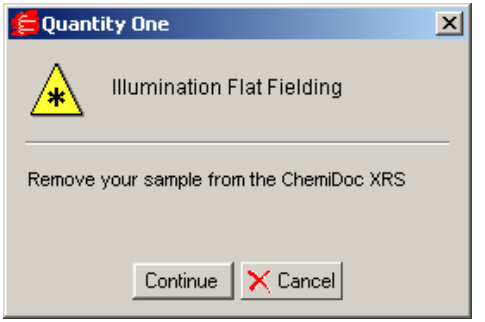


Fig.C-9. Illumination flat fielding reminder

A reference image of the plate will be acquired and saved on your computer hard drive. The reference image will be applied to the sample image to generate a flat field corrected exposure.

White Light Illumination Flat Fielding: When you first select the checkbox, and then acquire an image using the white light conversion screen or the white light transilluminator, you will be prompted to remove your sample, turn on the white light source, and collect an exposure of the white light screen/transilluminator (see the ChemiDoc XRS User Manual). A reference image of the screen/transilluminator will be acquired and saved on your computer hard drive. The reference image will be applied to the sample image to generate a flat field corrected exposure.

For subsequent UV or white light trans exposures, you will be prompted to either use the appropriate saved flat field image or acquire a new one.

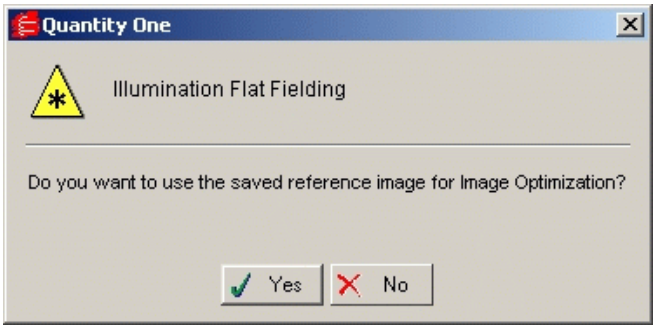


Fig.C-10. Using the saved reference image.

Note: Flat fielding is unavailable for White EPI illumination and chemiluminescence applications.

C.5 Options

Click on the Options button to open the Options dialog box. Here you can specify certain settings for your ChemiDoc XRS system.

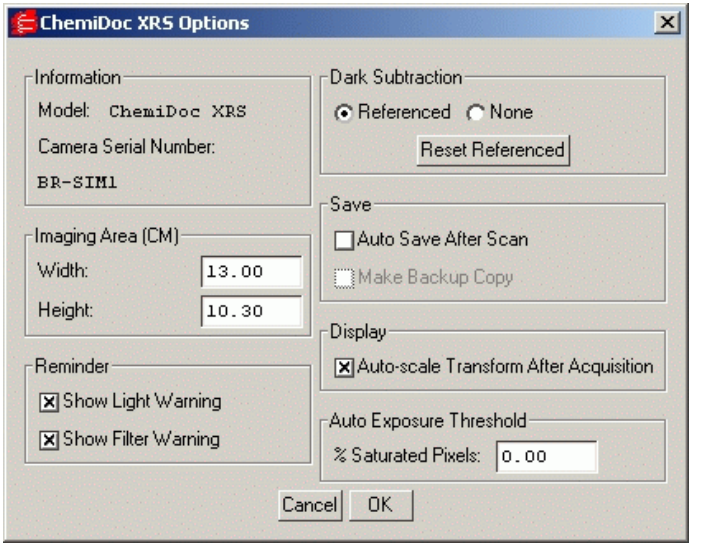


Fig.C-11. Available options in the ChemiDoc XRS acquisition window.

Click on OK to implement any changes you make in this box.

C.5.a. Imaging Area

These fields are used to specify the size of your imaging area in centimeters, which in turn determines the size of the pixels in your image (i.e., resolution). When you adjust one imaging area dimension, the other dimension will change to maintain the aspect ratio of the camera lens.

Note: Your imaging area settings must be correct if you want to do 1:1 printing. These are also important if you are comparing the size of objects (e.g., using the Volume Tools) between images.

C.5.b. Reminder

Show Light Warning - When this checkbox is selected, the software will warn you to turn off your transilluminator light when you exit the ChemiDoc XRS acquisition window or when your system is “idle” for more than 5 minutes.

Note: If you are performing experiments that are longer than 5 minutes (e.g., chemiluminescence), this should be deselected.

Show Filter Warning - When this checkbox is selected, the software will warn you to move the filter slider either to chemi or the UV/White position.

C.5.c. Dark Subtraction

At the time of installation a reference dark image file was generated and saved to your hard drive. The purpose of this file is to reduce dark current noise generated from the CCD. Dark current noise is typical of all CCDs and is a result of the accumulation of charge in the absence of light

The default time of the reference dark image is ten minutes. The default reference file is optimized for ChemiDoc XRS applications. If you select to change the reference dark image, ensure that the time of the reference dark image is of equal or greater duration than your typical sample image. When the sample image time is longer than the reference dark image time the ability to detect very faint samples may be compromised.

To reset the reference dark image time, select options in the ChemiDoc XRS acquisition window. Select Reset Referenced.

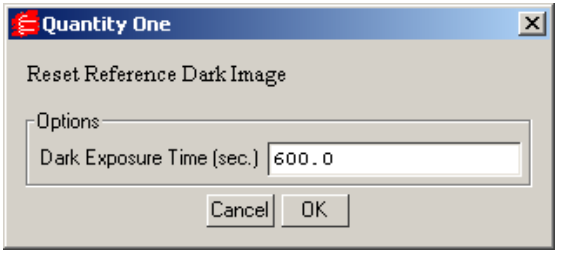


Fig.C-12. Reset Reference Dark pop-up box

Enter the desired time in seconds. Select OK. You will be prompted to close and re-open the acquisition window. Place the lens cap on the camera and close the door of the hood (See ChemiDoc XRS instruction manual). Select OK at the prompt and new reference dark images of the entered time interval will be taken for each of the five possible combinations of camera gain and bin settings. These will be retained as the reference dark images until the time is reset again.

C.5.d. Save Options

To automatically create a backup copy of any scan you create, select the Make Backup Copy checkbox.

With this checkbox selected, when you save a scan, a backup copy will be placed in the same directory as the scanned image. Windows backup files will have an ".sbk" extension. Macintosh backup files will have the word "backup" after the file name.

C.5.e. Auto-scale Transform

Auto-scale Transform after Acquisition allows the user the option of having the image automatically perform the Auto-Scale transform function upon completion of image acquisition. This eliminates the need to transform when the acquisition time was too short or the iris not opened enough.

To enable the auto-scale transform function, check the box labeled, Auto-Scale Transform after Acquisition in the Options dialog.

C.5.f. Auto Exposure Threshold

When you click on Auto Expose, the exposure time is determined by the percentage of saturated pixels you want in your image. This field allows you to specify that percentage.

Typically, you will want less than 1 percent of the pixels in your image saturated. Consequently, the default value for this field is 0.75 percent.

C.6 Other Features

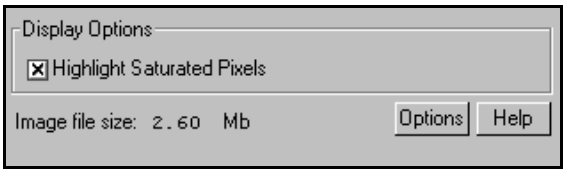


Fig.C-13. Other ChemiDoc XRS acquisition window features.

Highlight Saturated Pixels

When this box is checked, any saturated pixels in the image will appear highlighted in red in the scan window and in the pop-up image window. To view/hide saturated pixels in the pop-up image window, use the Image > Transform command.

File Size of Images

Image File Size shows the size of the image file you are about to create. This size is determined by the bin setting used for the selected application.

If you do not have enough computer memory for the specified file size, an error message will appear when you attempt to acquire an image. (Macintosh users can increase the application memory partition. See your Macintosh computer documentation for guidance.)

Appendix D
GS-700 Imaging Densitometer

Fig. D-1. GS-700 Imaging Densitometer

Before you can begin scanning images with the GS-700 Imaging Densitometer®, your instrument must be properly installed and connected with the host computer. See the hardware manual for installation, startup, and operating instructions.

PC Only: A Note About SCSI Cards

The GS-700 is connected to your computer by a Small Computer System Interface (SCSI) cable. To use the GS-700, you must have a SCSI card installed in your PC. If you have a PC with a Windows 98 or Windows ME operating system, you may also need to load the SCSI and WinASPI drivers that came with the card.

Simulation Mode

Any of the imaging device acquisition windows can be opened in “simulation mode.” In this mode, an acquisition window will open and the controls will appear active, but

instead of capturing real images, the window will create “dummy” images of manufactured data.

You do not need to be connected to an imaging device to open a simulated acquisition window. This is useful for demonstration purposes or practice scans.

To enter simulation mode, hold down the CTRL key and select the name of the device from the File menu. The title of the acquisition window will indicate that it is simulated.

Note: There is no simulated calibration for the GS-700 and GS-710 Imaging Densitometers.

D.1 GS-700 Acquisition Window

To acquire images using the GS-700, go to the File menu and select GS-700.... The acquisition window for the densitometer will open, displaying a control panel and a scanning window.

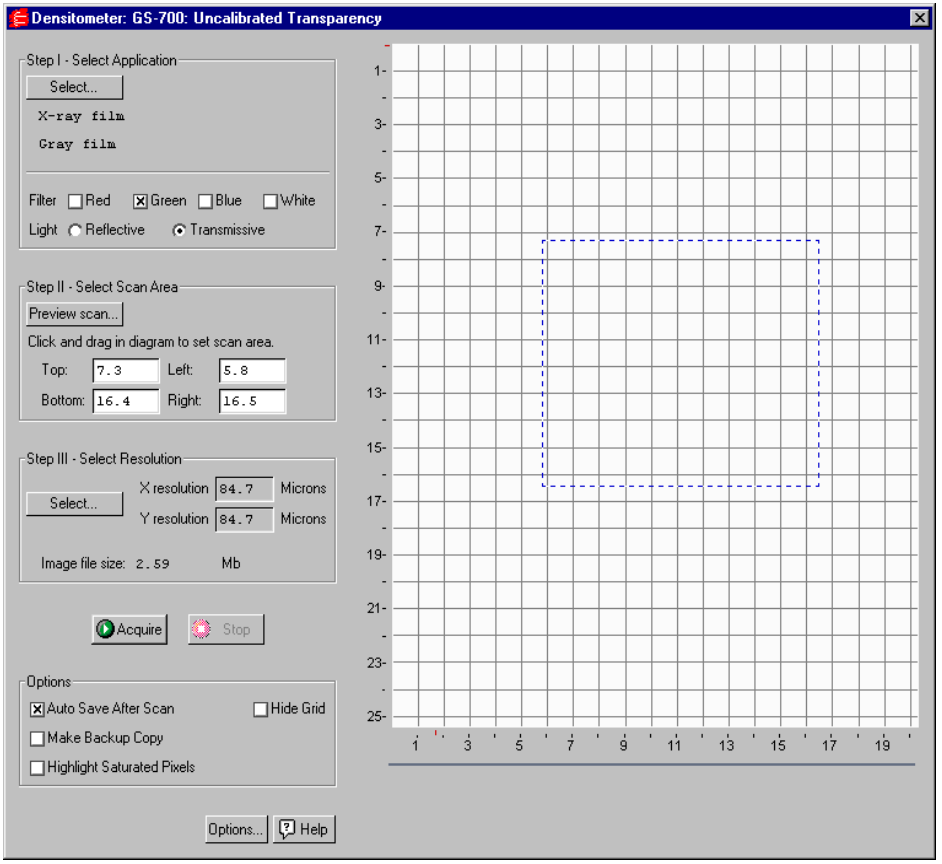


Fig. D-2. GS-700 acquisition window.

The scanning window is marked by grid lines that divide the area into square centimeters. These are numbered 1–35 top to bottom and 1–21 left to right if the light source is uncalibrated reflective, and 1–25 top to bottom and 1–20 left to right if the light source is uncalibrated transmissive (see below for details).

To hide the gridlines, click on the Hide Grid checkbox under Options.

The control panel has been arranged from top to bottom to guide you through the acquisition procedure. There are four basic steps to scanning an image using the GS-700:

1. Select the application.
2. Select the scan area.
3. Select the resolution of the scan.
4. Acquire the image.

D.2 Step I. Select Application

To set the parameters for your particular scan, you can:

1. Select from a list of possible applications, or
2. Choose your own filter and light source settings.

Selecting from the List of Applications

When you select from the list of applications, the software automatically sets the appropriate filter(s) and other parameters for that particular application.

To select from the list of applications, click on the Select button under Select Application.

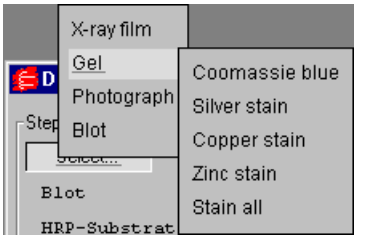


Fig. D-3. Example of the application tree in the GS-700 dialog box.

The applications are listed in a tree that expands from left to right. First you select the category of your application, then you select your particular application.

To exit the tree without selecting, press the ESC key.

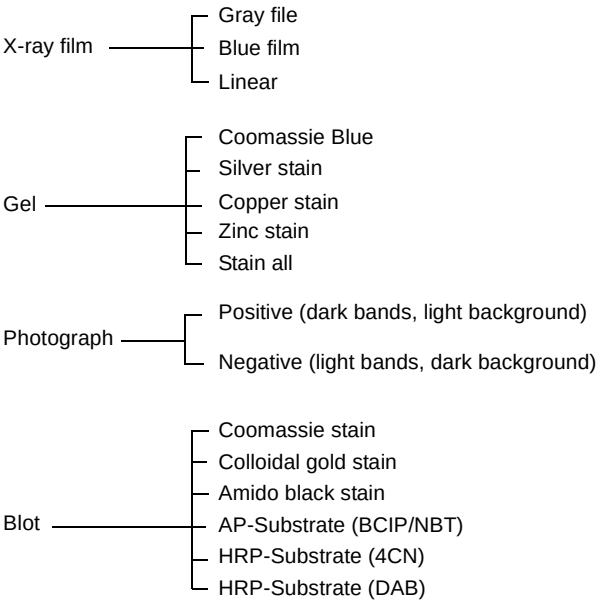


Fig. D-4. Applications available in the GS-700 acquisition window.

Choosing Your Own Settings

If you know the filter and light source settings you want, or want to experiment with different settings, you can choose them yourself.

Fig. D-5. GS-700 Custom Application controls.

Next to Filter, click on either the Red, Green, Blue, or White checkbox, or a combination of two of the first three (Red-Green, Green-Blue, Red-Blue).

Filter Color	Wavelength	Application Examples
Red	595–750 nm	Coomassie G-250, BCIP/NBT, Fast Green FCF, Methylene Blue
Green	520–570 nm	Coomassie R-250, Basic Fuchsin
Blue	400–530 nm	Crocein Scarlet
Gray Scale	400–750 nm	Silver Stains, Copper Stains, Film

Fig. D-6. Examples of filter colors and applications.

Next to Light, select the appropriate light source.

- Reflective mode is for scanning opaque mediums such as dried gels on filter paper, arrays, TLC plates, and photographs. The scanning dimensions in this mode are 21 cm x 35 cm (uncalibrated).
- Transmissive mode is for scanning transparent mediums such as films, gels, and slides. The scanning dimensions in this mode are 20 cm x 25 cm (uncalibrated).

D.3 Step II. Select Scan Area

Preview Scan

Before selecting the particular area to scan, you can preview the entire scanning area to determine the exact position of your sample.

Click on Preview Scan. A quick, low-resolution scan of the entire scanning area will appear in the scanning window. Your sample should be visible within a portion of this scan.

Selecting an Area

Using the preview scan as a guide, select your scan area by dragging your mouse within the scanning window. The border of the scan area you are selecting will be marked by a frame.

Note: The scan area you select must be at least 1 cm wide.

When you release the mouse button, the border changes to a dashed blue line, indicating a selected area.

- To *reposition* the scanning area box you have created, 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.

You can also select the scanning area by entering coordinates in the appropriate fields (Top, Bottom, Left, Right). As you enter a coordinate, the position of the scanning area box will change accordingly.

When selecting, be sure to include the entire area of interest, and be generous with borders. You can always crop the image later.

D.4 Step III. Select Resolution

To select from a list of possible scanner resolutions, click on the Select button under Select Resolution. This will open the Select Scan Resolution dialog box.

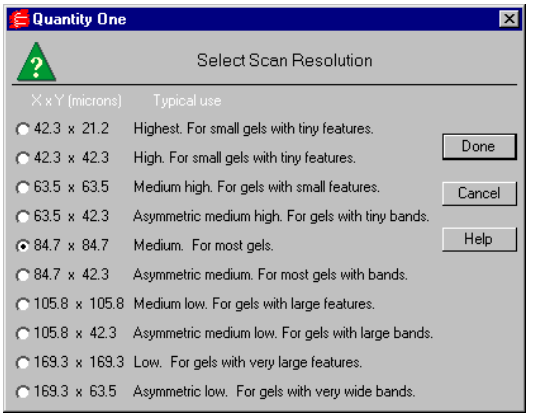


Fig. D-7. Select Scan Resolution dialog box.

Available resolutions are listed from highest to lowest in terms of the dimensions of the resulting pixels (in microns). Smaller pixels equal higher resolution. Each resolution is listed with its typical use.

In general, the size of your pixels should be one-tenth the height of your smallest object.

Some of the resolutions are asymmetrical, meaning that the resolution is higher in the vertical dimension (i.e., the pixels in the resulting image are larger in the horizontal dimension than in the vertical dimension). This is useful for gels with bands, where you are more interested in resolving in the y dimension to determine the size of bands and the spacing between them.

Specifying Your Own Resolution

If you select Oversample under More Options (see section D.7, Other Options, for details), you can specify your own resolution within the range of 43–169 microns (micrometers). With Oversample selected, enter values directly in the fields next to X resolution and Y resolution in the main acquisition window.

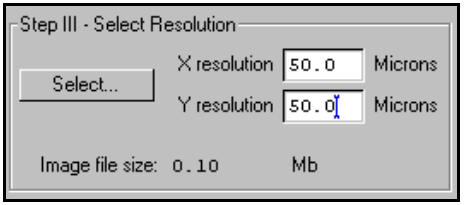


Fig. D-8. Entering a custom resolution (with Oversample selected).

Image File Size

Image File Size (under Select Resolution) shows the size of the scan file you are about to create. If you do not have enough computer memory for the specified file size, an error message will appear when you attempt to scan. If this happens, select a lower resolution or decrease the size of the area to be scanned. (Macintosh users can also increase the application memory partition. See your Macintosh computer documentation for guidance.)

D.5 Acquire the Image

If you want to calibrate your scans, read the following section on calibration before scanning.

To begin to scan, click on the Acquire button. The scanned image will begin to appear in the scanning window, line by line.

To interrupt a scan, click on the Stop button. A message will ask you to confirm the interrupt, and then you will be asked if you want to keep the partial scan. This feature is useful if you overestimated the size of the area you selected.

After the scan is complete, a window will open displaying the scan image, at which point you can analyze and save it.

Note: The image will open with a default file name that includes the date, time, and (if applicable) user name. However, unless you have selected Auto Save After Scan, the file will not be saved until you select Save or Save As from the File menu.

D.6 Calibration

If you have installed a calibration overlay, you can automatically calibrate your transmissive and reflective scans. (Calibration overlays for the GS-700 can be ordered from Bio-Rad.)

To set the automatic calibration settings, click on the More Options button in the GS-700 acquisition window. This will open the Densitometer Options dialog box.

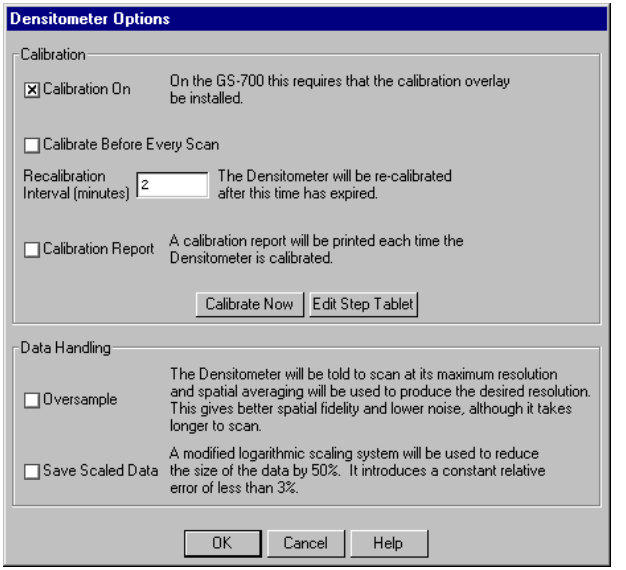


Fig. D-9. More Options in the GS-700 acquisition window.

To enable automatic calibration, click on the Calibration On checkbox.

Note: Before you can calibrate, you must enter the correct step tablet values for your transmissive calibration strip into the Step Tablet Values form, as described below. You do not need to change the values in the reflective step tablet form; you can use the default values.

With Calibration On selected, the other calibration settings become active.

Calibration Strip Window

When calibration is turned on, a calibration strip window will appear below the main scanning window and the length of the main scanning window will be reduced to 29 cm reflective and 23 cm transmissive.

Every time the densitometer calibrates, an image of the calibration strip will appear in the calibration strip window.

D.6.a Editing the Step Tablet

Before you can calibrate, you must enter the step tablet values for your transmissive calibration strip into the Step Tablet Values form.

In the package with your calibration strip, you will find a printout of the diffuse density values for each segment of your transmissive calibration strip. Those exact density values must be entered into the computer for the software to associate a correct density value with each step on the step tablet.

Note: Scanning in transmissive mode with incorrect step tablet values entered into the form can cause significant errors in the reported densities of your scans.

To enter the density values for your step tablet, click on the Edit Step Tablet button in the Densitometer Options box. The Step Tablet Values dialog box for the GS-700 will open.

