



Molecular Analyst™/PC

**Image Analysis Software
for the
Model GS-670
Densitometer**

Instruction Manual

**Catalog Number
170-7500**

For Technical Service
Call Your Local Bio-Rad Office or
in the U.S. Call **1-800-4BIORAD**
(1-800-424-6723)

BIO-RAD

Warranty

Bio-Rad Laboratories warrants that the Molecular Analyst/PC software shall substantially conform, in all operational features, to Bio-Rad's current specifications as published in Bio-Rad's user and installation guides and that, when properly installed, it will be free of material defects which affect system performance.

The Purchaser must notify Bio-Rad in writing, within 30 days of delivery of the software (not including delivery of any subsequent modifications to the software), of any defect. If the software is found to be defective by Bio-Rad, Bio-Rad's sole obligation under this warranty is to remedy the defect in a manner consistent with Bio-Rad's regular business practices. For a defect which adversely affects the performance of the software, Bio-Rad shall use its best efforts to cure such defect as soon as reasonably practicable after receipt of Purchaser's notice. For minor defects, Bio-Rad shall use its best efforts to correct such minor defects in the next release of its software. If, however, Bio-Rad is unable to cure a major defect within 90 days of receipt of Purchaser's notice, Purchaser shall have the option to cancel this agreement, whereupon Bio-Rad shall refund only the software fees paid.

The warranties set forth in this agreement are in lieu of all other representations and warranties, expressed or implied, including warranties of merchantability and fitness for a particular purpose and any other statutory or common-law warranty. Bio-Rad on its own behalf expressly disclaims and excludes any and all such other representations and warranties. Liability of Bio-Rad to Purchaser, if any, for breach of warranty, or any other claim relating to this agreement, shall be limited to the total amount of software fees paid by purchaser to Bio-Rad. In no event shall Bio-Rad be liable for incidental or consequential damages, loss of business or profits, special or indirect damages of any nature whatsoever. No amendment, waiver, or other alteration of the warranties in this agreement may be made except by mutual agreement in writing.

Purchaser agrees that Bio-Rad's liability arising out of contract, negligence, strict liability in tort or warranty shall not exceed the amount of software license fees paid by Purchaser.

This manual and the software (computer program) described in it are copyright Bio-Rad Laboratories, Inc. with all rights reserved worldwide. Under the copyright laws, this manual and the software program contained herein may not be copied, in whole or in part, without the prior written consent of Bio-Rad, except in the normal use of the software or to make a backup copy. This exception does not allow copies to be made for others, whether or not sold, but all of the materials purchased (with all backup copies) may be sold, given or loaned to another person. Under the law, copying includes translating into another language or format.

A multi-use license may be purchased to allow the software to be used on more than one computer owned by the purchaser, including a shared disk system.