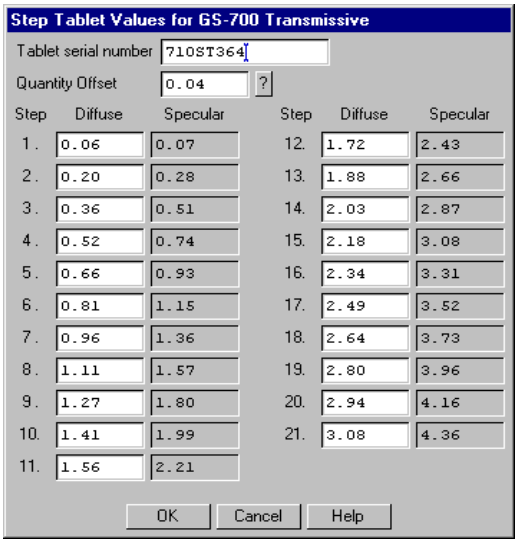


Fig. D-10. Step Tablet dialog box.

The dialog box will indicate whether the displayed form is for the transmissive or reflective step tablet, depending on whether you are in transmissive or reflective mode.

Note: In transmissive scanning, the scanner is calibrated against a transmissive step tablet. In reflective scanning, the scanner is calibrated against a reflective step tablet, unless the reflective calibration strip is uncalibrated. If the reflective calibration strip does not have step tablet values associated with it, you can use the default values in the form.

In the Tablet Serial Number field, you can type in the serial number for the step tablet values you are entering. (The reflective step tablet will probably not have a serial number.)

Your calibration strip will have a clear plastic cover. If the quantity offset value for the clear cover is included in your calibration strip package, you can enter this number in the Quantity Offset field. Otherwise, use a value of 0.045.

Finally, enter the values in the appropriate fields under the Diffuse column. After the step tablet is scanned, the software will associate each density value with its corresponding segment on the step tablet.

The step tablet density values do not need to be entered each time you calibrate. Once they have been entered and saved, they will be automatically recalled when the calibration strip is scanned. You only need to enter new values if you use a new step tablet.

When you finished filling out the Step Tablet Values form, click on OK.

Diffuse Versus Specular O.D.

In the step tablet form, you enter O.D. as diffuse density, and then the software automatically calculates the specular density.

Specular density is a measure of the light that passes directly through a medium. Diffuse density includes light that is scattered as it passes through the medium. Step tablet values are given in diffuse density, but are measured by the scanner in specular density, and therefore must be converted according to the specular/diffuse O.D. ratio. This conversion does not affect quantitation.

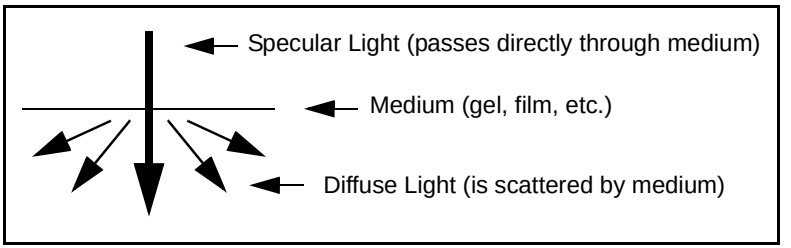


Fig. D-11. Specular and diffuse density

Diffuse density values are converted to specular optical density units according to the following formula:

$$Specular\ OD = 1.4 \cdot Diffuse\ OD$$

D.6.b Calibration Settings

After you have entered the step tablet values, you can immediately calibrate by clicking on the Calibrate Now button (in the Densitometer Options dialog box).

You can also specify how often you want the densitometer to automatically recalibrate. Either click on the Calibrate Before Every Scan checkbox or enter a Recalibration Interval (in minutes) in the appropriate field.

Note: With calibration turned on, the scanner will automatically recalibrate each time you change your filter or your reflective/transmissive setting. (If you select a different application with the same filter and light settings, it will not auto recalibrate.)

Calibration Report

To print out a calibration report each time the densitometer calibrates, click on the Calibration Report checkbox.

D.7 Other Options

Oversample

This feature allows you to scan at the maximum resolution of the GS-700 (42.3 x 42.3 microns) and then use spatial averaging to create an image with lower resolution. This can result in better images at lower resolution—however, it takes longer to scan.

To turn on oversampling, click on the More Options button in the acquisition window and click on the Oversample checkbox.

With oversampling on, you can specify your own resolution within the range of 43–169 microns by entering values directly in the fields next to X resolution and Y resolution in the main acquisition window.

Auto Save After Scan

To automatically save any scan you create, click on the Auto Save After Scan checkbox.

With this checkbox selected, when you click on Acquire, a Save As dialog box will open asking you to specify a file name and location for the image you are about to create. The scan will begin when you click on the Save button.

Make Backup Copy

If you have checked Auto Save After Scan, you can also automatically create a backup copy of any scan you create.

Click on the Make Backup Copy checkbox. With this checkbox selected, when a scan is created and saved, a backup copy will be placed in the same directory as the scanned image. Windows backup files will have an ".sbk" extension. Macintosh backup files will have the word "backup" after the file name.

This backup copy will be read-only, which means that you cannot make changes to it. You can open it like a normal file, but you must save it under a different file name before editing the image or performing analysis.

Highlight Saturated Pixels

When this box is checked, any saturated pixels in the image will appear highlighted in red in the scan window and in the pop-up image window. To view/hide saturated pixels in the pop-up image window, use the Image > Transform command.

Hide Grid

To hide the gridlines in the scanning area window, click on the Hide Grid checkbox.

Appendix E
GS-710 Imaging Densitometer

Fig. E-1. GS-710 Imaging Densitometer

Before you can begin scanning images with the GS-710 Imaging Densitometer®, your instrument must be properly installed and connected with the host computer. See the hardware manual for installation, startup, and operating instructions.

PC Only: A Note About SCSI Cards

The GS-710 is connected to your computer by a Small Computer System Interface (SCSI) cable. To use the GS-710, you must have a SCSI card installed in your PC. If you have a PC with a Windows 98 or Windows ME operating system, you may also need to load the SCSI and WinASPI drivers that came with the card.

Simulation Mode

Any of the imaging device acquisition windows can be opened in “simulation mode.” In this mode, an acquisition window will open and the controls will appear active, but

instead of capturing real images, the window will create “dummy” images of manufactured data.

You do not need to be connected to an imaging device to open a simulated acquisition window. This is useful for demonstration purposes or practice scans.

To enter simulation mode, hold down the CTRL key and select the name of the device from the File menu. The title of the acquisition window will indicate that it is simulated.

Note: There is no simulated calibration for the GS-700 and GS-710 Imaging Densitometers.

E.1 GS-710 Acquisition Window

To acquire images using the GS-710, go to the File menu and select GS-710.... The acquisition window for the densitometer will open, displaying a control panel and a scanning window.

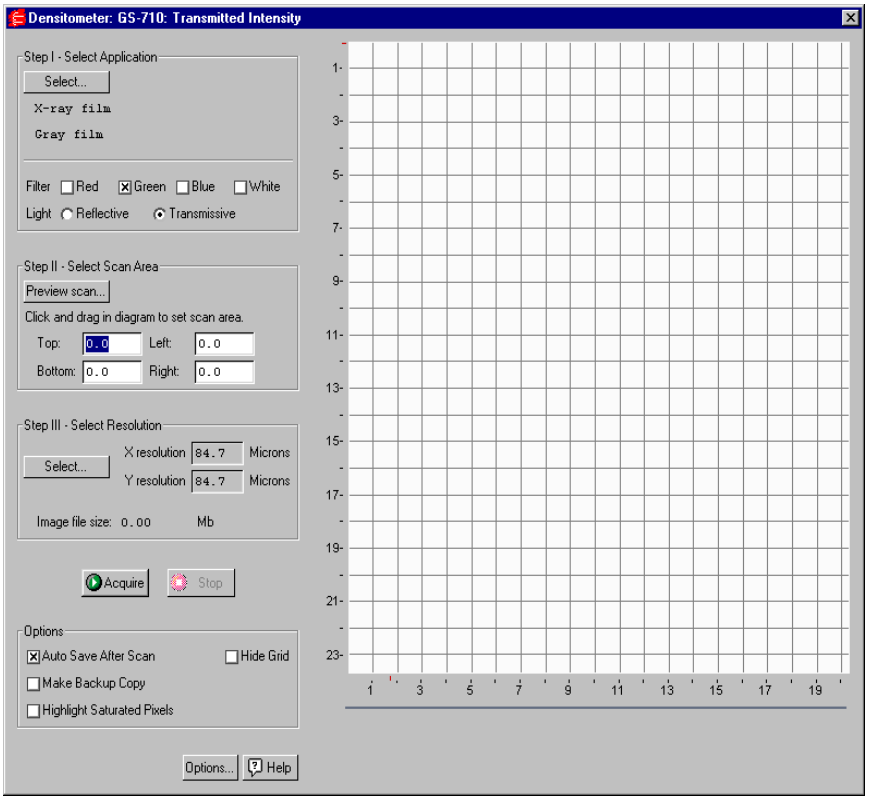


Fig. E-2. GS-710 acquisition window

The scanning window is marked by grid lines that divide the area into square centimeters. These are numbered 1–29 top to bottom and 1–21 left to right if the light source is reflective, and 1–23 top to bottom and 1–20 left to right if the light source is transmissive (see below).

To hide the gridlines, click on the Hide Grid checkbox under Options.

Below the main scanning window is the calibration strip window. Every time the densitometer calibrates, an image of the calibration strip will appear in this window.

The control panel has been arranged from top to bottom to guide you through the acquisition procedure. There are five basic steps to scanning an image using the GS-710:

1. Select the application.
2. Select the scan area.
3. Select the resolution of the scan.
4. Calibrate the instrument. (This is automatic, after you enter the step tablet values before you first scan after installation.)
5. Acquire the image.

E.2 Step I. Select Application

To set the parameters for your particular scan, you can:

1. Select from a list of possible applications, or
2. Choose your own filter and light source settings.

Selecting from the List of Applications

When you select from the list of applications, the software automatically sets the appropriate filter(s) and other parameters for that particular application.

To select from the list of applications, click on the Select button under Select Application.

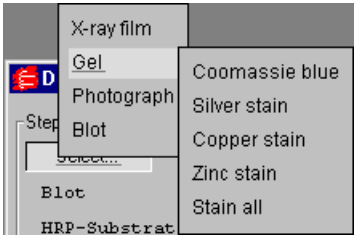


Fig. E-3. Example of the application tree in the GS-710 dialog box.

The applications are listed in a tree that expands from left to right. First you select the category of your application, then you select your particular application.

To exit the tree without selecting, press the ESC key.

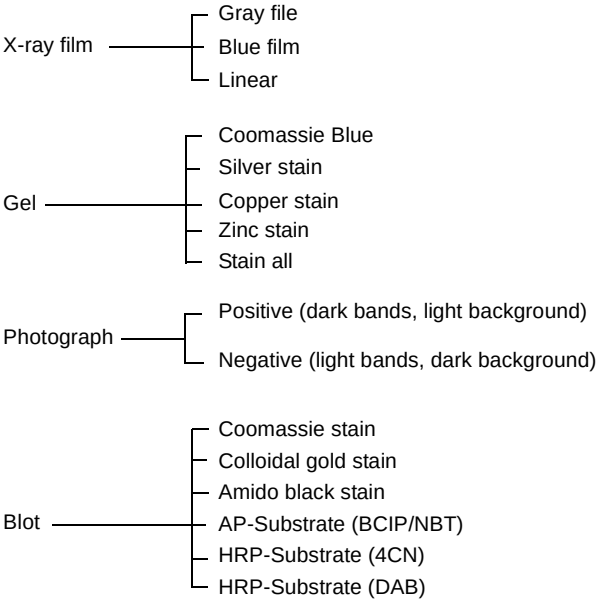


Fig. E-4. Applications available in the GS-710 acquisition window.

Choosing Your Own Settings

If you know the filter and light source settings you want, or want to experiment with different settings, you can choose them yourself.

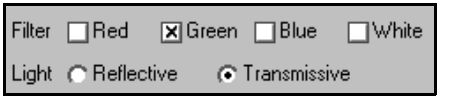


Fig. E-5. GS-710 Custom Application controls.

Next to Filter, click on either the Red, Green, Blue, or White checkbox, or a combination of two of the first three (Red-Green, Green-Blue, Red-Blue).

Filter Color	Wavelength	Application Examples
Red	595–750 nm	Coomassie G-250, BCIP/NBT, Fast Green FCF, Methylene Blue
Green	520–570 nm	Coomassie R-250, Basic Fuchsin
Blue	400–530 nm	Crocein Scarlet
Gray Scale	400–750 nm	Silver Stains, Copper Stains, Film

Fig. E-6. Examples of filter colors and applications.

Next to Light, select the appropriate light source.

- Reflective mode is for scanning opaque mediums such as dried gels on filter paper, arrays, or photographs. The scanning dimensions in this mode are 21 cm x 29 cm.
- Transmissive mode is for scanning transparent mediums such as films, gels, or slides. The scanning dimensions in this mode are 20 cm x 23 cm.

E.3 Step II. Select Scan Area

Preview Scan

Before selecting the particular area to scan, you can preview the entire scanning area to determine the exact position of your sample.

Click on Preview Scan. A quick, low-resolution scan of the entire scanning area will appear in the scanning window. Your sample should be visible within a portion of this scan.

Selecting an Area

Using the preview scan as a guide, select your scan area by dragging your mouse within the scanning window. The border of the scan area you are selecting will be marked by a frame.

Note: The scan area you select must be at least 1 cm wide.

When you release the mouse button, the border changes to a dashed blue line, indicating a selected area.

- To *reposition* the scanning area box you have created, 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.

You can also select the scanning area by entering coordinates in the appropriate fields (Top, Bottom, Left, Right). As you enter a coordinate, the position of the scanning area box will change accordingly.

When selecting, be sure to include the entire area of interest, and be generous with borders. You can always crop the image later.

E.4 Step III. Select Resolution

To select from a list of possible scanner resolutions, click on the Select button under Select Resolution. This will open the Select Scan Resolution dialog box.

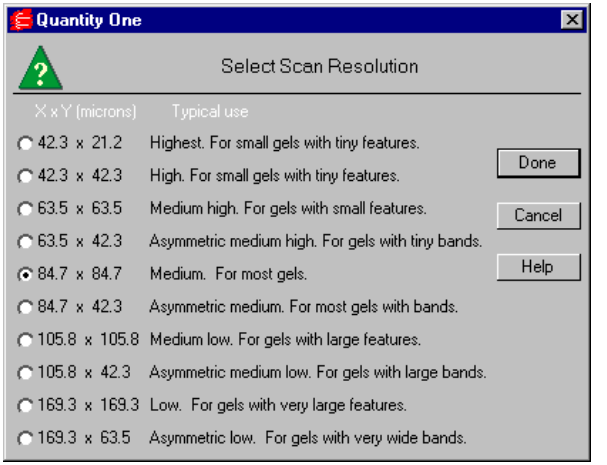


Fig. E-7. Select Scan Resolution dialog box.

Available resolutions are listed from highest to lowest in terms of the dimensions of the resulting pixels (in microns). Smaller pixels equal higher resolution. Each resolution is listed with its typical use.

In general, the size of your pixels should be one-tenth the height of your smallest object.

Some of the resolutions are asymmetrical, meaning that the resolution is higher in the vertical dimension (i.e., the pixels in the resulting image are larger in the horizontal dimension than in the vertical dimension). This is useful for gels with bands, where you are more interested in resolving in the y dimension to determine the size of bands and the spacing between them.

Specifying Your Own Resolution

If you select Oversample under More Options, you can specify your own resolution within the range of 43–169 microns (micrometers). With Oversample selected, enter values directly in the fields next to X resolution and Y resolution in the main acquisition window.

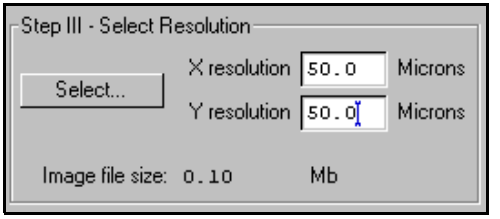


Fig. E-8. Entering a custom resolution (with Oversample selected).

Image File Size

Image File Size (under Select Resolution) shows the size of the scan file you are about to create. If you do not have enough computer memory for the specified file size, an error message will appear when you attempt to scan. If this happens, select a lower resolution or decrease the size of the area to be scanned. (Macintosh users can also increase the application memory partition. See your Macintosh computer documentation for guidance.)

E.5 Calibration

Images scanned using the GS-710 are automatically calibrated. The scanner plate has a built-in calibration overlay for both transmissive and reflective scanning.

- The transmissive calibration strip calibrates the densitometer from 0 to 3.0 O.D.
- The reflective calibration strip calibrates the densitometer to 2.0 O.D.

The first time you use the GS-710, you must select some calibration settings to ensure that your calibration is accurate.

To set the automatic calibration settings, click on the More Options button in the GS-710 acquisition window. This will open the Densitometer Options dialog box.

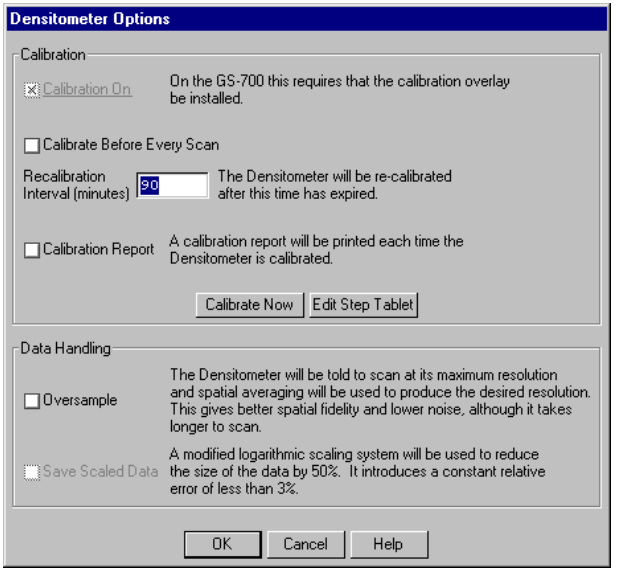


Fig. E-9. Densitometer Options dialog box.

E.5.a Editing the Step Tablet

Before you scan for the first time, you must enter the step tablet values for your transmissive calibration strip into the Step Tablet Values form.

Note: In reflective scanning, the scanner is calibrated against the reflective step tablet. However, you do not need to enter the values for this step tablet; you can use the default values in the Step Tablet Values form.

Attached to the outside of your GS-710, you will find a copy of the manufacturer's printout of the diffuse density values for each segment of your transmissive step tablet, as well as a serial number that identifies the step tablet to which those density values belong. Those exact density values must be entered into the computer for the software to associate a correct density value with each step on the step tablet.

Note: Scanning in transmissive mode with incorrect step tablet values entered into the computer can cause significant errors in the reported densities of your scans.

To enter the density values for your step tablet, click on the Edit Step Tablet button in the Densitometer Options box. The Step Tablet Values dialog box for the GS-710 will open.

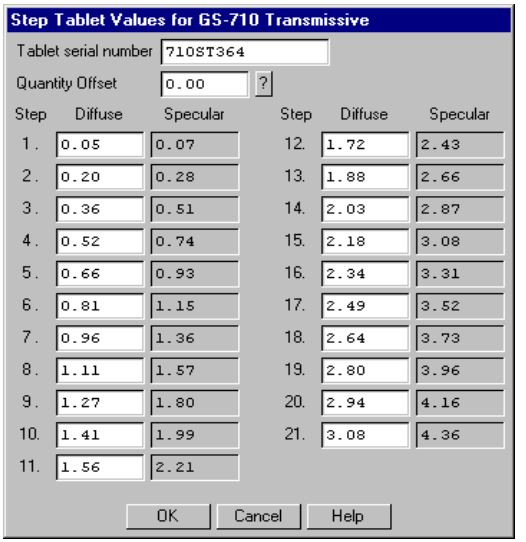


Fig. E-10. Step Tablet dialog box.

The dialog box will indicate whether the values are for the transmissive or reflective step tablet, depending on whether you are in transmissive or reflective mode.

If you are in reflective mode, you do not need to change the default step tablet values.

If you are in transmissive mode, you do need to enter the correct step tablet values that were shipped with your GS-710. You can type the serial number for these values into the Tablet Serial Number field.

The Quantity Offset field does not apply in the GS-710. This value should be set to zero.

Next, enter the values for the transmissive step tablet in the appropriate fields under the Diffuse column. After the step tablet is scanned, the software will associate each density value with its corresponding segment on the step tablet.

The step tablet density values do not need to be entered each time you calibrate. Once they have been entered and saved, they will be automatically recalled when the calibration strip is scanned.

When you are finished filling out the Step Tablet Values form, click on OK.

Diffuse Versus Specular O.D.

In the step tablet form, you enter O.D. as diffuse density, and then the software automatically calculates the specular density.

Specular density is a measure of the light that passes directly through a medium. Diffuse density includes light that is scattered as it passes through the medium. Step tablet values are given in diffuse density, but are measured by the scanner in specular density, and therefore must be converted according to the specular/diffuse O.D. ratio. This conversion does not affect quantitation.

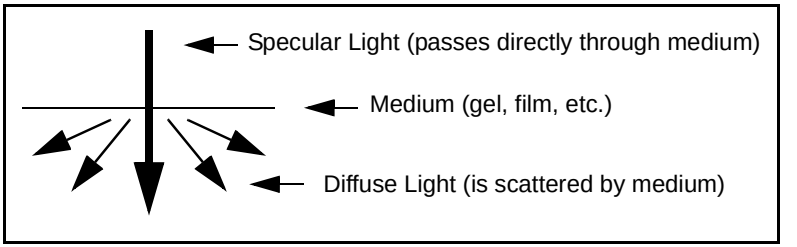


Fig. E-11. Specular and diffuse density

Diffuse density values are converted to specular optical density units according to the following formula:

$$Specular\ OD = 1.4 \cdot Diffuse\ OD$$

E.5.b Calibration Settings

After you have entered the step tablet values, you can immediately calibrate by clicking on the Calibrate Now button (in the Densitometer Options dialog box).

You can also specify how often you want the GS-710 to automatically recalibrate. Either click on the Calibrate Before Every Scan checkbox or enter a Recalibration Interval (in minutes) in the appropriate field.

Note: The scanner will automatically recalibrate each time you change your filter or your reflective/transmissive setting. (If you select a different application with the same filter and light settings, it will not auto recalibrate.)

Calibration Report

To print out a calibration report each time the densitometer calibrates, click on the Calibration Report checkbox.

E.6 Acquire the Image

Note: Before scanning in transmissive mode, make sure the white balance region of the scanning area is not covered or obstructed in any way.

To begin to scan, click on the Acquire button. The scanned image will begin to appear in the scanning window, line by line.

To interrupt a scan, click on the Stop button. A message will ask you to confirm the interrupt, and then you will be asked if you want to keep the partial scan. This feature is useful if you overestimated the size of the area you selected.

After the scan is complete, a window will open displaying the scan image, at which point you can analyze and save it.

Note: The image will open with a default file name that includes the date, time, and (if applicable) user name. However, unless you have selected Auto Save After Scan, the file will not be saved until you select Save or Save As from the File menu.

E.7 Other Options

Oversample

This feature allows you to scan at the maximum resolution of the GS-710 (42.3 x 42.3 microns) and then use spatial averaging to create an image with lower resolution. This can result in better images at lower resolution—however, it takes longer to scan.

To turn on oversampling, click on the More Options button in the acquisition window and click on the Oversample checkbox.

With oversampling on, you can specify your own resolution within the range of 43–169 microns by entering values directly in the fields next to X resolution and Y resolution in the main acquisition window.

Auto Save After Scan

To automatically save any scan you create, click on the Auto Save After Scan checkbox.

Note: In PDQuest, this option is preselected and cannot be turned off. All images must be automatically saved when acquired.

With this checkbox selected, when you click on Acquire, a Save As dialog box will open asking you to specify a file name and location for the image you are about to create. The scan will begin when you click on the Save button.

Make Backup Copy

If you have checked Auto Save After Scan, you can also automatically create a backup copy of any scan you create.

Click on the Make Backup Copy checkbox. With this checkbox selected, when a scan is created and saved, a backup copy will be placed in the same directory as the scanned image. Windows backup files will have an ".sbk" extension. Macintosh backup files will have the word "backup" after the file name.

This backup copy will be read-only, which means that you cannot make changes to it. You can open it like a normal file, but you must save it under a different file name before editing the image or performing analysis.

Highlight Saturated Pixels

When this box is checked, any saturated pixels in the image will appear highlighted in red in the scan window and in the pop-up image window. To view/hide saturated pixels in the pop-up image window, use the Image > Transform command.

Hide Grid

To hide the gridlines in the scanning area window, click on the Hide Grid checkbox.

Appendix F
GS-800 Imaging Densitometer

Fig.F-1. GS-800 Imaging Densitometer

Before you can begin scanning images with the GS-800 Imaging Densitometer®, your instrument must be properly installed and connected with the host computer. See the hardware manual for installation, startup, and operating instructions.

PC Only: A Note About SCSI Cards

The GS-800 is connected to your computer by a Small Computer System Interface (SCSI) cable. To use the GS-800, you must have a SCSI card installed in your PC. If

you have a PC with a Windows 98 or Windows ME operating system, you may also need to load the SCSI and WinASPI drivers that came with the card.

Simulation Mode

Any of the imaging device acquisition windows can be opened in "simulation mode." In this mode, an acquisition window will open and the controls will appear active, but instead of capturing real images, the window will create "dummy" images of manufactured data.

You do not need to be connected to an imaging device to open a simulated acquisition window. This is useful for demonstration purposes or practice scans.

To enter simulation mode, hold down the Ctrl key and select the name of the device from the File menu. The title of the GS-800 acquisition window will indicate that it is simulated.

Note: There is no simulated calibration for densitometers.

F.1 GS-800 Acquisition Window

To begin acquiring images, go to the File menu and select GS-800. The acquisition window for the densitometer will open, displaying a control panel and a scanning window.

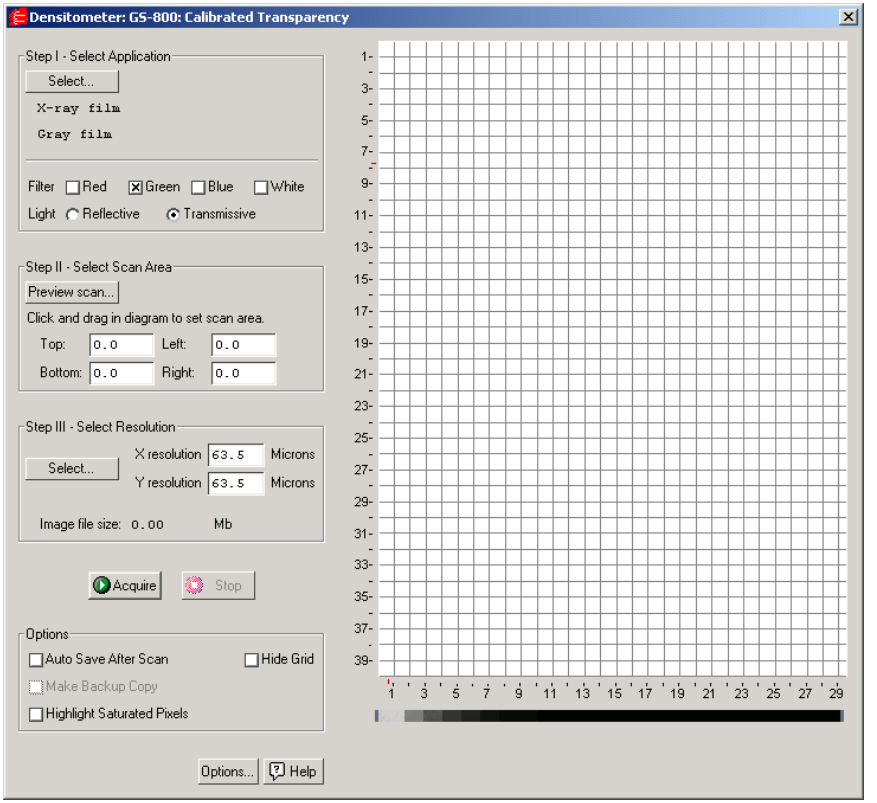


Fig. F-2. GS-800 acquisition window

The scanning window is marked by grid lines that divide the area into square centimeters. These are numbered 1-40 top to bottom and 1-30 left to right if the light source is reflective, and 1-40 top to bottom and 1-29 left to right if the light source is transmissive.

To hide the gridlines, select the Hide Grid checkbox under Options.

Below the main scanning window is the calibration strip window. Every time the densitometer calibrates, an image of the calibration strip will appear in this window.

The control panel has been arranged from top to bottom to guide you through the acquisition procedure. There are five basic steps to scanning an image using the GS-800:

1. Select the application.
2. Select the scan area.
3. Select the resolution of the scan.
4. Calibrate the instrument. (This is automatic, after you enter the step tablet values before you first scan after installation.)
5. Acquire the image.

F.2 Step I. Select Application

To set the parameters for your particular scan, you can:

1. Select from a list of possible applications, or
2. Choose your own filter and light source settings.

Selecting from the List of Applications

When you select from the list of applications, the software automatically sets the appropriate filter(s) and other parameters for that particular application.

To select from the list of applications, click on the **Select** button under *Step I. Select Application*.

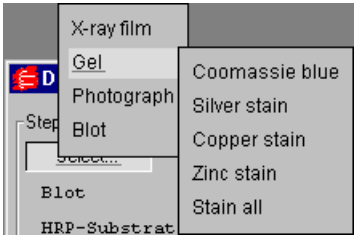


Fig. F-3. Example of the application tree in the GS-800 dialog box.

The applications are listed in a tree that expands from left to right. First you select the category of your application, then you select your particular application.

To exit the tree without selecting, press the **Esc** key.

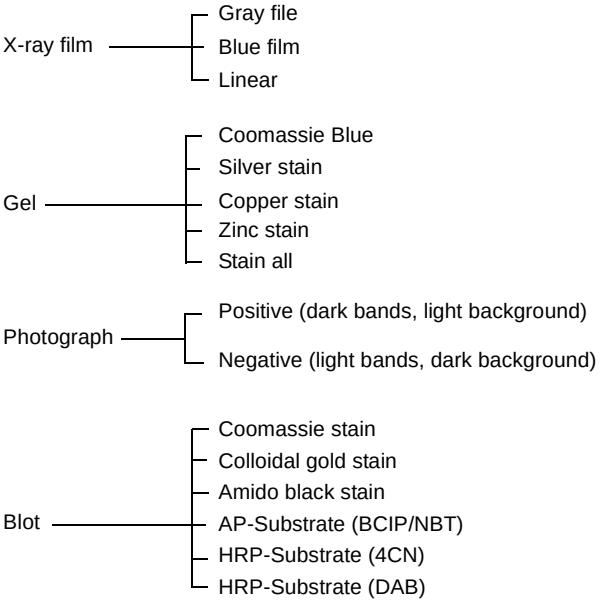


Fig. F-4. Applications available in the GS-800 acquisition window.

Choosing Your Own Settings

If you know the filter and light source settings you want, or want to experiment with different settings, you can choose them yourself.

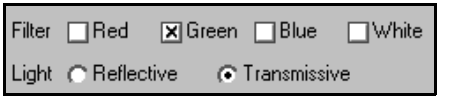


Fig. F-5. GS-800 Custom Application controls.

Next to *Filter*, click on either the **Red**, **Green**, **Blue**, or **White** checkbox, or a combination of two of the first three (**Red-Green**, **Green-Blue**, **Red-Blue**).

Filter Color	Wavelength	Application Examples
Red	595–750 nm	Coomassie G-250, BCIP/NBT, Fast Green FCF, Methylene Blue
Green	520–570 nm	Coomassie R-250, Basic Fuchsin
Blue	400–530 nm	Crocein Scarlet
White	400–750 nm	Silver Stains, Copper Stains, Film

Fig. F-6. Examples of filter colors and applications.

Next to *Light*, select the appropriate light source.

- Select **Reflective** for scanning opaque mediums such as dried gels on filter paper, arrays, or photographs. The scanning dimensions in this mode are 30 cm x 40 cm.
- Select **Transmissive** for scanning transparent mediums such as films, gels, or slides. The scanning dimensions in this mode are 29 cm x 40 cm.

F.3 Step II. Select Scan Area

Preview Scan

Before selecting the particular area to scan, you can preview the entire scanning area to determine the exact position of your sample.

Click on Preview Scan. A quick, low-resolution scan of the entire scanning area will appear in the scanning window. Your sample should be visible within a portion of this scan.

Selecting an Area

Using the preview scan as a guide, select your scan area by dragging your mouse within the scanning window. The border of the scan area you are selecting will be marked by a frame.

Note: The scan area you select must be at least 1 cm wide.

When you release the mouse button, the border changes to a dashed blue line, indicating a selected area.

- To reposition the scanning area box you have created, 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.

You can also select the scanning area by entering coordinates in the appropriate fields (Top, Bottom, Left, Right). As you enter a coordinate, the position of the scanning area box will change accordingly.

When selecting, be sure to include the entire area of interest, and be generous with borders. You can always crop the image later.

F.4 Step III. Select Resolution

To select from a list of possible scanning resolutions, click on the Select button under Step III. Select Resolution. This will open the Select Scan Resolution dialog box.

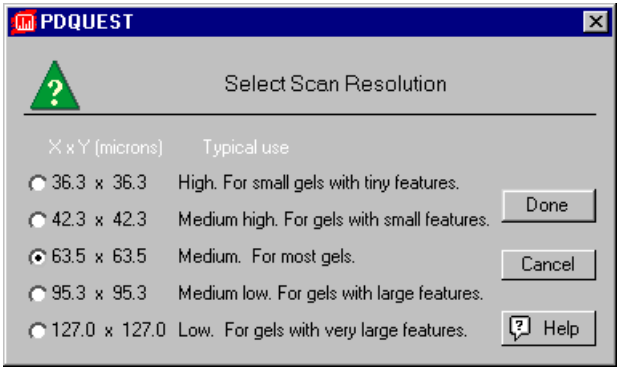


Fig. F-7. Select Scan Resolution dialog box.

Available resolutions are listed from highest to lowest in terms of the dimensions of the resulting pixels (in microns). Smaller pixels equal higher resolution. Each resolution is listed with its typical use.

In general, the size of your pixels should be one-tenth the height of your smallest object.

Specifying Your Own Resolution

If you select Oversample in the Options dialog box, you can specify your own resolution within the range of 32–169 micrometers. With Oversample selected, enter values directly in the fields next to X resolution and Y resolution in the main acquisition window.

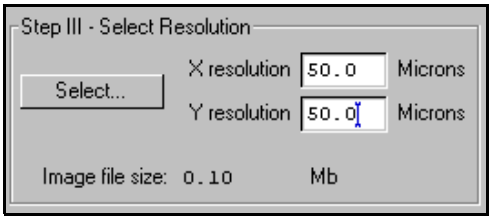


Fig. F-8. Entering a custom resolution (with Oversample selected).

Image File Size

The size of the scan file for the selected resolution is listed next to Image File Size. If you do not have enough computer memory for the specified file size, an error message will appear when you attempt to scan. If this happens, select a lower resolution or decrease the size of the area to be scanned. (Macintosh users can also increase the application memory partition. See your Macintosh computer documentation for guidance.)

F.5 Calibration

The GS-800 automatically calibrates the densities of scanned images to ensure accuracy and reproducibility of results. The GS-800 has built-in step tablets for both transmissive and reflective scanning.

- The transmissive step tablet calibrates transmissive scans from 0 to 3.0 O.D.
- The reflective step tablet targets reflective scans from 0 to 2.0 O.D.

The first time you use the GS-800, you must select some settings to ensure accurate calibration. Click on the Options button in the main acquisition window. This will open the Densitometer Options dialog box.

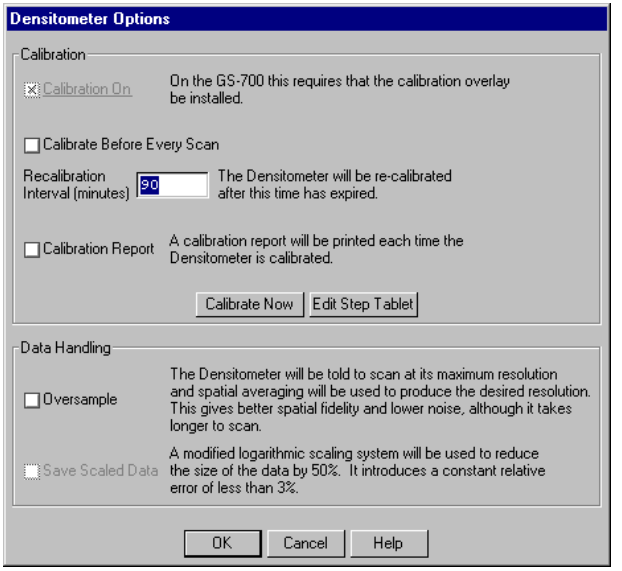


Fig. F-9. Densitometer Options dialog box.

Note that calibration is always on for the GS-800.

F.5.a Step Tablet Values

The built-in transmissive and reflective step tablets on your densitometer have specific density values. Before scanning for the first time, you must enter the values for your transmissive step tablet into the software. You can use the default values in the software for the reflective step tablet.

Transmissive Step Tablet

Before you scan in transmissive mode for the first time, you must specify the values for your transmissive step tablet.

First, make sure that the Transmissive checkbox is selected in the main acquisition window, then click on the Options button. In the Densitometer Options dialog, click on the Edit Step Tablet button. The Step Tablet Values for GS-800 Transmissive dialog box will open.

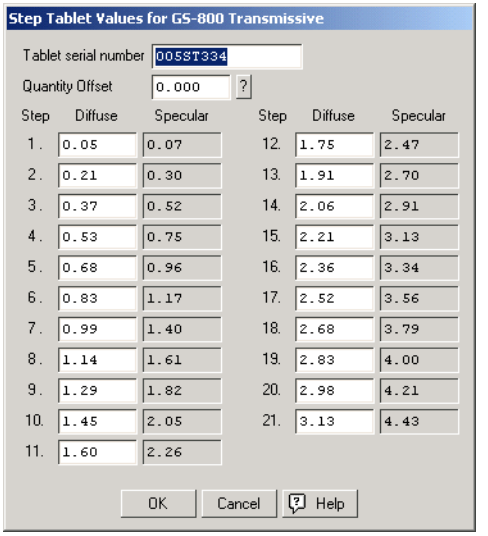


Fig. F-10. Step Tablet dialog box.

Attached to the outside of your GS-800, you will find a copy of the manufacturer's printout of the diffuse density values for each segment of the transmissive step tablet, as well as a serial number for the tablet. These exact density values must be entered into the software to associate a correct density value with each step on the step tablet.

Note: Scanning in transmissive mode with incorrect step tablet values can cause significant errors in the reported densities of your scans.

First, type the serial number for the tablet into the Tablet Serial Number field.

The Quantity Offset field does not apply in the GS-800 in transmissive mode. This value should remain at zero.

Next, enter the step tablet values into the appropriate fields under the Diffuse column. After the step tablet is scanned, the software will associate each density value with its corresponding segment on the step tablet. The density values do not need to be reentered each time you calibrate.

When you are finished entering the transmissive step tablet values, click on OK.

Reflective Step Tablet

For the reflective step tablet, it is recommended that you use the default target values in the software. Although you can edit these values based on your own measurements and testing, the default values have been preset based on careful testing and review.

To view or edit the default reflective values, make sure that the **Reflective** checkbox is selected in the main acquisition window, then click on the **Options** button. In the ***Densitometer Options*** dialog, click on the **Edit Step Tablet** button. The ***Step Tablet Values for GS-800 Reflective*** dialog box will open.

When you are finished viewing/editing the reflective step tablet values, click on **OK**.

Diffuse Versus Specular O.D.

In the step tablet form, you enter O.D. as diffuse density, and then the software automatically calculates the specular density.

Specular density is a measure of the light that passes directly through a medium. Diffuse density includes light that is scattered as it passes through the medium. Step tablet values are given in diffuse density, but are measured by the scanner in specular density, and therefore must be converted according to the specular/diffuse O.D. ratio. This conversion does not affect quantitation.

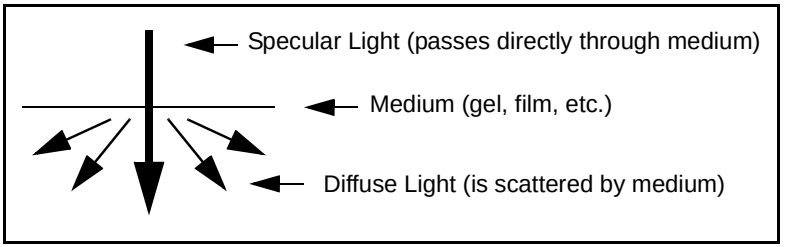


Fig. F-11. Specular and diffuse density

Diffuse density values are converted to specular optical density units according to the following formula:

$$Specular\ OD = 1.4 \cdot Diffuse\ OD$$

F.5.b Other Calibration Settings

After you have entered the step tablet values, you can immediately calibrate by clicking on the **Calibrate Now** button (in the ***Densitometer Options*** dialog box).

You can also specify how often you want the GS-800 to automatically recalibrate. Either click on the **Calibrate Before Every Scan** checkbox or specify a period between automatic recalibrations (in minutes) in the *Recalibration Interval* field.

Note: The scanner will automatically recalibrate each time you change your filter or your reflective/transmissive setting. (If you select a different application with the same filter and light settings, it will not auto recalibrate.)

To print out a calibration report each time the densitometer calibrates, click on the **Calibration Report** checkbox.

F.6 Acquire the Image

Note: Before scanning in transmissive mode, make sure the white balance region of the scanning area is not covered or obstructed in any way.

To begin to scan, click on the **Acquire** button. The scanned image will begin to appear in the scanning window, line by line.

To interrupt a scan, click on the **Stop** button. A message will ask you to confirm the interrupt, and then you will be asked if you want to keep the partial scan. This feature is useful if you overestimated the size of the area you selected.

After the scan is complete, a window will open displaying the scan image, at which point you can analyze and save it.

Note: The image will open with a default file name that includes the date, time, and (if applicable) user name. However, unless you have selected **Auto Save After Scan**, the file will not be saved until you perform a **Save** or **Save As** operation.

F.7 Other Options

Oversample

This feature allows you to scan at the maximum resolution of the scanner and then use spatial averaging to create an image with lower resolution. This can result in better images at lower resolution—however, it takes longer to scan.

To turn on oversampling, click on the **Options** button in the acquisition window and select the **Oversample** checkbox in the options dialog.

With oversampling on, you can specify your own resolution within the range of the densitometer by entering values directly in the fields next to X resolution and Y resolution in the main acquisition window.

Auto Save After Scan

To automatically save any scan you create, select the **Auto Save After Scan** checkbox.

Note: In PDQuest, this option is preselected and cannot be turned off. All images must be automatically saved when acquired.

With this checkbox selected, when you click on **Acquire**, a ***Save As*** dialog box will open asking you to specify a file name and location for the image you are about to create. The scan will begin when you click on the **Save** button.

Make Backup Copy

If you have selected **Auto Save After Scan**, you can also automatically create a backup copy of any scan you create.

Click on the **Make Backup Copy** checkbox. With this checkbox selected, when a scan is created and saved, a backup copy will be placed in the same directory as the scanned image. Windows backup files will have an "***.sbk**" extension. Macintosh backup files will have the word "**backup**" after the file name.

This backup copy will be read-only, which means that you cannot make changes to it. You can open it like a normal file, but you must save it under a different file name before editing the image or performing analysis.

Highlight Saturated Pixels

When this box is checked, any saturated pixels in the image will appear highlighted in red in the scan window and in the pop-up image window. To view/hide saturated pixels in the pop-up image window, use the **Transform** command on the main toolbar or *Image* menu.

Hide Grid

To hide the gridlines in the scanning area window, click on the **Hide Grid** checkbox.

Appendix G
Fluor-S MultiImager

Fig.G-1. Fluor-S MultiImager.

Before you can begin acquiring images using the Fluor-S® MultiImager, the imaging system must be properly installed and connected with the host computer. See the Fluor-S hardware manual for installation, startup, and operating instructions.

Note: Make sure that the temperature light on the Fluor-S is green before attempting to capture an image. If you are using a PC, the Fluor-S should be turned on and the initialization sequence completed *before* the host computer is turned on. See the hardware manual for more details.

PC Only: A Note About SCSI Cards

The Fluor-S is connected to your computer by a Small Computer System Interface (SCSI) cable. To use the Fluor-S, you must have a SCSI card installed in your PC. If you have a PC with a Windows 98 or Windows ME operating system, you may also need to load the SCSI and WinASPI drivers that came with the card.