Catalog No. _____

Date of Delivery _____

Warranty Period _____

Serial No. _____

Invoice No. _____

Purchase Order No. _____

Table of Contents

Section 1	Molecular Analyst™\PC Software	
1.1	Introduction	6
1.2	System requirements.....	7
1.3	Optional storage and output devices.....	7
1.4	Data Analysis System-PC4.....	8
1.5	Molecular Analyst/PC system setup.....	8
1.6	PC GPIB connection.....	9
1.7	Notational conventions.....	9
1.8	Installing the Molecular Analyst/PC Software	10
Section 2	Tutorial: Learning to use the Molecular Analyst/PC Software	
2.1	Lesson 1: Displaying and optimizing an image.....	13
	Part 1 Starting the program	
	Part 2 Displaying an image	
	Part 3 Windows manipulation	
	Part 4 Optimizing an image	
	Part 5 Saving an image	
	Part 6 Printing an image	
2.2	Lesson 2: Area integration.....	17
	Part 1 Creating a profile	
	Part 2 Optimizing the profile	
	Part 3 Enhancing the data	
	Part 4 Identifying and integrating peaks	
	Part 5 Saving the profile	
	Part 6 Printing reports and profiles	
2.3	Lesson 3: Molecular weight determination.....	22
	Part 1 Displaying and optimizing a standard profile	
	Part 2 Identifying peaks on the standard profile	
	Part 3 Creating a standard curve	
	Part 4 Calculating the molecular weights of an unknown	
	Part 5 Exporting profile reports and profile data points	
	Part 6 Moving within Profile Analysis and Molecular Analyst	
2.4	Lesson 4: Volume integration.....	28
	Part 1 Identifying spots	
	Part 2 Creating a volume integration report	
	Part 3 Generating a standard curve and calculating concentration	
	Part 4 Exporting volume data to other applications	
2.5	Lesson 5: Data acquisition and manipulation (Scanning).....	32
	Part 1 Setting up the GS-670 Imaging Densitometer	
	Part 2 Starting the Molecular Analyst/PC program	
	Part 3 Setting scan parameters	
	Part 4 Identifying the scanning area	
	Part 5 Starting the scan	
	Part 6 Saving the scan as an image	
	Part 7 Deleting image files	
2.6	Tutorial Summary	35
Section 3	Description of Palettes	
3.1	The Tool Palette	38
3.2	The Modify View Palette	40

Section 4 Description of Molecular Analyst Menus

4.1	The HELP menu	42
4.2	The FILE menu	43
4.3	The EDIT menu	44
4.4	The VIEW menu	45
4.5	The TOOLS menu	46
4.6	The ANALYSIS menu	47
4.7	The TRANSFORMATIONS menu	48
4.8	The WINDOW menu	51

Section 5 Molecular Analyst/PC Modules: Descriptions and Uses

5.1	Profile Analyst Module	
	Part 1 Introduction.....	52
	Part 2 Profile Analysis Tool Palette.....	52
	Part 3 Description of Profile Analysis Functions and Menus	
	Display Function.....	56
	A MODE menu.....	56
	B FILE menu.....	57
	C PROFILE menu.....	58
	D OPTIMIZE menu.....	60
	E ENHANCE menu.....	64
	F PEAKS menu.....	65
	G SCALE menu.....	69
	H OPERATION menu.....	69
	I SETUP menu.....	70
	J Right Mouse Button.....	71
	Molecular Weight Function.....	72
	A MODE menu.....	72
	B FUNCTIONS menu.....	73
	Report Function.....	79
	A MODE menu.....	79
	B FUNCTIONS menu.....	79
5.2	Volume Analyst Module	81
	Part 1 Introduction.....	81
	Part 2 Volume tool Palette.....	81
	Part 3 Automatic Spot Detection.....	85
	Part 4 Description of the volume report.....	86
	Part 5 Editing the volume report.....	87
	Part 6 Description of the volume analysis OPTIONS menu.....	90
	Part 7 Description of the standard curve.....	91

Section 6 Appendices

Appendix A:	Computer System Configuration.....	93
Appendix B:	Transferring Image Files and Data File Management.....	95
Appendix C:	Use of Estimated Molecular Weights.....	96
Appendix D:	File Size and Resolution.....	97
Appendix E:	Regression Analyses.....	98
Appendix F:	Edge Enhancement.....	100
Appendix G:	Troubleshooting.....	100
Appendix H:	Technical Support.....	101

Section 1 Molecular Analyst/PC Software

1.1 Introduction

The Molecular Analyst™/PC image analysis software is a powerful tool for controlling and analyzing images and data from the Model GS-670 Imaging Densitometer. Operating under Microsoft® Windows® 3.1 software, the Molecular Analyst/PC software uses a series of pull down menus, tool palettes, and mouse commands to allow you to easily scan, display, analyze, and output data from virtually any transparent or opaque sample. The determination of valuable information such as sample concentration or amount of macromolecule, relative mobility, and molecular weight, is easily performed with the Molecular Analyst/PC graphical interface.

It is recommended that the first-time user follow the tutorial section of this manual to become acquainted with some of the Molecular Analyst/PC functions and features. More detailed information about each function is contained in the sections following the tutorial.