Simulation Mode

Any of the imaging device acquisition windows can be opened in "simulation mode." In this mode, an acquisition window will open and the controls will appear active, but instead of capturing real images, the window will create "dummy" images of manufactured data.

You do not need to be connected to an imaging device to open a simulated acquisition window. This is useful for demonstration purposes or practice scans.

To enter simulation mode, hold down the CTRL key and select the name of the device from the File menu. The title of the acquisition window will indicate that it is simulated.

G.1 Fluor-S Acquisition Window

To acquire images using the Fluor-S, go to the File menu and select Fluor-S.... The acquisition window for the imager will open, displaying a control panel and an image display window.

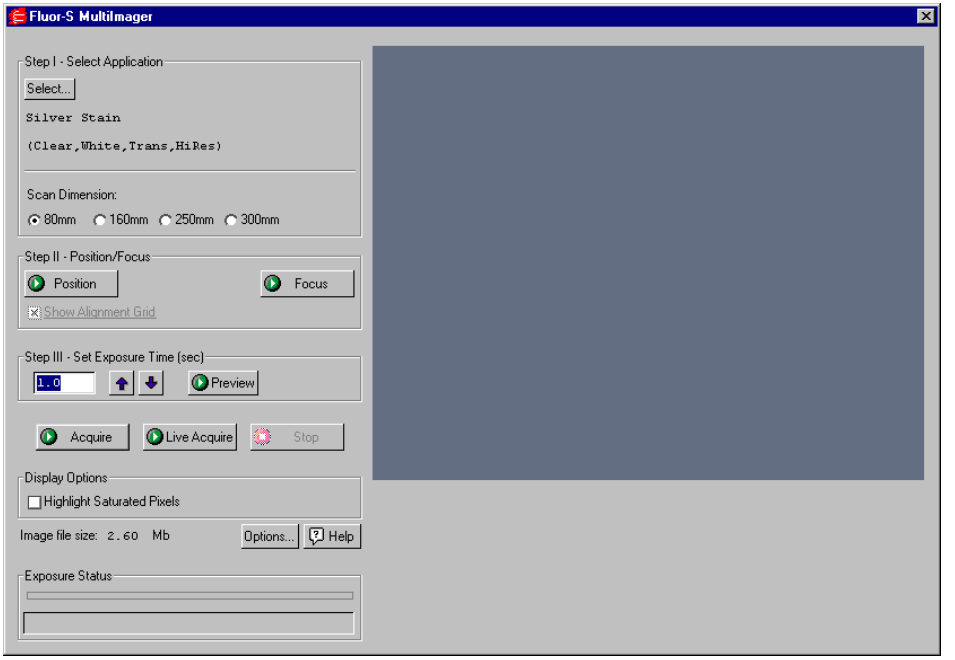


Fig.G-2. Fluor-S acquisition window

When the Fluor-S window first opens, no image will be displayed.

The control panel has been arranged from top to bottom to guide you through the acquisition procedure. There are four basic steps to acquiring an image using the Fluor-S:

1. Select the application.
2. Position and focus the gel or other object to be imaged.
3. Set the exposure time.
4. Acquire the image.

G.2 Step I. Select Application

To set the appropriate filter and other parameters for the type of object you are imaging, click on the Select button under Select Application. The available applications and their associated settings are listed in a tree that expands from left to right.

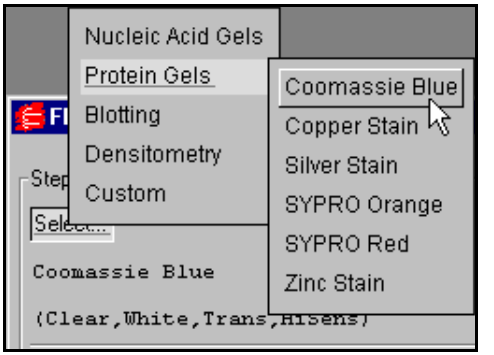


Fig.G-3. The application tree in the Fluor-S acquisition window.

First select your general application, then select the particular stain or medium you are using. If you select the chemiluminescent application under “Blotting,” you also have the option of selecting High Resolution or High Sensitivity (see below).

When you select an application, the software automatically sets the appropriate standard filter (520LP, 530DF60, 610LP, clear, or none), light type (UV, white, or none), and light source (trans, epi, or neither) in the Fluor-S for that particular application.

For applications involving trans illumination, you must also specify a scan dimension (see below).

Your selection will be displayed below the Select button. To exit the tree without selecting, press the ESC key.

Custom Applications

If your application is not listed, if you want to use a user-installed filter, or if you want to access High Sensitivity mode (see below), you can create and save your own custom application.

From the application tree, select Custom, then Create. This will open a dialog box in which you can name your application and select your settings.

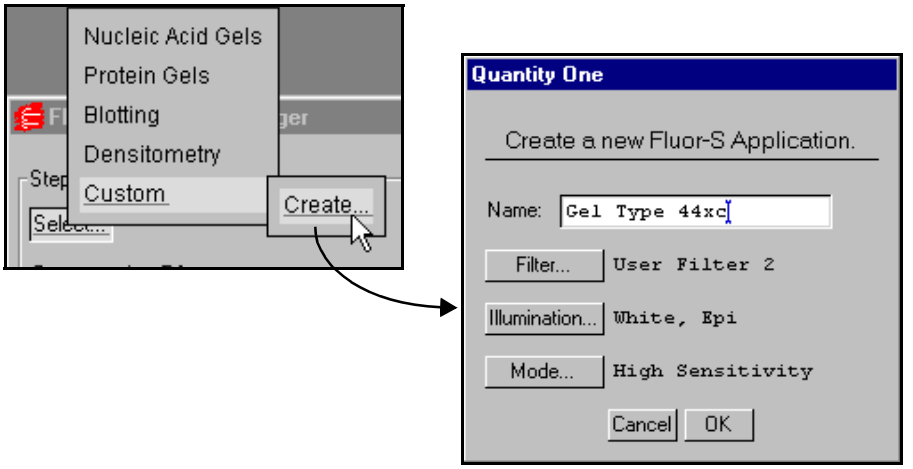


Fig.G-4. Creating a new custom application.

To select the filter (including user-defined), type of illumination, and camera mode, click on the appropriate buttons and select from the pop-up lists.

Note: Under Illumination, there is a listing for a spare UV light source. This selects the spare UV bulb in the Fluor-S. Select this light source if your main UV bulb fails.

Enter a name for your application in the Name field. 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.

Scan Dimension

If an application uses trans illumination, the Scan Dimension buttons become active. The scan dimension is the distance traveled by the transilluminating light source as it scans horizontally across the platen.

Fig.G-5. Scan dimension settings for trans illumination.

The full scanning range is 300 mm. Select a smaller range if your sample is small and you do not want to wait while the light source travels over the maximum scan width.

High Resolution Versus High Sensitivity

High Resolution and High Sensitivity are mutually exclusive options. High Resolution is the normal operating mode for the Fluor-S. High Sensitivity provides optimal sensitivity for low-light applications. It is the default selection for the chemiluminescence application, and may be selected for a custom application.

In High Resolution mode, images are captured at the maximum resolution of the camera.

In High Sensitivity mode, the pixels in the camera are “binned” (i.e., four pixels are combined into one) to increase the amount of signal per pixel. However, combining the pixels results in a reduction in the resolution of the image.

G.3 Step II. Position/Focus

Note: When you click on the Position or Focus button, the light inside the Fluor-S box automatically turns on. To turn the light off while positioning or focusing, hold down the SHIFT key when clicking on the button.

Position

After you have selected your application, you are ready to center your gel or other object within the camera frame. To do so, click on the Position button. The Fluor-S will begin capturing a “live” image and updating it every second.

With the Position button selected, look at the image in the acquisition window while you position your object in the center of the platen. If you have a zoom lens on the camera, you can adjust the magnification while you position.

You can select the Show Alignment Grid checkbox to facilitate positioning.

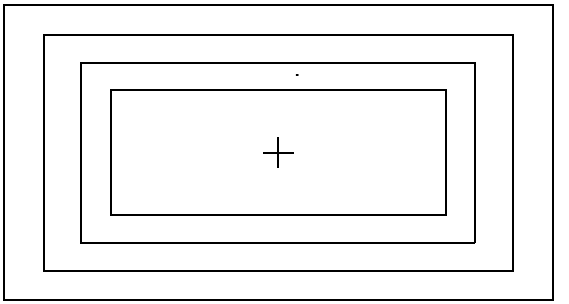


Fig.G-6. Fluor-S alignment grid.

When you are finished positioning, click on the Stop button.

Focus

Note: Before focusing, you should adjust the f-stop on the camera to the lowest setting (i.e., the maximum aperture). This reduces the depth of field, allowing you to more accurately focus the camera. Then, after focusing, increase the f-stop to the desired setting. (See the following table on recommended f-stops.)

After you have positioned your sample, click on the Focus button and look at the image in the acquisition window while aligning the two focusing arrows on the camera lens. While focusing, the camera will limit its focus to a small portion of the sample (this will not affect any zoom lens adjustments you may have made.)

When you are finished focusing, click on the Stop button.

G.4 Step III. Set Exposure Time

When you are ready to capture an image, you will need to select an exposure time. “Exposure” refers to the integration of image captures on the CCD over a set period of time. The effect is analogous to exposing photographic film to light.

The exposure time you select should be based on your application and your “best guess” as to what exposure will give you the best image.

Note: The minimum exposure time in trans illuminated mode is 1 second. The minimum exposure time in epi illuminated mode is 0.1 second.

You can enter an exposure time (in seconds) directly in the field, or use the Arrow buttons to adjust the exposure time in 10 percent increments.

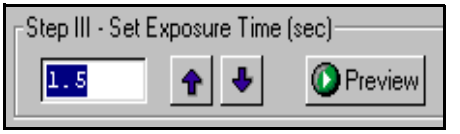


Fig.G-7. Selecting an exposure time.

The following table provides recommended exposure times for various applications.

Recommended Exposure Times and Lenses

Sample	Recommended Exposure[1]	Lens & Filter[2]	Accessories Used
Fluorescent Stain Gel	3–40 sec.	Zoom/IR	None
Fluorescence End-Label Gel	30 sec.–3 min.	Zoom/IR	None
Fluorescent Blot	0.5–5 sec.	Zoom/IR	None
Chemifluorescent Blot	0.5–5 sec.	Zoom/IR	None
Colorimetric Gel	1–10 sec.	Zoom/IR	White Diffusion Plate
Colorimetric Blot	0.5–20 sec.	Zoom/IR	None
X-ray film	1–10 sec.	Zoom/IR	White Diffusion Plate
Weak Chemiluminescence[3]	5–20 min.	50 mm	Chemi Tray (if sample is small)
Strong Chemiluminescence[3]	30 sec.–2 min.	50 mm	Chemi Tray (if sample is small)

[1]Increase exposure time two fold for each step increase in f-stop.

[2]For sharper focusing, close the f-stop down 1–2 stops from full open while focusing.

[3]For chemi applications, the 50mm lens is recommended. Always remove the 660 filter.

For most applications, you can select an exposure time, capture an image, study it, then adjust the exposure time accordingly. Repeat this procedure as many times as necessary to obtain a good image.

For chemiluminescent samples, which degrade over time and emit low levels of light, you can select a high exposure time initially and use Live Acquire mode to save intermediate exposures (see following section).

Preview

For shorter exposures, you can use Preview to test different exposure times. Click on the Preview button create a preview exposure and display it in the acquisition window.

A preview scan takes only half as long to create as a real scan, because the preview scan does not capture a “dark” image (see the following section on Options). The progress of the exposure will be displayed in the Exposure Status bar at the bottom of the dialog box.

You cannot save preview scans.

If you want to stop a preview scan that is in progress, click on the Stop button.

G.5 Acquire the Image

The Fluor-S gives you the option of simply acquiring and displaying a fully exposed image, or preserving intermediate exposures.

Acquire

To acquire and display a fully exposed image, click on the Acquire button. An exposure will be taken based on the time selected in Step III. This is appropriate for most short exposures.

The progress of the exposure will be displayed in the Exposure Status bar at the bottom of the acquisition window.

Fig.G-8. Exposure Status bar when acquiring an image.

Depending on which dark subtraction type you have selected, a dark count may be acquired immediately following image acquisition. See Dark Subtraction Type under Options, below.

If you want to stop a scan that is in progress, click on the Stop button. The acquisition will be terminated.

After an image has been acquired, a separate window will pop up containing the new image. The window will have a default file name that includes the date, time, and user (if known). To save the image, select Save or Save As from the File menu.

You can then analyze the image using the analysis functions.

Live Acquire

Live Acquire allows you to view and preserve intermediate exposures leading up to a full exposure. This is useful for longer exposure times, such as those required by chemiluminescent samples, where there is the potential for image saturation.

When you click on the Live Acquire button, the exposure time you selected is divided by the number of exposure counts set in the Fluor-S Options dialog box (see Options, below). For example, if you enter an exposure time of 10 minutes and an exposure count of 20, then 20 intermediate exposures will be produced at 30-second intervals. Each intermediate exposure will be displayed in the scan window. The final, full exposure will be displayed in a separate image window.

Note: The first intermediate exposure will take longer than 30 seconds to display if dark subtraction is performed.

You can automatically save your intermediate exposures as separate files for later review using the Auto Save After Scan option. See Options, below.

If you see an intermediate exposure that you like in the scan window, click on the Stop button. Live Acquire mode will end and the last intermediate exposure to be completed will open in a separate image window. You can then save it for analysis.

G.6 Options

Click on the Options button to open the Fluor-S Options dialog box.

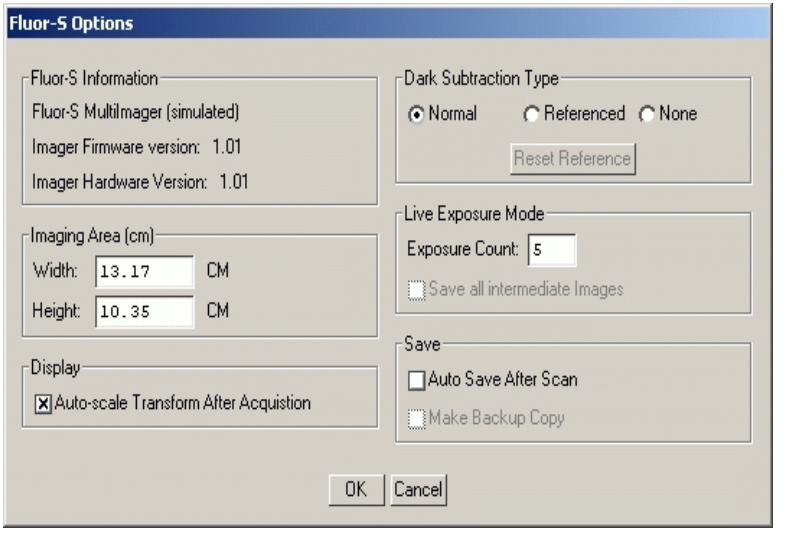


Fig.G-9. Options dialog box.

G.6.a Dark Subtraction Type

All CCD cameras accumulate electrons that produce a signal that is indistinguishable from light. This “dark count” adds to the noise in your images. In most cases, you will want to subtract this dark count from your images.

Normal

The Normal option button selects the default dark subtraction type. In this mode, after you acquire an image, a “dark” image of the same exposure length will be taken, and this will be subtracted from your image.

The progress of the dark exposure will be displayed in the Exposure Status bar following the regular image exposure.

Note: In Normal mode, a dark image is only acquired the first time you perform a scan with particular application and exposure settings. If you perform subsequent scans with the same settings, no dark exposure will be taken.

Referenced

If you do not want to perform a dark exposure with each acquisition, you can take a "reference" dark exposure that will be saved and subtracted from all subsequent acquisitions. Click on the Referenced button to activate this feature.

The first time you acquire an image after selecting this option, the Fluor-S will take a 60-second dark exposure that will be saved and used to subtract the dark count from all subsequent acquisitions.

For image exposures of greater or less than 60 seconds, the reference dark will be scaled accordingly and then subtracted. You can change the default reference dark exposure time using the Reset Reference button (see below).

If you deselect the Referenced button and then reselect it, the old reference dark exposure will still be available.

Note: Separate reference dark exposures will be taken for High Resolution mode and High Sensitivity mode. Once you have created a reference dark in each of these modes, each reference dark will be used according to the mode you are in.

Reset Reference

If you would like a reference dark with an exposure time that more closely matches that of your typical scans, click on the Reset Reference button.

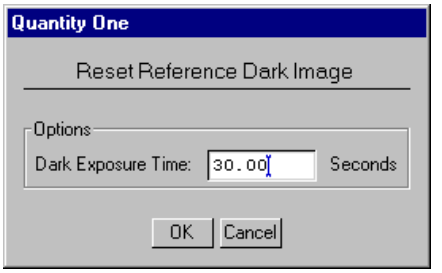


Fig.G-10. Reset Reference Dark pop-up box.

A pop-up box will prompt you to enter a new reference dark exposure time in seconds. Click on OK to implement your change. The new reference dark will be created when you acquire your next image.

Note: Because of the high sensitivity of the CCD, fluctuations in background radiation and/or temperature in the room can affect the level of dark count. If you feel that radiation/temperature conditions have changed in the room since your last reference dark was created, use the Reset Reference button to delete your old reference and create a new one under current conditions.

None

If you do not want to perform dark subtraction, select None. No dark exposure will be acquired or subtracted.

G.6.b Live Exposure Mode

Exposure Count

If you are using the Live Acquire function (see previous section), you need to specify how many intermediate exposures you want to view/save during acquisition. Enter this number in the Exposure Count field.

The total exposure time will be divided by the number you enter in the Exposure Count field. If you enter an exposure time of 10 minutes and a count of 10, you will create 10 intermediate exposures at 1 minute intervals.

Note: Do not enter a count that will result in an intermediate exposure time that is less than the minimum exposure time for the mode you are in. The minimum exposure time in trans illuminated mode is 1 second, and the minimum exposure time in epi illuminated mode is 0.1 second. (Example: For a trans illuminated application, an exposure time of 20 seconds and an exposure count of 21 would result in an error.)

Save All Intermediate Images

If Auto Save After Scan is selected (see following section), the Save All Intermediate Images checkbox will become active. If you select this checkbox, all your intermediate exposures will be saved as separate files. These files will have the same

root name appended by a number indicating the exposure sequence. The final, full exposure will have the root name only, with no exposure number.

G.6.c Save

Auto Save After Scan

To automatically save any image you create, click on the Auto Save After Scan checkbox.

Note: In PDQuest, this option is preselected and cannot be turned off. All images must be automatically saved when acquired.

With this checkbox selected, when you click on Acquire or Live Acquire, a Save As dialog box will open asking you to specify a file name and location for the image you are about to create. The scan will begin when you click on the Save button.

Note that in Live Acquire mode you can save your intermediate exposures by selecting Auto Save After Scan and then Save All Intermediate Images.

Make Backup Copy

You can automatically create a backup copy of any scan you create. To do so, first select Auto Save After Scan (see above), then select the Make Backup Copy checkbox.

With this checkbox selected, when you save a scan, a backup copy will be placed in the same directory as the scanned image. Windows backup files will have an ".sbk" extension. Macintosh backup files will have the word "backup" after the file name.

This backup copy will be read-only, which means that you cannot make changes to it. You can open it like a normal file, but you must save it under a different file name before editing the image or performing analysis.

G.6.d Imaging Area Size

The imaging area is the area of the sample that is captured by the camera and displayed in the scan window. To specify the size of this area, enter a dimension in the appropriate field under Imaging Area.

When you change one imaging area dimension, the other will change to maintain the aspect ratio of the camera lens.

The imaging area will change depending on your zoom factor. For example, if you have zoomed in on a area that is 4.5 x 3.5 cm, then you would enter 4.5 for the width (3.5 for the height would be calculated automatically).

Note: Your imaging area settings must be correct if you want to do 1:1 printing. They must also be correct if you want to compare the quantities of objects (e.g., using the Volume Tools) in different images.

The imaging area dimensions also determine the size of the pixels in your image (i.e., resolution). A smaller imaging area will result in a higher resolution.

G.6.e Auto-scale Transform

Auto-scale Transform after Acquisition allows the user the option of having the image automatically perform the Auto-Scale transform function upon completion of image acquisition. This eliminates the need to transform an image or re-scan an image when the acquisition time was too short or the iris not opened enough.

To enable the auto-scale transform function, check the box labeled, Auto-Scale Transform after Acquisition in the Options dialog.

G.7 Other Features

Fig.G-11. Other Fluor-S acquisition window features.

Highlight Saturated Pixels

When this box is checked, any saturated pixels in the image will appear highlighted in red in the scan window and in the pop-up image window. To view/hide saturated pixels in the pop-up image window, use the Image > Transform command.

File Size of Images

Image File Size shows the size of the image file you are about to create. This size is determined by whether the image was created in High Resolution or High Sensitivity mode.

If you do not have enough computer memory for the specified file size, an error message will appear when you attempt to acquire an image. (Macintosh users can increase the application memory partition. See your Macintosh computer documentation for guidance.)

Appendix H
Fluor-S MAX MultiImager

Fig.H-1. Fluor-S MAX MultiImager.

Before you can begin acquiring images using the Fluor-S® MAX MultiImager, the imaging system must be properly installed and connected with the host computer. See the Fluor-S MAX hardware manual for installation, startup, and operating instructions.

Note: Make sure that the temperature light on the Fluor-S MAX is green before attempting to capture an image. If you are using a PC, the Fluor-S MAX should be turned on and the initialization sequence completed *before* the host computer is turned on. See the hardware manual for more details.

PC Only: A Note About SCSI Cards

The Fluor-S MAX is connected to your computer by a Small Computer System Interface (SCSI) cable. To use the Fluor-S MAX, you must have a SCSI card installed in your PC. If you have a PC with a Windows 98 or Windows ME operating system, you may also need to load the SCSI and WinASPI drivers that came with the card.

Simulation Mode

Any of the imaging device acquisition windows can be opened in "simulation mode." In this mode, an acquisition window will open and the controls will appear active, but instead of capturing real images, the window will create "dummy" images of manufactured data.

You do not need to be connected to an imaging device to open a simulated acquisition window. This is useful for demonstration purposes or practice scans.

To enter simulation mode, hold down the CTRL key and select the name of the device from the File menu. The title of the acquisition window will indicate that it is simulated.

H.1 Fluor-S MAX Acquisition Window

To acquire images using the Fluor-S MAX, go to the File menu and select Fluor-S MAX.... The acquisition window for the imager will open, displaying a control panel and an image display window.

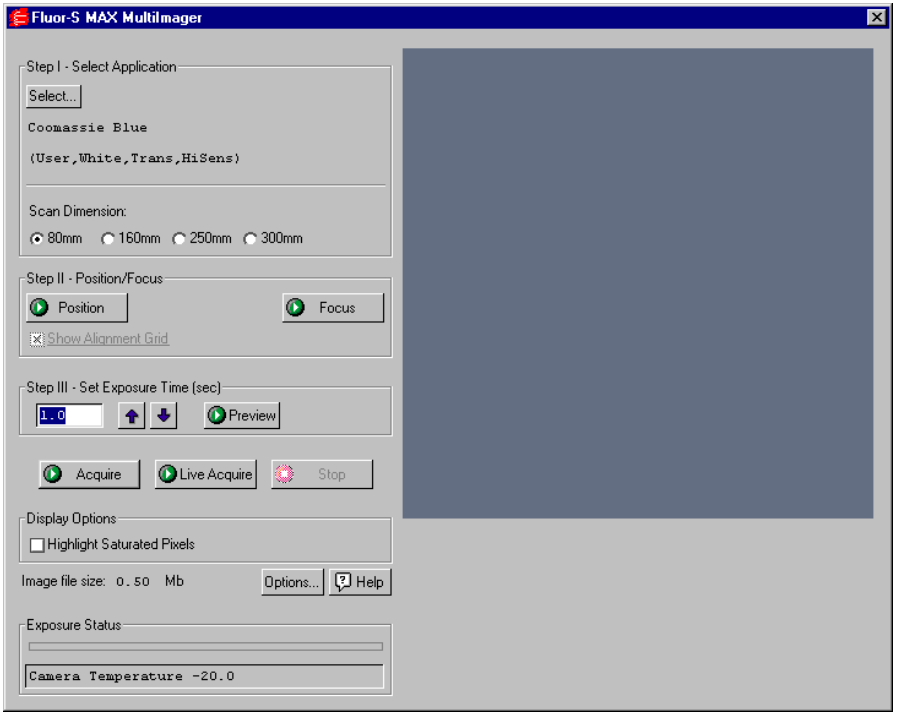


Fig.H-2. Fluor-S MAX acquisition window.

When the Fluor-S MAX window first opens, no image will be displayed.

The control panel has been arranged from top to bottom to guide you through the acquisition procedure. There are four basic steps to acquiring an image using the Fluor-S MAX:

1. Select the application.
2. Position and focus the gel or other object to be imaged.
3. Set the exposure time.
4. Acquire the image.

H.2 Step I. Select Application

To set the appropriate filter and other parameters for the type of object you are imaging, click on the Select button under Select Application. The available applications and their associated settings are listed in a tree that expands from left to right.

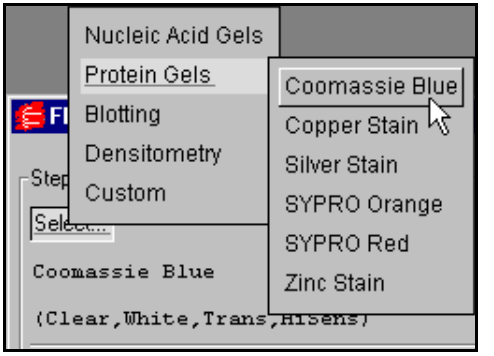


Fig.H-3. The application tree in the Fluor-S MAX acquisition window.

First select your general application, then select the particular stain or medium you are using. If you select the chemiluminescent application under “Blotting,” you also have the option of selecting High Sensitivity or Ultra Sensitivity (see below).

When you select an application, the software automatically sets the appropriate standard filter (520LP, 530DF60, 610LP, clear, or none), light type (UV, white, or none), and light source (trans, epi, or neither) in the Fluor-S MAX for that particular application.

For applications involving trans illumination, you must also specify a scan dimension (see below).

Your selection will be displayed below the Select button. To exit the tree without selecting, press the ESC key.

Custom Applications

If your application is not listed, if you want to use a user-installed filter, or if you want to access Ultra Sensitivity mode (see below), you can create and save your own custom application.

From the application tree, select Custom, then Create. This will open a dialog box in which you can name your application and select your settings.

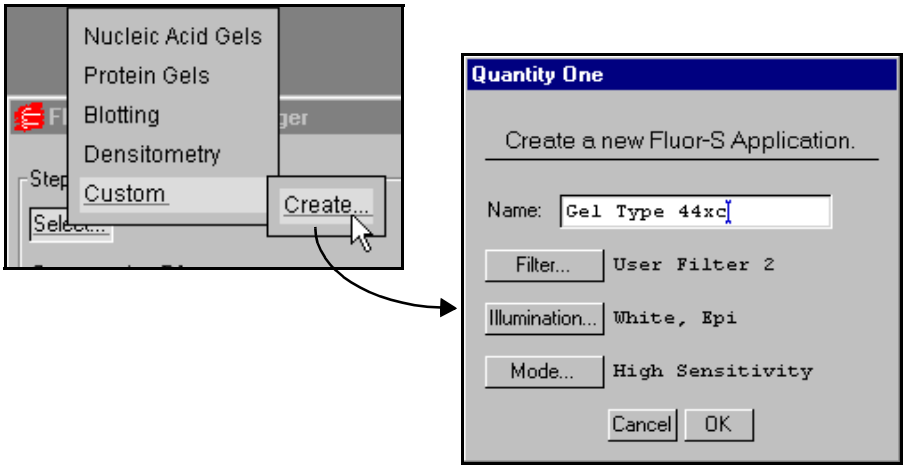


Fig.H-4. Creating a new custom application.

To select the filter (including user-defined), type of illumination, and camera mode, click on the appropriate buttons.

Note: Under Illumination, there is a listing for a spare UV light source. This selects the spare UV bulb in the Fluor-S MAX. Select this light source if your main UV bulb fails.

Enter a name for your application in the Name field. 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.

Scan Dimension

If an application uses trans illumination, the Scan Dimension buttons become active. The scan dimension is the distance traveled by the transilluminating light source as it scans horizontally across the platen.

Fig.H-5. Scan dimension settings for trans illumination.

The full scanning range is 300 mm. Select a smaller range if your sample is small and you do not want to wait while the light source travels over the maximum scan width.

High Sensitivity and Ultra Sensitivity

High Sensitivity and Ultra Sensitivity are different camera modes. In Ultra Sensitivity mode, the Fluor-S MAX camera is cooled to –35.0 degrees C. In High Sensitivity mode, the camera is cooled to –20.0 degrees C. The current camera temperature is displayed at the bottom of the acquisition window.

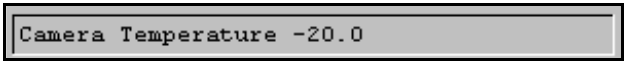


Fig.H-6. Camera Temperature display.

High Sensitivity is the normal operating mode of the Fluor-S MAX. Ultra Sensitivity provides optimal sensitivity for low-light applications. It is the default selection for the chemiluminescence application, and may be selected for a custom application.

Note: When you change from High to Ultra Sensitivity or visa versa, there will be a delay of several minutes while the Fluor-S MAX camera cools down or warms up. If you attempt to acquire an image during this period, you will be notified of the changing temperature. If you do not want to wait, you can cancel the mode change.

H.3 Step II. Position/Focus

Note: When you click on the Position or Focus button, the light inside the Fluor-S MAX box automatically turns off. This is because if you focus with the camera at maximum aperture (see note below), leaving the light on would make it difficult to view the image. To turn the light on while positioning or focusing, hold down the SHIFT key when clicking on the button.

Position

The next step in acquiring an image is centering your gel or other object within the camera frame. To do this, click on the Position button. The Fluor-S MAX will begin capturing a "live" image and updating it every second.

With the Position button selected, look at the image in the acquisition window while you position your object in the center of the platen. If you have a zoom lens on the camera, you can adjust the magnification while you position.

You can select the Show Alignment Grid checkbox to facilitate positioning.

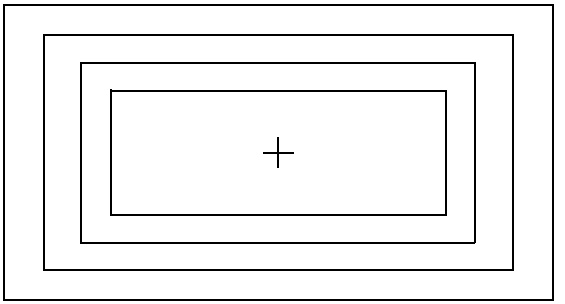


Fig.H-7. Fluor-S MAX alignment grid.

When you are finished positioning, click on the Stop button.

Focus

Note: Before focusing, you should adjust the f-stop on the camera to the lowest setting (i.e., the maximum aperture). This reduces the depth of field, allowing you to

more accurately focus the camera. Then, after focusing, increase the f-stop to the desired setting.

After you have positioned your sample, click on the Focus button and look at the image in the acquisition window while aligning the two focusing arrows on the camera lens. While focusing, the camera will limit its focus to a small portion of the sample (this will not affect any zoom lens adjustments you may have made.)

When you are finished focusing, click on the Stop button.

H.4 Step III. Set Exposure Time

When you are ready to capture an image, you will need to select an exposure time. “Exposure” refers to the integration of image captures on the CCD over a set period of time. The effect is analogous to exposing photographic film to light.

The exposure time you select should be based on your application and your “best guess” as to what exposure will give you the best image.

Note: The minimum exposure time in trans illuminated mode is 1 second. The minimum exposure time in epi illuminated mode is 0.1 second.

You can enter an exposure time (in seconds) directly in the field, or use the Arrow buttons to adjust the exposure time in 10 percent increments.

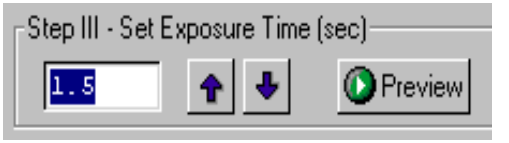


Fig.H-8. Selecting an exposure time.

The following table provides recommended exposure times for various applications.

Recommended Exposure Times and Lenses

Sample	Recommended Exposure	Lens & Filter[1]	Accessories Used
Fluorescent Stain Gel	1–20 sec.	Zoom/IR	None
Fluorescence End-Label Gel	10 sec.–2 min.	Zoom/IR	None
Fluorescent Blot	0.1–3 sec.	Zoom/IR	None
Chemifluorescent Blot	0.1–3 sec.	Zoom/IR	None
Colorimetric Gel	1–5 sec.	Zoom/IR	White Diffusion Plate
Colorimetric Blot	0.2–10 sec.	Zoom/IR	None
X-ray film	0.1–5 sec.	Zoom/IR	White Diffusion Plate
Weak Chemiluminescence[2]	2–10 min.	50 mm	Chemi Tray (if sample is small)
Strong Chemiluminescence[2]	5 sec.–1 min.	50 mm	Chemi Tray (if sample is small)

[1]For sharper focusing, close the f-stop down 1–2 stops from full open while focusing.
[2]For chemi applications, the 50mm lens is recommended. Always remove the 660 filter.

For most applications, you can select an exposure time, capture an image, study it, then adjust the exposure time accordingly. Repeat this procedure as many times as necessary to obtain a good image.

For chemiluminescent samples, which degrade over time and emit low levels of light, you can select a high exposure time initially and use Live Acquire mode to save intermediate exposures (see following section).

Preview

For shorter exposures, you can use Preview to test different exposure times. Click on the Preview button create a preview exposure and display it in the acquisition window.

A preview scan takes only half as long to create as a real scan, because the preview scan does not capture a “dark” image (see below). The progress of the exposure will be displayed in the Exposure Status bar at the bottom of the dialog box.

You cannot save preview scans.

If you want to stop a preview scan that is in progress, click on the Stop button.

H.5 Acquire the Image

The Fluor-S MAX gives you the option of simply acquiring and displaying a fully exposed image, or preserving intermediate exposures.

Acquire

To acquire and display a fully exposed image, click on the Acquire button. An exposure will be taken based on the time selected in Step III. This is appropriate for most short exposures.

The progress of each exposure will be displayed in the Exposure Status bar at the bottom of the acquisition window.

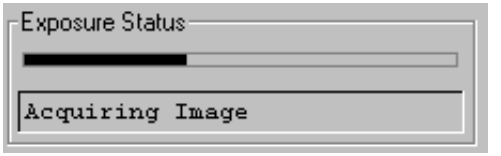


Fig.H-9. Exposure Status bar when acquiring an image.

Depending on which dark subtraction type you have selected, a dark count may be acquired immediately following image acquisition. See Dark Subtraction Type under Options, below.

If you want to stop a scan that is in progress, click on the Stop button. The acquisition will be terminated.

After an image has been acquired, a separate window will pop up containing the new image. The window will have a default file name that includes the date, time, and user (if known). To save the image, select Save or Save As from the File menu.

You can then analyze the image using the standard analysis functions.

Live Acquire

Live Acquire allows you to view and preserve intermediate exposures leading up to a full exposure. This is useful for longer exposure times, such as those required by chemiluminescent samples, where there is the potential for image saturation.

When you click on the Live Acquire button, the exposure time you selected is divided by the number of exposure counts set in the Options dialog box (see Options, below). For example, if you enter an Exposure Time of 10 minutes and an Exposure Count of 20, then 20 intermediate exposures will be produced at 30-second intervals. Each intermediate exposure will be displayed in the scan window. The final, full exposure will be displayed in a separate image window.

Note: The first intermediate exposure will take longer than 30 seconds to display if dark subtraction is performed.

You can automatically save your intermediate exposures as separate files for later review using the Auto Save After Scan option. See Options, below.

If you see an intermediate exposure that you like in the scan window, click on the Stop button. Live Acquire will end and the last intermediate exposure to be completed will open in a separate image window. You can then save it for analysis.

H.6 Options

Click on the Options button to open the Options dialog box.

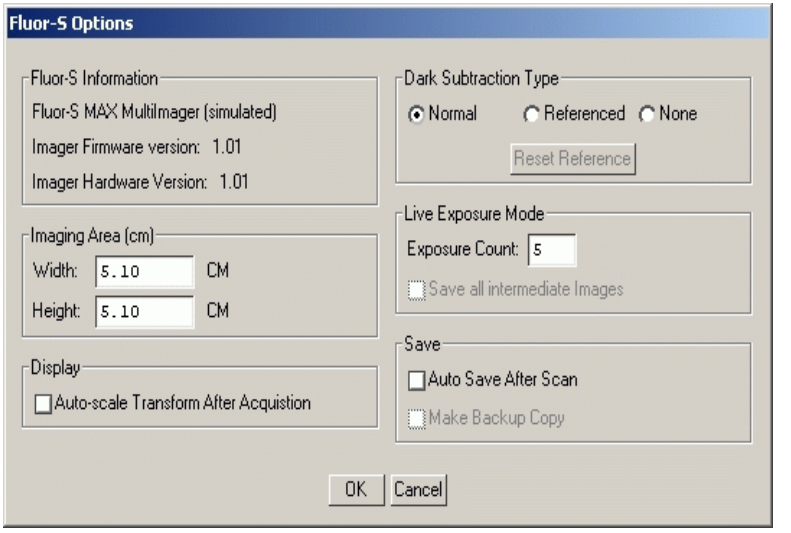


Fig.H-10. Options dialog box.

H.6.a Dark Subtraction Type

All CCD cameras accumulate electrons that produce a “signal” that is indistinguishable from light. This “dark count” adds to the noise in your images. In most cases, you will want to subtract this dark count from your images.

Normal

The Normal option button selects the default dark subtraction type. In this mode, after you acquire an image, a “dark” image of the same exposure length will be taken, and this will be subtracted from your image.

The progress of the dark exposure will be displayed in the Exposure Status bar following the regular image exposure.

Note: In Normal mode, a dark image is only acquired the first time you perform a scan with particular application and exposure settings. If you perform subsequent scans with the same settings, no dark exposure will be taken.

Referenced

If you do not want to perform a dark exposure with each acquisition, you can take a "reference" dark exposure that will be saved and subtracted from all subsequent acquisitions. Click on the Referenced button to activate this feature.

The first time you acquire an image after selecting this option, the Fluor-S MAX will take a 300-second dark exposure that will be saved and used to subtract the dark count from all subsequent acquisitions.

For image exposures of greater or less than 300 seconds, the reference dark will be scaled accordingly and then subtracted. You can change the default reference dark exposure time using the Reset Reference button (see below).

If you deselect the Referenced button and then reselect it, the old reference dark exposure will still be available.

Note: Separate reference dark exposures will be taken for High Sensitivity mode and Ultra Sensitivity mode. Once you have created a reference dark in each of these modes, each reference dark will be used according to the mode you are in.

Reset Reference Button

If you would like a reference dark with an exposure time that more closely matches that of your typical scans, click on the Reset Reference button.

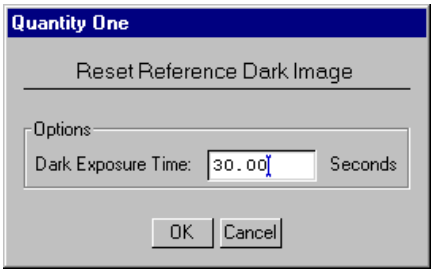


Fig.H-11. Reset Reference Dark pop-up box.

A pop-up box will prompt you to enter a new reference dark exposure time in seconds. Click on OK to implement your change. The new reference dark will be created when you acquire your next image.

Note: Because of the high sensitivity of the CCD, fluctuations in background radiation and/or temperature in the room can affect the level of dark count. If you feel that radiation/temperature conditions have changed in the room since your last reference dark was created, use the Reset Reference button to delete your old reference and create a new one under current conditions.

None

If you do not want to perform dark subtraction, select None. No dark exposure will be acquired or subtracted.

H.6.b Live Exposure Mode

Exposure Count

If you are using the Live Acquire function (see previous section), you need to specify how many intermediate exposures you want to view/save during acquisition. Enter this number in the Exposure Count field.

The total exposure time will be divided by the number you enter in the Exposure Count field. If you enter an exposure time of 10 minutes and a count of 10, you will create 10 intermediate exposures at 1 minute intervals.

Note: Do not enter a count that will result in an intermediate exposure time that is less than the minimum exposure time for the mode you are in. The minimum exposure time in trans illuminated mode is 1 second, and the minimum exposure time in epi illuminated mode is 0.1 second. (Example: For a trans illuminated application, an exposure time of 20 seconds and an exposure count of 21 would result in an error.)

Save All Intermediate Images

If Auto Save After Scan is selected (see following section), the Save All Intermediate Images checkbox will become active. If you select this checkbox, all your intermediate exposures will be saved as separate files. These files will have the same

root name appended by a number indicating the exposure sequence. The final, full exposure will have the root name only, with no exposure number.

H.6.c Save

Auto Save After Scan

To automatically save any image you create, click on the Auto Save After Scan checkbox.

Note: In PDQuest, this option is preselected and cannot be turned off. All images must be automatically saved when acquired.

With this checkbox selected, when you click on Acquire or Live Acquire, a Save As dialog box will open asking you to specify a file name and location for the image you are about to create. The scan will begin when you click on the Save button.

Note that in Live Acquire mode you can save your intermediate exposures by selecting Auto Save After Scan and then Save All Intermediate Images.

Make Backup Copy

You can automatically create a backup copy of any scan you create. To do so, first select Auto Save After Scan (see above), then select the Make Backup Copy checkbox.

With this checkbox selected, when you save a scan, a backup copy will be placed in the same directory as the scanned image. Windows backup files will have an ".sbk" extension. Macintosh backup files will have the word "backup" after the file name.

This backup copy will be read-only, which means that you cannot make changes to it. You can open it like a normal file, but you must save it under a different file name before editing the image or performing analysis.

H.6.d Imaging Area Size

The imaging area is the area of the sample that is captured by the camera and displayed in the scan window. To specify the size of your imaging area, enter a dimension in the appropriate field. When you change one imaging area dimension, the other will change to maintain the aspect ratio of the camera lens.

The imaging area will change depending on your zoom factor. For example, if you have zoomed in on a area that is 4.5 x 3.5 cm, then you would enter 4.5 for the width (3.5 for the height would be calculated automatically).

Note: Your imaging area settings must be correct if you want to do actual-size printing. They must also be correct if you want to compare the quantities of objects (e.g., using the Volume Tools) in different images.

The imaging area dimensions also determine the size of the pixels in your image (i.e., resolution). A smaller imaging area will result in a higher resolution.

H.6.e Auto-scale Transform

Auto-scale Transform after Acquisition allows the user the option of having the image automatically perform the Auto-Scale transform function upon completion of image acquisition. This eliminates the need to transform an image or re-scan an image when the acquisition time was too short or the iris not opened enough.

To enable the auto-scale transform function, check the box labeled, Auto-Scale Transform after Acquisition in the Options dialog.

H.7 Other Features

Fig.H-12. Other Fluor-S MAX acquisition window features.

Highlight Saturated Pixels

When this box is checked, any saturated pixels in the image will appear highlighted in red in the scan window and in the pop-up image window. To view/hide saturated pixels in the pop-up image window, use the Image > Transform command.

File Size of Images

Image File Size (below Options) shows the size of the image file you are about to create. This size is determined by whether the image was created in High Sensitivity or Ultra Sensitivity mode.

If you do not have enough computer memory for the specified file size, an error message will appear when you attempt to acquire an image. (Macintosh users can increase the application memory partition. See your Macintosh computer documentation for guidance.)

Appendix I
Personal Molecular Imager FX

Fig.I-1. Personal Molecular Imager FX

Before you can begin acquiring images using the Personal Molecular Imager® FX, the instrument must be properly installed and connected with the host computer. See the Personal FX hardware manual for installation, startup, and operating instructions.

Note: The Personal FX should be turned on and the initialization sequence completed *before* the host computer is turned on (except in the case of certain Power Macintosh configurations). See the hardware manual for more details.

PC Only: A Note About SCSI Cards

The Personal FX is connected to your computer by a Small Computer System Interface (SCSI) cable. To use the Personal FX, you must have a SCSI card installed in your PC. If you have a PC with a Windows 98 or Windows ME operating system, you may also need to load the SCSI and WinASPI drivers that came with the card.