The Molecular Analyst/PC software version 1.1 offers the following features:

Image Acquisition

- Scanning control of the Model GS-670 Imaging Densitometer

Image Display

Molecular Analyst/PC

- Color and gray scale display
- Rapid brightness and contrast adjustment
- High frequency noise filtering and dynamic range selection
- Image rotation and flipping
- Image cropping

Volume Analysis

- Numerous object tools for simplified volume integration
- 96, 48, or custom grid matrices
- Automatic spot and band detection
- Report table with sorting and exporting capabilities
- Standard curve generation and calculation of unknown concentrations, molecular weights, and pIs

Profile Analysis

- Multiple profile display
- High frequency noise filtering
- Variable zoom tool to magnify any section of the profile
- Integration tool to manually determine the area under the profile curve
- Automatic identification and integration based on user criteria
- Gaussian curve fitting of overlapping peaks
- Print profile graphs at any point in the analysis
- Print peak report list integrals, peaks, and standards

Molecular Weight Determination

- Generate molecular weight standard calibration curves for best fit determination
- Calculate unknown molecular weight values

1.2 System Requirements

The Molecular Analyst/PC software requires the use of an external AT compatible 16 bit PC computer system compatible with DOS version 5.0 or better and Microsoft Windows version 3.1. The following are the recommended PC computer system requirements:

	<u>Recommended</u>
Central Processing Unit:	80486 AT compatible computer
Floppy Disk Drives	3.5", 1.44 mb disk drive
Hard Disk Drive	240 + mb
RAM Memory	16 + mb
Windows:	Version 3.1
Monitor:	256 gray level color monitor
Graphics Adapter:	ATI 8514 Ultra 256 Gray Level Graphics Engine or equivalent
Keyboard:	Extended keyboard
Mouse:	Windows 3.1 compatible mouse
Interface	Standard GPIB interface cable
Interface Adapter	GPIB-PCI/A adapter

Due to the large potential file sizes generated by the Model GS-670 Imaging Densitometer system, Bio-Rad recommends Data Analysis System PC4 (Bio-Rad catalog number 170-7363) to insure Molecular Analyst/PC

optimum instrument and software performance. Compatibility and performance of all other computers not purchased from Bio-Rad are the sole responsibility of the user. Please check with your local Bio-Rad representative regarding compatibility of your specific brand of computer.

1.3 Optional Storage and Output Devices

Optional storage and output devices are available through Bio-Rad Laboratories to improve image storage, manipulation, and output.

Storage Devices:

- Iomega 90 mb Transportable/Removable Media Disk Drive-PC (Bio-Rad cat. no. 170-7339)
- 90 mb Cartridges (Bio-Rad cat. no. 170-7341)
- Sony 128 mb or 650 mb rewritable optical drive (Bio-Rad cat. no. 170-7420, 128mb; 170-7421, 650mb)
- PC or Mac rewritable optical drive interface kit (Bio-Rad cat. no. 170-7422, PC; 170-7423, Mac)
- 128 mb or 650 mb rewritable optical disk (Bio-Rad cat. no. 170-7424, 128mb; 170-7425, 650mb)

Output Devices:

- GCC ColorFast Digital Film Recorder (Bio-Rad cat. no. 170-7345)
- GCC ColorFast PC Kit (Bio-Rad cat. no. 170-7346)
- Mitsubishi 256 Gray Level Video Printer (Bio-Rad cat. no. 170-7350)
- VGA, PC Cable for the Mitsubishi Video Printer (Bio-Rad cat. no. 170-7411)

1.4 Bio-Rad's Data Analysis System-PC4

If purchased from Bio-Rad Laboratories, the Data Analysis System-PC4 computer platform (catalog number 170-7363) should arrive complete with the following:

<u>Qty</u>	<u>Item</u>
1	AST® Premia 486/50 Hz CPU, 16 mb RAM, 340 mb hard disk (Includes 5.25" & 3.5" disk drives, local bus graphics engine, Microsoft Windows version 3.1)
1	NEC 3FGX 15" Color Monitor
1	Extended Keyboard
1	Logitech® Mouse

If your system is missing any of these items, immediately contact your local Bio-Rad representative for a prompt replacement.