Simulation Mode

Any of the imaging device acquisition windows can be opened in "simulation mode." In this mode, an acquisition window will open and the controls will appear active, but instead of capturing real images, the window will create "dummy" images of manufactured data.

You do not need to be connected to an imaging device to open a simulated acquisition window. This is useful for demonstration purposes or practice scans.

To enter simulation mode, hold down the CTRL key and select the name of the device from the File menu. The title of the acquisition window will indicate that it is simulated.

I.1 Personal FX Acquisition Window

To acquire images using the Personal FX, go to the File menu and select Personal FX.... The acquisition window for the imager will open, displaying a control panel and the scanning area window.

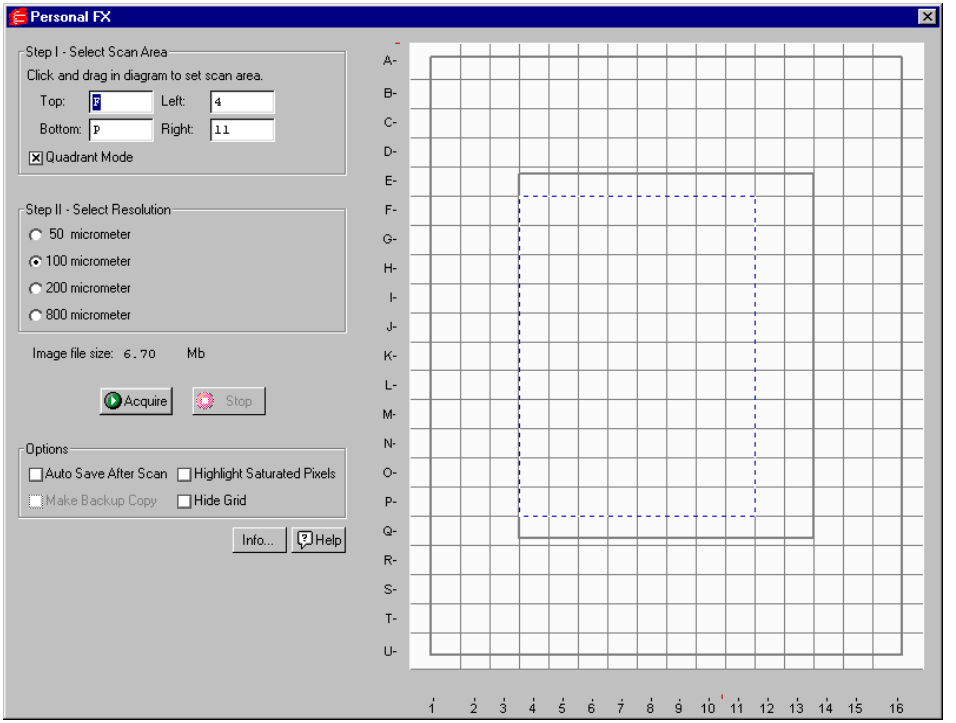


Fig.I-2. Personal FX acquisition window

The default scanning window is marked by grid lines that divide the area into quadrants. There is also an outer box and inner box marked by thicker lines. The quadrants are numbered 1–16 left to right and lettered A–U top to bottom.

If you prefer a scanning window measured in centimeters, deselect the Quadrant Mode checkbox in the control panel by clicking on it. To hide the gridlines, click on the Hide Grid checkbox under Options.

The control panel has been arranged from top to bottom to guide you through the acquisition procedure. There are three basic steps to scanning an image using the Personal FX:

1. Select the scan area

2. Select the resolution
3. Acquire the image

I.2 Step I. 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.

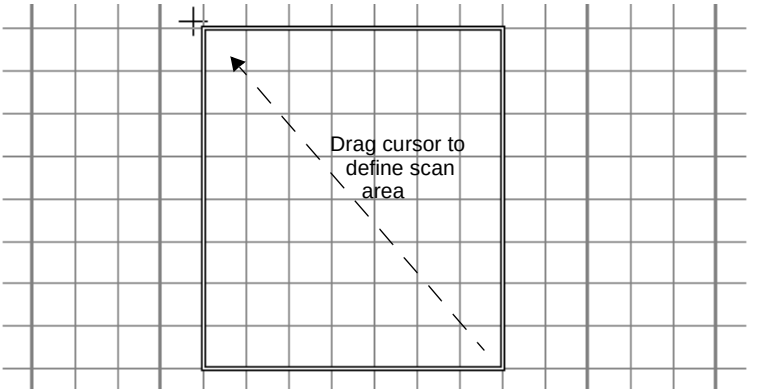


Fig.I-3. Selecting a scan area.

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.

You can also select the scanning area by entering coordinates in the appropriate fields (Top, Bottom, Left, Right). After you enter a coordinate, the position of the scanning area box will change accordingly.

When selecting, be sure to include the entire area of interest, and be generous with borders. You can always crop the image later.

I.3 Step II. Select Resolution

The Personal FX acquisition window allows you to scan at 50, 100, 200, or 800 micrometers. These resolutions are listed as option buttons in the control panel.

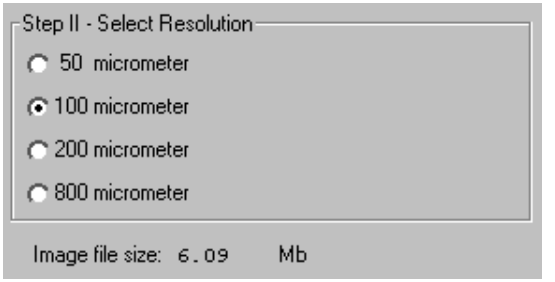


Fig.I-4. Resolution option buttons.

The resolution you select should be based on the size of the objects (e.g., bands, spots) you are interested in. For example:

- 50 micrometer resolution should be reserved for images requiring the highest level of detail, e.g., high density in situ samples, 1,536-well microplates, high density arrays, samples with very closely spaced bands. Files scanned at 50 micrometers can be very large.
- 100 micrometer resolution should be used for typical gels and arrays.
- 200 micrometer resolution is useful for gels with large bands and dot blots.
- 800 micrometer resolution should be reserved for very large objects, such as CAT assays.

File Size of Images

Image File Size (below Select Resolution) shows the size of the scan file you are about to create. If you do not have enough computer memory for the specified file size, an error message will appear when you attempt to scan. If this happens, select a lower resolution or decrease the size of the area to be scanned. (Macintosh users can also increase the application memory partition. See your Macintosh computer documentation for guidance.)

I.4 Acquire the Image

Once you have selected your scan area and resolution, you ready to acquire an image.

Click on the Acquire button. There may be a short delay while the image laser warms up; then the scanned image will begin to appear in the scanning window, line by line.

To interrupt a scan, click on the Stop button. A message will ask you to confirm the interrupt, and then you will be asked if you want to keep the partial scan. This feature is useful if you overestimated the size of the area you selected.

Note: If the image you are scanning has more than 10 saturated pixels, you will receive a warning message.

Saving the Image

After the scan is complete, a message will appear asking you if you want to keep the scan. If you select Yes, a separate window will pop up containing the new image.

You can then save and analyze the image using the standard menu and toolbar functions.

I.5 Options

Auto Save After Scan

To automatically save any scan you create, click on the Auto Save After Scan checkbox.

Note: In PDQuest, this option is preselected and cannot be turned off. All images must be automatically saved when acquired.

With this checkbox selected, when you click on Acquire, a Save As dialog box will open asking you to specify a file name and location for the image you are about to create. The scan will begin when you click on the Save button.

Make Backup Copy

You can automatically create a backup copy of any scan you create. To do so, first select Auto Save After Scan (see above), then select the Make Backup Copy checkbox.

With this checkbox selected, when you save a scan, a backup copy will be placed in the same directory as the scanned image. Windows backup files will have an ".sbk" extension. Macintosh backup files will have the word "backup" after the file name.

This backup copy will be read-only, which means that you cannot make changes to it. You can open it like a normal file, but you must save it under a different file name before editing the image or performing analysis.

Highlight Saturated Pixels

When this box is checked, any saturated pixels in the image will appear highlighted in red in the scan window and in the pop-up image window. To view/hide saturated pixels in the pop-up image window, use the Image > Transform command.

Hide Grid

To hide the gridlines in the scanning area window, click on the Hide Grid checkbox.

Disable Default Filter

To disable the default filter in the FX, click the Disable Default Filter checkbox.

Warn on Saturated Pixels

The Warn on Saturated Pixels option is checked by default. Uncheck this box to disable this warning.

Appendix J
Molecular Imager FX Family (FX Pro, FX Pro Plus and Molecular FX)

Fig. J-1. Molecular Imager FX

Before you can begin acquiring images using any of the Molecular Imager FX family of products, the particular instrument must be properly installed and connected with the host computer. See the corresponding FX hardware manual for installation, startup, and operating instructions.

Note: The FX should be turned on and the initialization sequence completed *before* the host computer is turned on (except in the case of certain Power Macintosh configurations). See the hardware manual for more details.

PC Only: A Note About SCSI Cards

The FX is connected to your computer by a Small Computer System Interface (SCSI) cable. To use the FX, you must have a SCSI card installed in your PC. If you have a PC with a Windows 98 or Windows ME operating system, you may also need to load the SCSI and WinASPI drivers that came with the card.

Simulation Mode

Any of the imaging device acquisition windows can be opened in "simulation mode." In this mode, an acquisition window will open and the controls will appear active, but instead of capturing real images, the window will create "dummy" images of manufactured data.

You do not need to be connected to an imaging device to open a simulated acquisition window. This is useful for demonstration purposes or practice scans.

To enter simulation mode, hold down the CTRL key and select the name of the device from the File menu. The title of the acquisition window will indicate that it is simulated.

J.1 FX Acquisition Window

To acquire images using the FX, go to the File menu and select FX.... The acquisition window for the imager will open, displaying the control panel for the imager and the scanning area window.

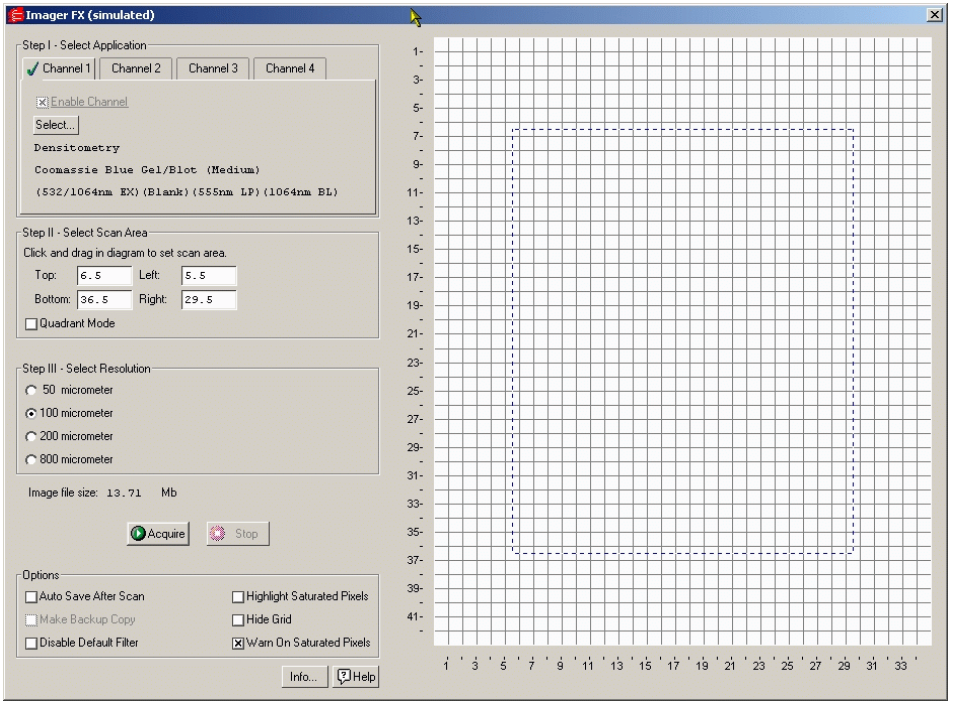


Fig. J-2. FX acquisition window

The default scanning window is marked by grid lines that divide the area into quadrants. There is also an outer box and inner box marked by thicker lines. The quadrants are numbered 1–16 left to right and lettered A–U top to bottom.

If you prefer a scanning window measured in centimeters, deselect the Quadrant Mode checkbox in the control panel by clicking on it. To hide the gridlines, click on the Hide Grid checkbox under Options.

The control panel has been arranged from top to bottom to guide you through the acquisition procedure. There are four basic steps to scanning an image using the FX:

1. Select the application(s).

2. Select the scan area.

3. Select the resolution.
4. Acquire the image.

J.2 Step I. Select Application

In Step 1, you select the appropriate filters and other scanning parameters for the type of gel, blot, plate, or other object that you are imaging. Each set of parameters you select is called an “application.”

The FX now supports multi-channel sequential scanning. This allows you to automatically scan the same object using up to four different applications (e.g., to detect different types of stains on the same gel).

First you select a channel, then you select the application under that channel.

J.2.a Selecting a Channel

The four channels are accessed using the tabs under Step 1. Channel 1 is always enabled—that is, the FX will always scan using the application settings selected under Channel 1 first.

To enable any of the remaining channels, click on a channel tab, then select the Enable Channel checkbox and select the application for that channel as described in the following section.

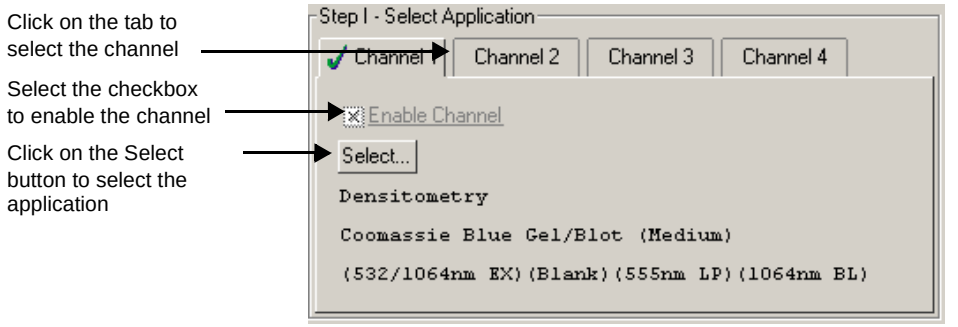


Fig. J-3. Enabling Channel 2.

Enabled channels have a green check mark on their tabs.

You do not need to enable Channels 2–4 in sequence. For example, you can set up your four most common applications using the different channels, but only enable Channel 4 for a particular gel. Channel 1 would be scanned first, Channel 4 second.

Enabled channels are scanned sequentially. The separate scans are displayed and saved as separate images. The total scanning time depends on the number and type of enabled applications.

If you have selected Auto Save After Scan, the image created using Channel 1 will have the base file name, and images created using subsequent channels will have the base file name plus a version number. (v. 2, v. 3, v. 4). Note that the image version number does not necessarily correspond to the channel number. For example, if you scanned an image using only Channels 1 and 4, the image created using Channel 4 will be saved as version 2 (v. 2).

J.2.b Selecting an Application

To select an application (i.e., the appropriate filter and other parameters for the type of object you are imaging), click on the Select button under a channel tab.

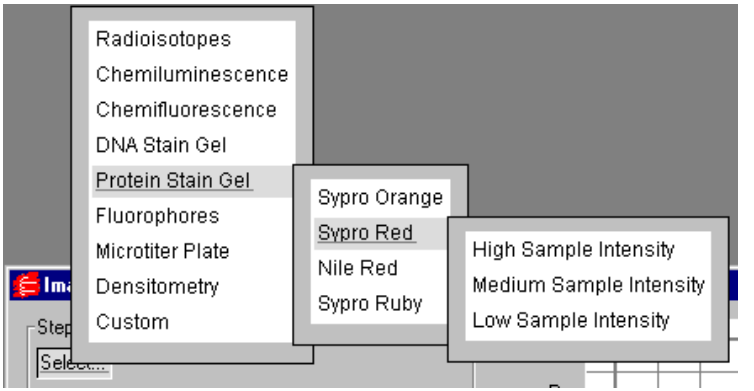


Fig. J-4. Example of an application tree: Ethidium Bromide gel.

Standard Applications

The standard applications and associated settings are listed in a tree that expands from left to right. When you select a standard application, the software automatically selects the appropriate filter(s) in the FX for that particular application. Some applications are inactive in the FX Pro and FX Pro Plus systems.

Standard FX Applications

Category	Application
Radioisotopes	CS- or BI-Screen (Bio-Rad)[1] K-Screen (Kodak) Fuji-Screen
Chemiluminescence	Chemi-Screen (Bio-Rad)[1]
Chemifluorescence	ECL-Plus Attophos
DNA Stain Gel	Ethidium Bromide Sybr Green I & II Sybr Gold

Standard FX Applications

Protein Stain Gel	Sypro Orange Sypro Red Nile Red Sypro Ruby
Fluorophores	Alexa 488 Alexa 532 Alexa 546 FITC FAM CY3 HEX R6G Texas Red
Microtiter Plate	DNA (Sybr Green I) Protein (Nano Orange) ssDNA (Oligreen) DNA (Picogreen) B-Gal (FDG) GUS (FDG)
Densitometry	Coomassie Blue Gel/Blot Copper Stain Gel/Blot Silver Stain Gel/Blot X-Ray Film (Grey Type)

[1]Not supported in the FX Pro and FX Pro Plus systems.

First select your general application, next select the particular stain or medium you are using, and finally (if appropriate) select the intensity of your samples.

Note: Some applications require an external laser. If you choose one of these without having an external laser attached, you will receive a warning.

To exit the tree without selecting, press the ESC key.

Your selection and settings will be displayed below the Select button.

Sample Intensity

Many FX applications require that you select a sample intensity (High, Medium, or Low) from the application tree. This is simply a rough estimate of how much sample is visible in your gel or other object.

If you are unsure of the level of intensity of your sample, you can always select a level, capture an image, then adjust the level and capture another image.

For example, if you select Low Sample Intensity and the resulting image has too many saturated pixels, you will receive a warning message. Simply change the setting to Medium Sample Intensity and rescan. If you select High Sample Intensity and the resulting image is too faint, select Medium or Low and rescan.

Custom Applications

If your application is not listed, if you want to use user-installed filters, or if you want to use an external laser, you can create and save your own custom application.

From the application tree, select Custom, then Create. This will open a dialog box in which you can name your application and select your settings.

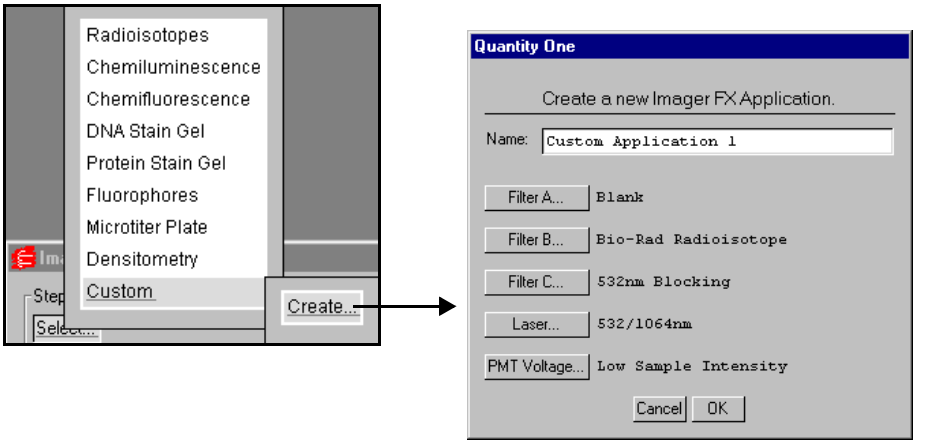


Fig. J-5. Creating a custom application.

To select a filter (including user-defined) or filter combination, click on the buttons for Filters A, B, and C, and make your choice from each pop-up list.

Note: Filter wheel C is not available in the FX Pro or the FX Pro Plus systems.

Note: The user-defined filters (User1, User2, etc.) cannot be renamed in the pop-up list, so be sure to remember which filter you insert into each position in the FX (i.e. 690 nm filter, 605 nm BPfilter).

To use an external laser, click on the Laser button and select it from the pop-up list (i.e. 488 nm or 635 nm laser). Otherwise, use the default internal laser (532/1064nm).

Note: The dual laser (532/1064 nm) is not available in the FX Pro or the FX Pro Plus systems.

Click on the PMT Voltage button to select a standard voltage for your custom application or create a custom PMT voltage.

To select a custom voltage, click on the Custom option. In the dialog box, adjust the slider to select a PMT voltage as a percentage of the maximum.

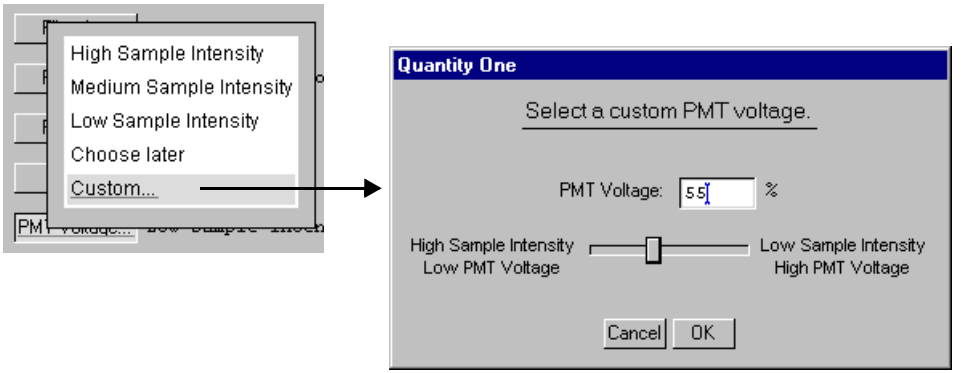


Fig. J-6. Selecting a custom PMT voltage.

Note: For voltages above 80% of maximum, you will receive a warning message that the high voltage could damage the PMT.

If you select Choose Later from the list of PMT voltages, the choices of sample intensity will be displayed when you select your custom application.

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.

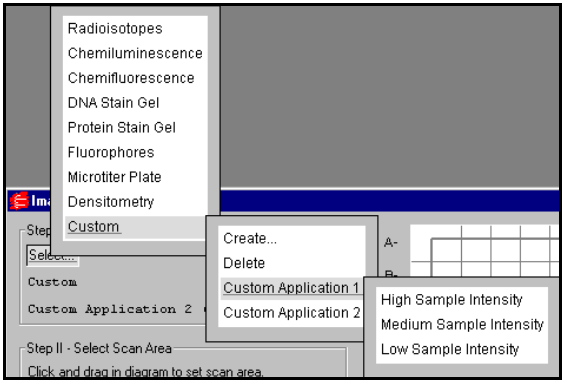


Fig. J-7. Selecting a custom application.

You can edit a custom application by selecting **Custom**, **Edit**, and the name of the application**.** You can also use this feature to create a new custom application from an existing one**.**

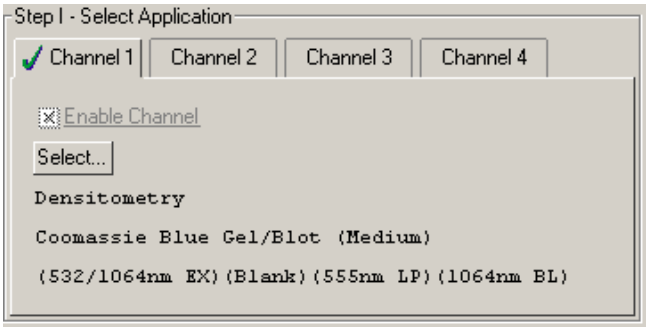


Fig. J-8. Application selection and settings.

Once you select an application, the application name and settings appear below the Select button.

J.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.

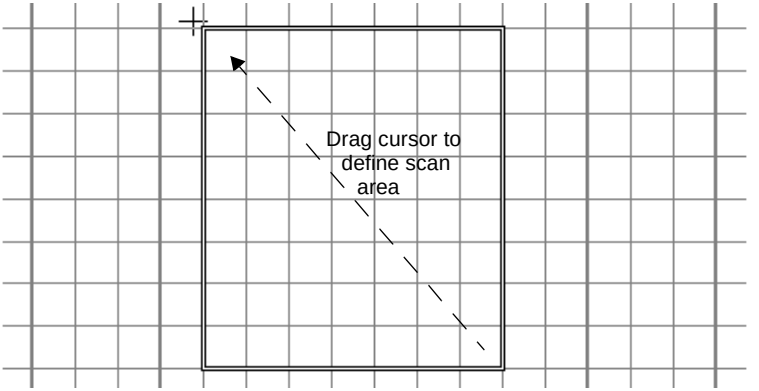


Fig. J-9. Selecting a scan area.

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.

You can also select the scanning area by entering coordinates in the appropriate fields (Top, Bottom, Left, Right). After you enter a coordinate, the position of the scanning area box will change accordingly.

When selecting, be sure to include the entire area of interest, and be generous with borders. You can always crop the image later.

J.4 Step III. Select Resolution

The FX acquisition window allows you to scan at 50, 100, 200, or 800 micrometers. These resolutions are listed as option buttons in the control panel.

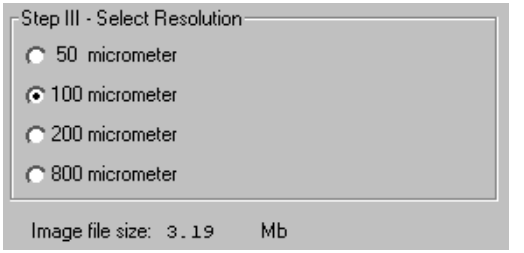


Fig. J-10. Resolution option buttons.

The resolution you select should be based on the size of the objects (e.g., bands, spots) you are interested in. For example:

- 50 micrometer resolution should be reserved for images requiring the highest level of detail, e.g., high density in situ samples, 1,536-well microplates, high density arrays, samples with very closely spaced bands. Files scanned at 50 micrometers can be very large.
- 100 micrometer resolution is useful for typical gels and arrays.
- 200 micrometer resolution is useful for gels with large bands and dot blots.
- 800 micrometer resolution should be reserved for very large objects, such as CAT assays.

File Size of Images

Image File Size (below Select Resolution) shows the size of the scan file you are about to create. If you do not have enough computer memory for the specified file size, an error message will appear when you attempt to scan. If this happens, select a lower resolution or decrease the size of the area to be scanned. (Macintosh users can also increase the application memory partition. See your Macintosh computer documentation for guidance.)

J.5 Acquire the Image

Once you have selected your application, scan area, and resolution, you are ready to acquire an image.

Click on the Acquire button. There may be a short delay while the image laser warms up; then the scanned image will begin to appear in the scanning window, line by line.

To interrupt a scan, click on the Stop button. A message will ask you to confirm the interrupt, and then you will be asked if you want to keep the partial scan. This feature is useful if you overestimated the size of the area you selected.

Note: If the image you are scanning has more than 10 saturated pixels, you will receive a warning message. If this happens, you can go back and select a higher sample intensity in the application tree.

Saving the Image

After the scan is complete, a message will appear asking you if you want to keep the scan. If you select Yes, a separate window will pop up containing the new image.

You can then save and analyze the image using the standard menu and toolbar functions.

J.6 Options

Auto Save After Scan

To automatically save any scan you create, click on the Auto Save After Scan checkbox.

With this checkbox selected, when you click on Acquire, a Save As dialog box will open asking you to specify a file name and location for the image you are about to create. The scan will begin when you click on the Save button.

If you are scanning using multiple channels, the image created using Channel 1 will be saved using the base file name, and images created using subsequent channels will have the base file name plus a version number. (v. 2, v. 3, v. 4). Note that the image version number does not necessarily correspond to the channel number. For example, if you scanned an image using only Channels 1 and 4, the image created using Channel 4 will still be saved as version 2 (v. 2).

Make Backup Copy

You can automatically create a backup copy of any scan you create. To do so, first select Auto Save After Scan (see above), then select the Make Backup Copy checkbox.

With this checkbox selected, when you save a scan, a backup copy will be placed in the same directory as the scanned image. Windows backup files will have an ".sbk" extension. Macintosh backup files will have the word "backup" after the file name.

This backup copy will be read-only, which means that you cannot make changes to it. You can open it like a normal file, but you must save it under a different file name before editing the image or performing analysis.

Highlight Saturated Pixels

When this box is checked, any saturated pixels in the image will appear highlighted in red in the scan window and in the pop-up image window. To view/hide saturated pixels in the pop-up image window, use the Image > Transform command.

Hide Grid

To hide the gridlines in the scanning area window, click on the Hide Grid checkbox.

Disable Default Filter

To disable the default filter in the FX, click the Disable Default Filter checkbox.

Warn on Saturated Pixels

The Warn on Saturated Pixels option is checked by default. Uncheck this box to disable this warning.

Appendix K
VersaDoc

Fig.K-1. VersaDoc.

Before you can acquire images using the VersaDoc Imaging System, you need to install the Roper Scientific interface adapter and its associated device drivers in your computer. The driver installation procedures for Windows operating systems inlcude pre-installation and use of the Windows Hardware Wizards.

Note: *Important!* Please read and follow the driver installation procedures contained in the README documents before you install the VersaDoc camera interface adapter. You can find the README documents in the VersaDoc Drivers folders on The Discovery Series (TDS) CD.

The CD also contains Roper Scientific camera interface driver installation instructions for Macintosh computers.

See the VersaDoc hardware manual for further installation, startup, and operating instructions for the VersaDoc Imaging System.

K.1 VersaDoc Acquisition Window

To acquire images using the VersaDoc, go to the File menu and select VersaDoc.... The acquisition window for the instrument will open, The acquisition window opens, displaying a control panel and an image display window.

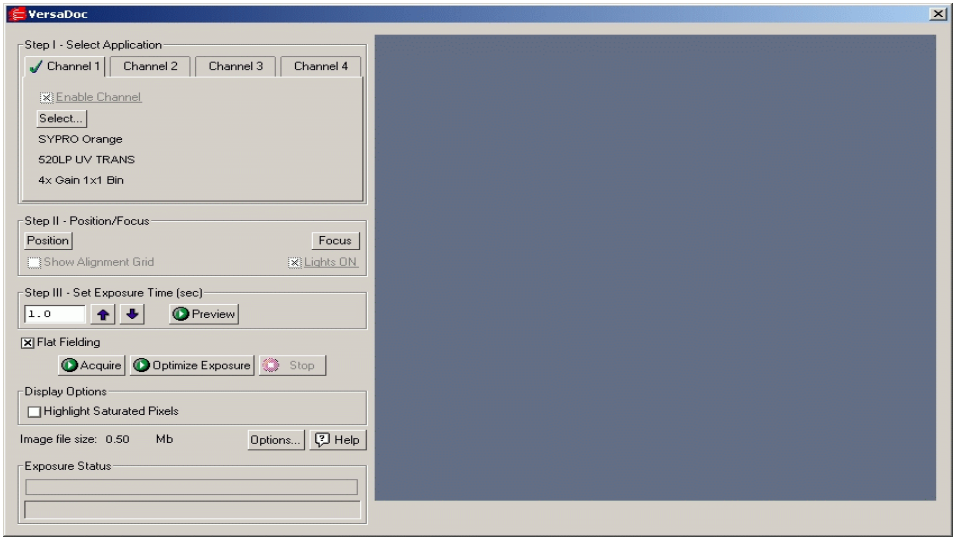


Fig.K-2. VersaDoc acquisition window.

When the VersaDoc window first opens, no image will be displayed.

The control panel has been arranged from top to bottom to guide you through the acquisition procedure. There are four basic steps to acquiring an image using the VersaDoc:

1. Select the application.
2. Position and focus the object to be imaged.
3. Set the exposure time.
4. Acquire the image.

K.2 Step I. Select Application

In Step 1, you select the appropriate filters and other imaging parameters for the type of gel, blot, plate, or other object that you are imaging. Each set of parameters you select is called an application.

The VersaDoc supports multichannel sequential imaging. This allows you to automatically image the same object using up to four different applications (e.g., to detect different types of stains on the same gel).

First you select a channel, then you select the application under that channel.

K.2.a. Selecting a Channel

Note: Illumination Flat Fielding will be disabled if you use multiple channels.

The four channels are accessed using the tabs under Step 1. Channel 1 is always enabled—that is, the VersaDoc will always capture an image using the application settings selected under Channel 1 first.

To enable any of the remaining channels, click on a channel tab, then select the Enable Channel checkbox and select the application for that channel as described below.

Enabled channels have a green check mark on their tabs.

Note: If you use the Optimize Exposure feature, only the application under the selected channel tab will be imaged; other channels, even if enabled, will be ignored.

You do not need to enable Channels 2–4 in sequence. For example, you can set up your four most common applications using the different channels, but only enable Channel 4 for a particular gel. Channel 1 would be scanned first, Channel 4 second.

Enabled channels are imaged sequentially. The separate scans are displayed and saved as separate images. The total imaging time depends on the number and type of enabled applications.

If you have selected Auto Save After Scan, the image created using Channel 1 will have the base file name, and images created using subsequent channels will have the base file name plus a version number. (v. 2, v. 3, v. 4). Note that the image version number does not necessarily correspond to the channel number. For example, if you captured an image using only Channels 1 and 4, the image created using Channel 4 will be saved as version 2 (v. 2).

K.2.b. Selecting an Application

To select an application (i.e., the appropriate filter and illumination source for the type of object you are imaging), click on the Select button under a channel tab.

Standard Applications

The standard applications and associated settings are listed in a tree that expands from left to right. When you select an application, the software automatically sets the appropriate filter in the VersaDoc for that particular application.

First select your general application, then select the particular stain or medium you are using. When you select the stain or medium, the software automatically sets the appropriate filter (520LP, 530DF60, 610LP, clear, or none), light type (UV, white, or none), and light source (Trans, Epi, or neither) in the VersaDoc for that particular application.

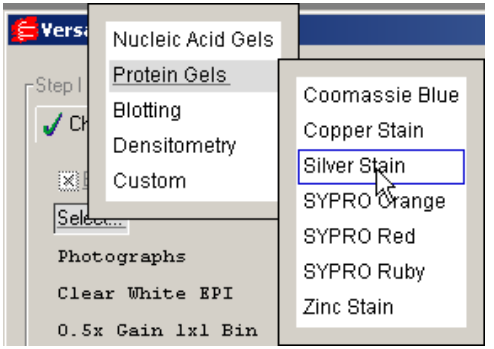


Fig.K-3. The application tree in the VersaDoc acquisition window.

Note: If you select an application that requires trans white illumination, you will need to place the *white light conversion screen* on the sample stage area.

To exit the tree without selecting, press the Esc key or click outside the dialog box.

Your selection will be displayed below the Select button.

Custom Applications

If your application is not listed, if you want to use a user-installed filter, or if you want to set the gain and bin settings manually, you can create and save your own custom applications.

From the application tree, select Custom, then Create. This will open a dialog box in which you can name your application and select your settings.

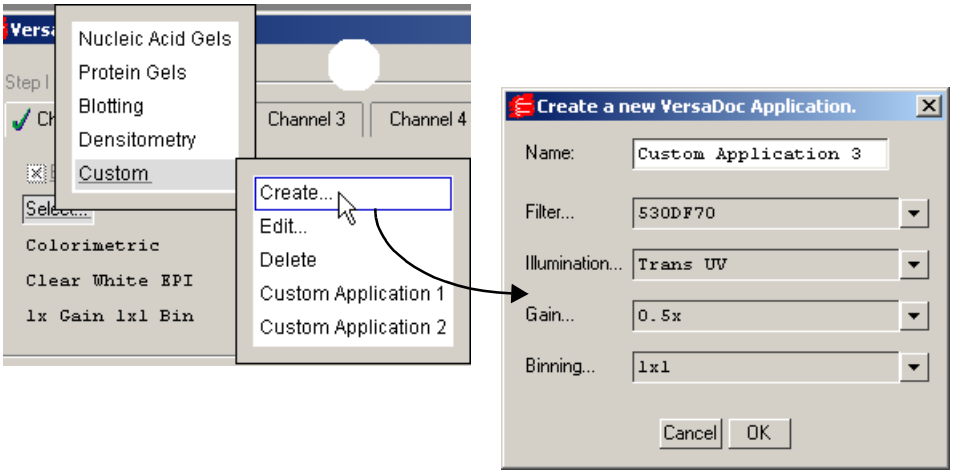


Fig.K-4. Creating a new custom application.

Enter a name for your application in the Name field.

Next, click on the buttons next to the Filter, Illumination, Gain and Binning fields to change these settings.

Note: Note that if you select trans white illumination, you will need to place the *white light conversion screen* on the sample stage.

A higher Binning setting (2x2, 3x3) provides optimal sensitivity for low-light applications such as chemiluminescence. In this mode, the pixels in the camera are "binned" (e.g., four pixels are combined into one) to increase the amount of signal per pixel without increasing noise. Note that combining the pixels results in a reduction in the resolution of the image.

Selecting a higher Gain (4x) provides higher sensitivity without reduced resolution; however, noise will also increase. This is useful for faint signals (bright spots will saturate). Selecting a lower gain (0.5x) is useful for brighter images that tend to saturate. A gain setting of 1x provides the greatest dynamic range.

Click 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 edit a custom application by selecting Custom, Edit, and the name of the application. You can also use this feature to create a new custom application from an existing one.

You can delete a custom application by selecting Custom, Delete, and the name of the application.

K.3 Step II. Position/Focus

Before positioning and focusing the image, select the application that you will be using under Step I.

Note that the optimal position and focus will be different for a filtered image versus an unfiltered one. (All standard applications except Chemiluminescence use a filter.)

Position

After you have selected your application, you are ready to center your gel or other object within the camera frame. To do so, click on the Position button. The VersaDoc will begin capturing a "live" image and updating it every second.

With the Position button selected, study the image in the acquisition window while you position your object in the center of the sample stage. If you have a zoom lens on the camera, you can adjust the magnification while you position. (See the VersaDoc User Manual for details on positioning.)

While you are positioning, you can select the Show Alignment Grid checkbox to display a target grid overlay on the image.

When you click on Position, the light inside the camera box automatically switches on. To turn the light off while positioning, deselect the Light On checkbox.

When you are finished positioning, click on the Stop button.

Focus

Note: Before focusing, you should adjust the f-stop on the camera to the lowest setting (i.e., the maximum aperture). This reduces the depth of field, allowing you to more accurately focus the camera. Then, after focusing, increase the f-stop to the

desired setting. See Table 1, “Recommended Exposure Times and Lenses,” on page 9.

After you have positioned your sample, click on the Focus button and look at the image in the acquisition window while adjusting the focus on the camera lens. While focusing, the camera will limit its focus to a small portion of the sample (this will not affect any zoom lens adjustments you may have made.) (See the VersaDoc User Manual for details on focusing.)

When you click on Focus, the light inside the camera box automatically switches on. To turn the light off while positioning, deselect the Light On checkbox.

When you are finished focusing, click on the Stop button.

K.4 Step III. Set Exposure Time

The “exposure time” is the period of time an image is integrated on the CCD. The effect is analogous to exposing photographic film to light.

Setting an Exposure Time

Different applications have different optimal exposure times. If you are imaging using multiple channels, you can select a different exposure time for each channel. First select the channel using the tabs, then select the appropriate exposure time for that channel.

See Table 1, “Recommended Exposure Times and Lenses,” on page 9.

You can enter an exposure time (in seconds) directly in the field, or use the Arrow buttons to adjust the exposure time in 10 percent increments.

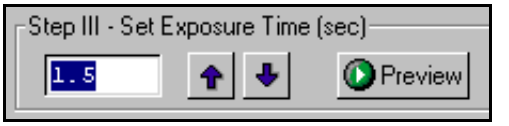


Fig.K-5. Selecting an exposure time.

The following table provides recommended exposure times for various applications

Table 1: Recommended Exposure Times and Lenses

Sample	Recommended Exposure	Lens & Filter	Accessories Used
Fluorescent Stain Gel	3–30 sec.	Zoom/IR	None
Fluorescence End-Label Gel	30 sec.–5 min.	Zoom/IR	None
Fluorescent Blot	0.5–5 sec.	Zoom/IR	Sample/Chemi Tray (if sample is small)
Chemifluorescent Blot	0.5–5 sec.	Zoom/IR	None
Colorimetric Gel	0.1–1 sec.	Zoom/IR	White light conversion screen
Colorimetric Blot	0.1–1 sec.	Zoom/IR	Sample/Chemi Tray (if sample is small)
X-ray film	0.1–1 sec.	Zoom/IR	White light conversion screen
Weak Chemiluminescence[1]	5–10 min.	50 mm	Sample/Chemi Tray (if sample is small)
Strong Chemiluminescence[1]	10 sec.–2 min.	50 mm	Sample/Chemi Tray (if sample is small)

[1]For chemi applications, the 50mm lens is recommended. Always remove the 660 filter.

Note: For most applications, you can select an exposure time, capture an image, study it, and then adjust the exposure time accordingly. Repeat this procedure as many times as necessary to obtain a good image. For chemiluminescent samples, which degrade over time and emit low levels of light, select a high exposure time initially or use the Optimize Exposure command described on page 12.

Preview

For shorter exposures, you can use Preview to test different exposure times. Click on the Preview button to create a preview exposure and display it in the acquisition window.

Note: The camera on the VersaDoc must be at the correct operating temperature before capturing images. The temperature adjustment can take several minutes after the camera is turned on, depending on your VersaDoc model and the ambient room temperature. See your hardware manual for details.

A preview scan takes only half as long to create as a real scan, because the preview scan does not capture a “dark” image. The progress of the exposure will be displayed in the Exposure Status bar at the bottom of the dialog box.

You cannot save preview scans.

If you want to stop a preview scan that is in progress, click on the Stop button.

K.5 Acquire the Image

You can acquire a single exposure for each channel based on the time selected in Step III (Set Exposure Time), or take a series of exposures for a particular channel over a specified interval (Optimize Exposure).

Illumination Flat Fielding

For applications using the UV or white light transillumination, you should use the appropriate reference plate to ensure a uniform intensity in the image. This will compensate for normal variations in image pixel intensity that occur with a transilluminating light source.

To enable this feature, select the Flat Fielding checkbox.

Note: Flat fielding is only available in single channel, single exposure mode. If you are using multiple channels, or take multiple exposures using the Optimize Exposure feature described below, Flat Fielding will be disabled.

UV Illumination Flat Fielding: When you first select the Flat fielding checkbox, and then acquire an image using the UV transilluminator, you will be prompted to remove your sample and place the fluorescent reference plate on the VersaDoc sample stage (see the VersaDoc User Manual). A reference image of the plate will be acquired and saved on your computer hard drive. The reference image will be applied to the sample image to generate a Flat Field corrected image.

White Illumination Flat Fielding: When you first select the checkbox, and then acquire an image using the white light conversion screen, you will be prompted to remove your sample and collect an exposure of the conversion screen. A reference image of the screen will be acquired and saved on your computer hard drive. The reference image will be applied to the sample image to generate a Flat Field corrected image.

For subsequent UV or white light trans exposures, you will be prompted to either use the appropriate saved Flat Fielding image or acquire a new one. Any changes in light source, filter, or lens setting will require the acquisition of a new Flat Fielding reference image.

Acquire

Click on the Acquire button to capture a single image for each enabled channel. An exposure will be taken for each enabled channel based on the time selected for that channel in Step III.

The progress of each exposure will be displayed in the Exposure Status bar at the bottom of the acquisition window.

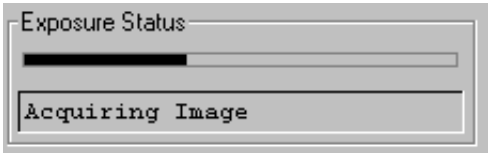


Fig.K-6. Exposure Status bar when acquiring an image.

Depending on which dark subtraction type you have selected under Options (see section K.6, Options), a dark image may be acquired immediately following image acquisition.

If you want to stop an acquisition that is in progress, click on the Stop button. The current acquisition will be terminated. If multiple channels are selected, you must click on the Stop button once per channel to stop all acquisitions.

After an image has been acquired, a separate window will pop up containing the new image. You can then analyze the image using the analysis functions.

Optimize Exposure

Optimize Exposure allows you to specify an interval over which a series of progressively longer exposures are taken. All exposures are then displayed on the screen, and you can choose the one that provides the best image.

Note: Multiple exposures will be taken for only the selected multichannel tab. Other channels, even if enabled, will not be used. Illumination Flat Fielding will be disabled if you are using Optimize Exposure.