1.5 Molecular Analyst/PC System Setup

System Hardware Configuration

Refer to your computer owner's manual for connection of mouse, keyboard, and printer.

NOTE: TURN OFF ALL EQUIPMENT PRIOR TO MAKING ANY CONNECTIONS!

1. Position the computer on a hard, flat location that is readily accessible to the GS-670 Imaging Densitometer.

2. Attach the monitor interface cable to the ATI® 8514 Ultra 256 gray level graphics card. Computers not purchased through Bio-Rad require a ATI® 8514 Ultra 256 gray level graphics card, equivalent 256 gray level graphics card, or equivalent 256 gray level local bus graphics chip (See appendix A, I for graphics card configuration). NOTE: With Bio-Rad's Data Analysis System-PC4 or computers with local bus, attach the monitor interface cable to the monitor port.

3. Attach the mouse cable to serial port 1. **NOTE: With Bio-Rad's Data Analysis System-PC4, do not attach the mouse cable to the circular port labeled "Mouse".**

4. Attach the keyboard interface cable to the circular port labeled "Keyboard".

The CPU and monitor power cords should be plugged into a high quality surge suppresser to avoid damage from A/C voltage fluctuations.

System Software Configuration

For detailed configuration instructions, please refer to your computer system's users manual.

1. Please refer to your computer owner's manual for system and printer setup.

2. Your system's DOS directory should include TEMP directory, ATI® 8514 Ultra 256 gray level graphic engine(if present), GPIB, and Windows 3.1 driver directories (See appendix A, II) .

3. The AUTOEXEC.BAT, CONFIG.SYS, and Windows Setup files should be appropriately configured for a system using a Microsoft Windows environment with enabled cache memory (See appendix A, III - V)

1.6 PC GPIB Connection

The PC to GPIB connection requires that a GPIB-PCIIA adapter and GPIB software be installed in the IBM AT compatible 16 bit PC. The GPIB-PCIIA adapter and software is available when purchasing the GS-670 system from Bio-Rad (170-7500). See appendix A, VI for a quick guide to installing the GPIB software.

To connect the computer to the Model GS-670 Imaging Densitometer, first **turn off** all equipment prior to making any connections. Next, plug one end of the standard GPIB connector into the densitometer's GPIB port. Now connect the other end of the GPIB connector to the GPIB adapter in your IBM AT compatible 16 bit PC. **Note: Start your densitometer before starting your computer.**

1.7 Notational Conventions

Keyboard

Throughout this manual, characters enclosed in brackets <> stand for a key on the keyboard. For example, <ENTER> designates that the large key labeled **Enter** be pressed. Whenever you are supposed to type something at the keyboard, the instructions will say: "Type: ". For example, Type: EXAMPLE <ENTER> instructs you to type in the characters EXAMPLE and then press the **Enter** key.

Mouse

Unless otherwise specified, all "clicking" using the mouse is performed with the **left mouse button** (LMB). The center mouse button, if present is not functional with the Molecular Analyst/PC software. The right mouse button (RMB) is active in Profile Analyst and is only functional when the mouse cross hair is on top of a graph containing an active trace.

Objects

Throughout this manual we refer to objects within the image window. Objects can be generated and positioned on an image using either the Text, Profile or Volume tools from the tool palette. Text objects are used to annotate images or number objects. Profile objects (Line, Multi-Segment, and Averaging Rectangle) are used to create profiles for area integration and molecular weight determination. Volume objects (Circle, Ellipse, Rectangle, Square, and Grid) are used to create volume reports for volume integration and concentration, molecular weight, and pI calculation. All of the objects can be moved and manipulated within the image window.

Active Objects

The only objects which are currently "live" and can have any function or operation performed upon them. Active objects are denoted by their color. Active objects and text will be *Fuschia* colored unless this active color is changed within the *Preferences Setup* command under the FILE menu. To make an individual object active when multiple objects are displayed, simply double click on the desired object. To make multiple objects active use the block select tool. Multiple active objects will generate multiple profiles within one graph.