Click on the Optimize Exposure button. A settings dialog box will open in which you can specify the total exposure time, starting exposure time, and number of exposures. (The specified number of exposures will be taken at regular intervals between the starting exposure time and the total exposure time.)

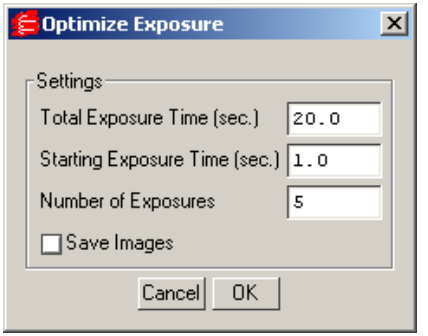


Fig.K-7. Optimize Exposure dialog.

Note: You should specify no more than 10 exposures in the Optimize Exposure dialog, to avoid excessive build up of image background in later exposures. The fewer the exposures, the less background will be added to the image. See the Release Notes for additional instructions on reducing background in images captured using Optimize Exposure.

Select the Save Images checkbox if you want to automatically save each exposure as it is taken.

Click on OK in the settings dialog to begin taking exposures. If you selected Save Images, a Save dialog box will open in which you can specify the base file name and location of the exposure files. When you click on Save, the exposures will be taken.

The specified number of exposures will be taken at equal intervals between the starting exposure time and total exposure time. The exposure status bar will show the progress of each exposure.

Depending on which dark subtraction type you have selected under Options, a dark image may be acquired immediately following each exposure.

When each exposure is complete, an image window containing that exposure will open in front of the VersaDoc window. Subsequent exposures are tiled in front of the VersaDoc window.

Note that the first exposure will have the base file name; the default base file name is the computer user name and a time stamp. Each subsequent exposure will have a version number (v2, v3, v4, etc.) appended to the base file name. (If you are using the

default base file name, the time stamp may change in the course of the series of exposures; in this case, the base file name will change and the version numbering will reset for subsequent exposures.)

The highest version number will be the final exposure. If you did not elect to auto-save the exposures as they were created, then each image will be unsaved.

To stop the image acquisitions, click on the Stop button.

Note: Exposures captured before stopping will be displayed in image windows.

Study the different images and select the best exposure(s) to keep.

K.6 Options

Click on the Options button to open the Options dialog box.

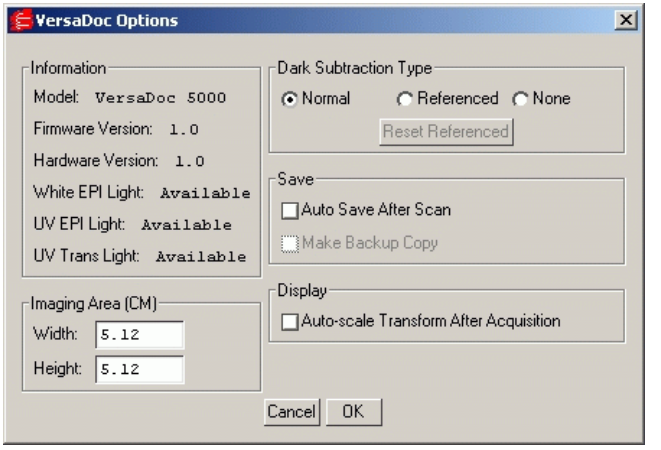


Fig.K-8. Options dialog box.

K.6.a. Dark Subtraction Type

All CCD cameras accumulate electrons that produce a signal that is indistinguishable from light. This “dark current” adds to the noise in your images, particularly for long

exposures. In most cases, you will want to subtract this dark current from your images.

The settings for subtracting the dark current are in the Options dialog box. Click on the Options button in the acquisition window to open this dialog.

Normal

The Normal option button selects the default dark subtraction type. In this mode, after you acquire an image, a “dark” image of the same exposure length will be taken, and this will be subtracted from your image.

The progress of the dark exposure will be displayed in the Exposure Status bar following the regular image exposure.

In Normal mode, a dark image is only acquired the first time you perform a scan with particular application and exposure settings. If you perform subsequent scans with the same settings, no dark exposure will be taken.

Reference

If you do not want to perform a dark exposure with each acquisition, you can take a “reference” dark exposure that will be saved and subtracted from all subsequent acquisitions. Click on the Referenced button to activate this feature.

The first time you acquire an image after selecting this option, the VersaDoc will take a dark exposure that will be saved and used to subtract the dark current from all subsequent acquisitions.

Note: The VersaDoc 1000 will take a 60-second reference dark exposure; the VersaDoc 3000 and 5000 will take a 180-second reference dark exposure.

For image exposures that are longer or shorter than the reference dark, the reference dark will be scaled accordingly and then subtracted. It is recommended that the reference dark exposure time be equal to or greater than the sample exposure time. You can change the default reference dark exposure time using the Reset Reference button (see below).

If you deselect the Referenced button and then reselect it, the old reference dark exposure will still be available.

Separate reference dark exposures will be taken for images that have different levels of binning or gain. Once you have created a reference dark for each level of binning or gain, the appropriate reference dark will be used according to the settings of your selected application.

Reset Reference

If you would like a reference dark with an exposure time that more closely matches that of your typical scans, click on the Reset Reference button.

A pop-up box will prompt you to enter a new dark exposure time in seconds.

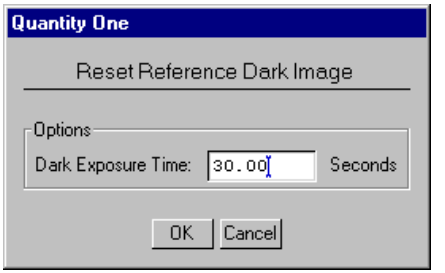


Fig.K-9. Reset Reference Dark pop-up box.

Click on OK to implement your change. The new reference dark will be created when you acquire your next image.

Because of the high sensitivity of the CCD, fluctuations in background radiation and/or temperature in the room can affect the level of dark count. If you feel that radiation/temperature conditions have changed in the room since your last reference dark was created, use the Reset Reference button to delete your old reference and create a new one under current conditions.

None

If you do not want to perform dark subtraction, select None. No dark exposure will be acquired or subtracted.

K.6.b. Save

Auto Save After Scan

To automatically save any image you acquire using the Acquire button, click on the Auto Save After Scan checkbox in the Options dialog box.

With this checkbox selected, when you click on Acquire, a Save As dialog box will open asking you to specify a file name and location for the image you are about to create. The scan will begin when you click on the Save button.

Make Backup Copy

You can automatically create a backup copy of any scan you create. To do so, first select Auto Save After Scan, then select the Make Backup Copy checkbox.

With this checkbox selected, when you save a scan, a backup copy will be placed in the same directory as the scanned image. Windows backup files will have an .sbk extension. Macintosh backup files will have the word backup after the file name.

K.6.c. Imaging Area Size

The imaging area is the area of the sample (in centimeters) that is captured by the camera and displayed in the scan window. To specify the size of this area, enter a dimension in the appropriate field under Imaging Area in the Options dialog box. Click on the Options button in the acquisition window to open this dialog.

When you change one imaging area dimension, the other will change to maintain the aspect ratio of the camera lens.

The imaging area will change depending on your zoom factor. For example, if you have zoomed in on a area that is 4.5 x 3.5 cm, then you would enter 4.5 for the width (3.5 for the height would be calculated automatically).

Note: Your imaging area settings must be correct if you want to do 1:1 printing. They must also be correct if you want to compare the quantities of objects (e.g., using the Volume Tools) in different images.

The imaging area dimensions also determine the size of the pixels in your image (i.e., resolution). A smaller imaging area will result in a higher resolution.

K.6.d. Auto-scale Transform

Auto-scale Transform after Acquisition allows the user the option of having the image automatically perform the Auto-Scale transform function upon completion of image acquisition. This eliminates the need to transform an image or re-scan an image when the acquisition time was too short or the iris not opened enough.

To enable the auto-scale transform function, check the box labeled, Auto-Scale Transform after Acquisition in the Options dialog.

K.7 Other Features

Fig.K-10. Other VersaDoc acquisition window features.

Highlight Saturated Pixels

When this box is checked, any saturated pixels in the image will appear highlighted in red in the scan window and in the pop-up image window. To view/hide saturated pixels in the pop-up image window, use the Transform command.

File Size of Images

Image File Size shows the size of the image file you are about to create. This size is determined by the resolution of the camera and any binning you perform when capturing the image.

If you do not have enough computer memory for the specified file size, an error message will appear when you attempt to acquire an image.

Macintosh users can increase the application memory partition. See your Macintosh computer documentation for guidance.

Appendix L
Cross-Platform File Exchange

It is possible to move image data between Discovery Series software applications on different platforms. There are different protocols depending on the platform (PC or Macintosh) you are transferring from and to.

L.1 Macintosh to PC

To transfer a file from a Discovery Series application running on a Macintosh to a Discovery Series application running on a PC, you need to tag the file name with the suffix appropriate to the image file type (e.g., 1-D scan).

For 1-D gels (Diversity Database, Quantity One) use the suffix: .1sc

For DNA Scans (DNA Code) use the suffix: .dsc

L.2 PC to Macintosh

PC versions of Discovery Series files can be read directly by Macintosh applications, with no required modifications.

Appendix M
Other Features

The following features are available in Quantity One, but have more utility in its more powerful companion application, Diversity Database. You can examine your gel images in Quantity One and then database them using Diversity Database.

M.1 Categories and Attributes

User-defined <category> buttons are available in the Standards dialog box, Matched Band Set dialog box, and Gel Layout dialog box. They allow you to categorize the characteristics of your particular gel or any related gel to which you might apply the same set of standards.

To define a new category, click on one of the <category> buttons. A dialog will pop up in which you can select from a list of categories or create a new one.

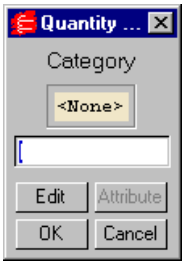


Fig.M-1. Category pop-up box.

To create a new category, click on the Edit button. This will open another pop-up box in which you can enter the name of your new category.

Fig.M-2. Edit Category dialog box.

Type the name of the new category (e.g., “Color”) next to the Category prompt and list attributes of that category in the Attribute fields (e.g., “Red,” “Green,” “Blue,” etc.). The form will automatically sort your attributes alphabetically within the Attribute fields. Categories and attributes can be defined for any characteristics of your gel that would be useful to sort by. Typical categories might be “Enzyme,” “Primer,” “Probe,” “Type,” “Who,” etc.

Once you’ve created a category and attributes, you can use them in the dialog box. Once again, click on a <category> button.

Select the category to be applied from the available categories on the list (or select <none>). Click on the Attribute button to specify an attribute. Click OK to apply your selection to the Standards box.

M.2 Gel Layout

In Diversity Database, you can use the Gel Layout dialog box to compare samples across multiple gel images. In Quantity One, you can use it to enter general information about your image.

To open the Gel Layout form, select Gel Layout from the Edit menu.

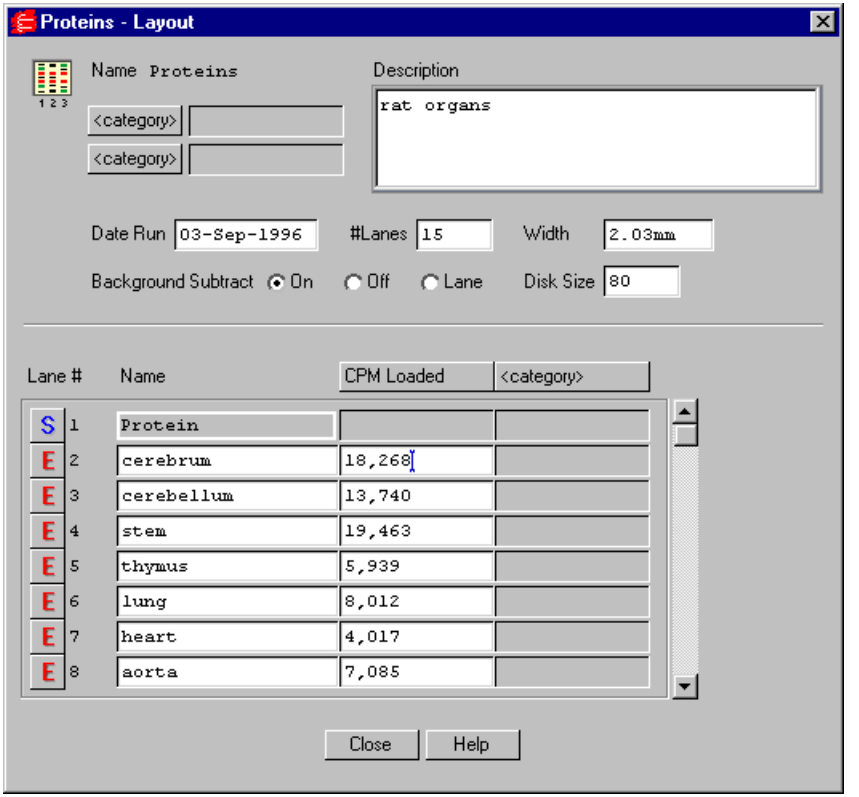


Fig.M-3. Gel Layout dialog box.

The following information about your gel can be specified at the top of the Gel Layout form:

- Category and attribute information (see above) for the whole gel.
- A brief description of the gel.
- Gel run date.
- Number of lanes.
- Lane width.

- Lane-based background subtraction and disk size.

Each lane in your gel has its own line on the Gel Layout form. The following information can be specified for a lane.

- Band set type (standard or experimental).
- Lane number.
- Band set name.
- Sample name.
- Category/attributes information (see above) for individual lanes.

The pop-up buttons on the left side of the form offer a number of choices pertaining to the individual lanes on your gel. Clicking on one will open the Lane Choices list.

Lane Report will open a customizable lane report form that can be printed or exported.

Assign Band Set allows you to select the specific band set that is to be applied to that lane.

Unassign Band Set allows you to remove the band set that is currently applied to that lane.

View Band Set displays the band set form for the band set that is currently applied to that lane.

Index

Numerics

A

B

C

D

E

F

G

H

I

K

L

M

N

O

P

Q

R

S

T

U

V

W

Z

The Discovery Series™ Image Analysis Software
Software License Agreement

READ THIS! THIS IS A LEGAL AGREEMENT BETWEEN YOU, THE CUSTOMER, AND BIO-RAD LABORATORIES, INC. ("BIO-RAD") CONCERNING THE ENCLOSED COMPUTER PROGRAM ("THE PROGRAM"). YOU SHOULD REVIEW THE FOLLOWING TERMS AND CONDITIONS OF THIS AGREEMENT CAREFULLY BEFORE OPENING THIS PACKAGE. BY OPENING THIS PACKAGE YOU INDICATE YOUR ACCEPTANCE OF SUCH TERMS AND CONDITIONS. IF YOU DO NOT AGREE WITH ANY OF THEM, YOU SHOULD PROMPTLY RETURN THE PACKAGE UNOPENED, YOUR MONEY WILL BE REFUNDED.

LICENSE

Pursuant to the license granted to you by this Agreement, you may (a) use the Program on a single computer; (b) copy the Program into any computer readable or printed form for back-up or modification purposes in support of your use of the Program on the single computer (certain programs, however, may include mechanisms to limit or inhibit copying; they are marked "copy protected"); (c) modify the Program and/or merge it into another program for your use on the single computer, and (d) transfer the Program and license to another party if the other party agrees to accept the terms and conditions of this Agreement.

Any portion of the Program merged into another program shall continue to be subject to the terms and conditions of this Agreement. If you must transfer the Program, you must at the same time either transfer all copies whether in printed or computer readable form to the same part or destroy any copies not transferred, including without limitations all modifications and portions of the Program contained or merged into other programs. You must also reproduce and include the copyright notice on any copy, modification or portion merged into another program.

YOU MAY NOT USE, COPY, MODIFY OR TRANSFER THE PROGRAM OR ANY COPY, MODIFICATION, OR MERGED PORTION, IN WHOLE OR IN PART, EXCEPT AS EXPRESSLY PROVIDED FOR IN THIS AGREEMENT. If you transfer possession or any copy modification, or merged portion of the Program to another party, your license is automatically terminated.

TERM

The license is effective until terminated. You may terminate it at any other time by destroying the Program together with all copies, modifications and merged portions in any form. It shall also terminate upon conditions set forth elsewhere in this Agreement or if you fail to comply with any term or condition of this Agreement. Upon such termination, you must destroy the Program together with all copies, modifications, and merged portions in any form.

LIMITED WARRANTY AND REMEDIES

BIO-RAD warrants the media on which the Program is furnished to be free from defects in materials and workmanship under normal use for a period of ninety (90) days from the date of delivery to you as evidenced by a copy of your receipt. This warranty is limited to you and is not transferable.

During the 90 day warranty period, BIO-RAD shall (a) replace any media item not meeting the foregoing warranty and which is returned to BIO-RAD or an authorized BIO-RAD dealer ("Authorized Dealer") with a copy of your receipt; or (b) if BIO-RAD of the Authorized Dealer is unable to deliver a replacement media item which is free of defects in materials or workmanship, you may terminate this Agreement by returning the Program, and you money shall be refunded.

The following warranty does not extent to any media item which has been damaged as a result of accident, misuse, abuse, or as a result of service or modification by anyone other than BIO-RAD or an Authorized Dealer.

EXCEPT AS EXPRESSLY SET FORTH ABOVE, THE PROGRAM IS PROVIDED "AS IS" AND NO OTHER WARRANTIES EITHER EXPRESSED OR IMPLIED, ARE MADE WITH RESPECT TO THIS PROGRAM, INCLUDING, WITHOUT LIMITATION THE IMPLIED WARRANTIES OF MECHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE. BIO-RAD WITHOUT LIMITATIONS EXPRESSLY DISCLAIMS ALL WARRANTIES NOT STATED HEREIN. YOU ASSUME THE ENTIRE RISK AS TO THE QUALITY AND PERFORMANCE OF THE PROGRAM SHOULD THE PROGRAM PROVE DEFECTIVE. YOU AND NOT BIO-RAD OR AN AUTHORIZED DEALER ASSUME THE ENTIRE COST OF NECESSARY SERVICING REPAIR OR CORRECTION. SOME STATES DO NOT ALLOW THE EXCLUSION OF IMPLIED WARRANTIES, SO THE ABOVE EXCLUSION MAY NOT APPLY TO YOU. THIS WARRANTY GIVES YOU SPECIFIC LEGAL RIGHTS, AND YOU MAY ALSO HAVE OTHER RIGHTS WHICH VARY FROM STATE TO STATE.

BIO-RAD DOES NOT WARRANT THAT THE FUNCTIONS CONTAINED IN THE PROGRAM WILL MEET YOUR REQUIREMENTS OR THAT THE OPERATION OF THE PROGRAM WILL BE UNINTERRUPTED OR ERROR-FREE. YOU ASSUME RESPONSIBILITY FOR THE SELECTION OF THE PROGRAM TO ACHIEVE YOUR INTENDED RESULTS, AND FOR THE INSTALLATION, USE AND RESULTS OBTAINED FROM THE PROGRAM.

YOUR SOLE REMEDIES AND BIO-RAD'S ENTIRE LIABILITY ARE SET FORTH ABOVE, IN NO EVENT WILL BIO-RAD BE LIABLE TO YOU OR ANY OTHER PERSON FOR ANY DAMAGES, INCLUDING WITHOUT LIMITATION ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, EXPENSES, LOST PROFITS, LOST SAVINGS OR OTHER DAMAGES ARISING OUT OF THE USE OR INABILITY TO USE

SUCH PROGRAM EVEN IF BIO-RAD OR AN AUTHORIZED DEALER HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES, OR FOR ANY CLAIM BY ANY OTHER PARTY.

SOME STATES DO NOT ALLOW THE LIMITATION OR EXCLUSION OF INCIDENTAL OR CONSEQUENTIAL DAMAGES FOR CONSUMER PRODUCTS, SO THE ABOVE LIMITATIONS OR EXCLUSIONS MAY NOT APPLY TO YOU.

GENERAL

You may not sublicense, assign, or transfer the license or the Program except as expressly provided in this Agreement. Any attempt to otherwise sublicense, assign or transfer any of the rights, duties or obligations hereunder is null and void and not merely voidable.

If you have any questions concerning this Agreement, including warranty service, you should contact BIO-RAD LABORATORIES, INC., 1000 Alfred Nobel Drive, Hercules, CA 94547.

This Agreement shall be governed by the laws of the state of California as applicable to contracts between residents of California which are entered into and performed entirely within California.

YOU HAVE ACKNOWLEDGED THAT YOU HAVE READ THIS AGREEMENT, UNDERSTAND IT, AND AGREE TO BE BOUND BY ITS TERMS AND CONDITIONS. YOU FURTHER AGREE THAT IT IS THE COMPLETE AND EXCLUSIVE STATEMENT OF THE AGREEMENT BETWEEN US WHICH SUPERCEDES ANY PROPOSAL OR PRIOR AGREEMENT, ORAL OR WRITTEN, AND ANY OTHER COMMUNICATIONS BETWEEN US RELATING TO THE SUBJECT MATTER OF THIS AGREEMENT.

Bio-Rad Laboratories, Inc.

Life Science Group

Web site www.bio-rad.com **USA** (800) 4BIORAD **Australia** 02 9914 2800 **Austria** (01)-877 89 01 **Belgium** 09-385 55 11 **Brazil** 55 21 507 6191
Canada (905) 712-2771 **China** (86-21) 63052255 **Denmark** 45 44 52-1000 **Finland** 358 (0)9 804 2200 **France** 01 47 95 69 65 **Germany** 089 318 84-177
Hong Kong 852-2789-3300 **India** (91-124) 6398112/113/114, 6450092/93 **Israel** 03 951 4127 **Italy** 39 02 216091 **Japan** 03-5811-6270
Korea 82-2-3473-4460 **Latin America** 305-894-5950 **Mexico** 52 5 534 2552 to 54 **The Netherlands** 0318-540666 **New Zealand** 64-9-4152280
Norway 47-23-38-41-30 **Portugal** 351-21-472-7700 **Russia** 7 095 721 1404 **Singapore** 65-2729877 **South Africa** 00 27 11 4428508 **Spain** 590 5200
Sweden 46 (0)8-55 51 27 00 **Switzerland** 061 717-9555 **United Kingdom** 0800-181134