Active profile

Active profiles are denoted by their color (Fuschia unless the default colors have been changed). When more than one graph is displayed on the screen, the active graph will be the graph containing the active profile. To make a different graph active, simply double click on one of the profiles within that graph.

View Modification

A view modification is a modification which affects only the display or viewing of the data, but does not actually modify the data itself.

Data Modification

A data modification is a modification which not only affects the viewing of the data, but actually transforms the data itself.

1.8 Installing the Molecular Analyst/PC Software

Check the contents of the Molecular Analyst/PC package for the following items:

1. Molecular Analyst/PC Software
2. Bio-Rad Software Registration Card
3. Molecular Analyst/PC Users Manual

Please take this time to completely fill out your product registration card. As a registered user, you will be notified regarding software upgrades, improvements and disk replacements.

The Molecular Analyst software is supplied on two 1.44 mb 3.5" microfloppy disks. One disk is labeled "Setup" and one disk is labeled "Program".

If not already installed, install Windows 3.1 software on to your hard drive as described in the Microsoft Windows 3.1 Instruction Manual. Molecular Analyst software is a Windows 3.1 application and is designed to run within the Windows 3.1 (Enhanced Mode) operating environment.

Install the Molecular Analyst/PC Program as follows:

1. Insert the Molecular Analyst software 3.5" floppy disk labeled "**Setup**" into disk drive A
2. Double click on the File Manager program icon found in the "Main" group of Program Manager.
3. Click on the disk drive icon A
4. Double click on the file: SETUP.EXE

A Molecular Analyst Setup window appears. Select <continue> to begin the setup process

Install to: automatically appoints the C:\ drive as the program destination. The Setup program also determines the necessary space required for installation of the Molecular Analyst/PC program file and image files; and the space available on Drive C. To designate drive C for installation, use the mouse to select <Install>. To designate another location, select <Set Location> and choose an alternate path. For more information select <Help>.

- At the message prompt, insert the floppy disk labeled "Program" into drive A and click <OK>
- The setup program will indicate if the setup was successful. If setup was successful click <OK>.
- The Molecular Analyst icon will appear in the Molecular Analyst group box.
- Start the Molecular Analyst/PC program by double clicking on the Molecular Analyst icon.

Note: Make sure that the entire content of the Molecular Analyst/PC program is copied to the hard drive. Contact your local Bio-Rad representative if you are experiencing difficulties with installation. In the U.S.A., call Bio-Rad Technical Service at 1-800-4BIORAD.

Section 2

Molecular Analyst/PC

Learning to Use the Molecular Analyst/PC Software

Using the Molecular Analyst/PC program you can quickly direct the Model GS-670 Imaging Densitometer to scan in transmittance or reflectance modes, display the image, optimize the image, perform volume or area integration, calculate the molecular weights or concentrations of the unknown substances, and print the results. This tutorial introduces the basic skills that you will need to perform these functions. An on-line help feature is available, under the Help menu in the main menu bar, to provide additional assistance as you go through the program. For more information about the help feature, refer to Section 3.1.

Lesson 1: Display and Optimization

In Lesson 1 of this tutorial you will learn to display and optimize an example image provided with the software.

Lesson 2: Area Integration

In Lesson 2 you will learn how to use the different tools to perform area integration. You will learn how to extract a profile from an example image; identify and quantitate peaks from the profile; optimize the profile by subtracting background and reducing noise; and finally, integrate profile peaks.

Lesson 3: Molecular Weight Determination

In Lesson 3 you will learn how to find the molecular weight of an unknown sample. A standard curve will be generated using the standard profile data, and the resulting scatter plot will be analyzed for best fit determination. Finally, you'll determine the molecular weight of peaks identified in an unknown sample profile.

Lesson 4: Volume Integration

In Lesson 4 you will learn how to use the different volume tools to identify bands, perform volume integration, and generate volume reports. A standard curve will be generated and the concentrations of the unknowns calculated.

Lesson 5: Data Acquisition and Manipulation

In Lesson 5 you will learn how control and acquire data from the GS-670 Imaging Densitometer.

2.1 Lesson 1: Displaying and Optimizing an Image

In this lesson, we will work with the example image file provided with the Molecular Analyst/PC software. We will first call the image up for display. Next, we will optimize and smooth the image to reduce high frequency noise and to enhance visualization.

Part 1: Starting the Densitometer System and Molecular Analyst Program-

With both the densitometer and host computer off, start the densitometer. Next, wait approximately 30 seconds and then start your computer. Start the Molecular Analyst/PC program by double clicking on the Molecular Analyst icon. The program will load. After the title screen, the Image window will appear.

Part 2: Displaying an Image-

To display the example image provided, click on the FILE menu, and drag the mouse to *Open*. Double-click on the file name "EXAMPLE.IMG". The image will then be displayed with metric rulers in the image window at 100% scale. Hide the rulers using the *Hide Rulers* command under the VIEW menu to display the image as in Figure 1.

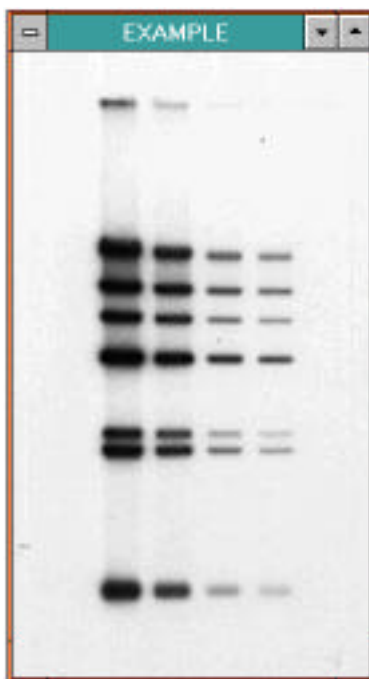


Figure 1. Example image in Image Window

Click on the VIEW menu and drag the mouse to the command *Scale to Fit* . This command allows the image to always fill the entire display window. As the display window is enlarged or reduced by a 2:1 ratio, the image is altered proportionately to correspond to the new window size.

Part 3: Windows Manipulation

The Molecular Analyst/PC software allows windows of different images, profiles, tools, or reports to be displayed, moved, and sized. Multiple windows may be arranged on the screen to suit your needs.

Moving Windows:

Windows which do not fill the entire screen may be easily relocated on the screen. Moving a window can reveal portions of other windows that are partially or totally hidden from view. To move a particular window, point to the title bar of the window, click the mouse, and drag the title bar to its new location. A gray outline will show the progress of the move. When you release the button on the mouse, the window will be re drawn. Move EXAMPLE.IMG to the center of the screen.

Sizing a Window:

If a window does not fill the entire screen, its size may be adjusted by the user. To change the size of the window, first point to the border of the window you wish to size. The mouse arrow will be replaced by two opposing arrows. Drag the border of the window in the desired direction. A gray outline will show the progress of the sizing. When you release the button on the mouse, the window will be sized according to the new dimensions. An image window will not size if the command *Scale to Fit* has not been selected from the VIEW menu. Additionally, the size of an image will only increase or decrease in proportion to the original scanned sample size, ie. a thin rectangular sample cannot be sized into a large square display window. Click on the border of EXAMPLE.IMG and increase and decrease the size of the display window.

Minimizing a Window:

An ICON is a compact representation of a window. Because no interaction can take place with a window in this form, an icon is usually used to suspend operation of a program (without exiting the software), so that the user can interact with another program. Click on the minimize box, the downward-pointing arrow in the upper right corner of the window, of the EXAMPLE.IMG. Notice that the image has been reduced to an icon near the bottom of the screen. This feature allows other images, tools, and objects to be opened and readily available. Double click on the Icon to restore the image.

Maximizing a Window:

A window may be maximized by pointing and clicking on the upward-pointing arrow in the upper right corner of the window. To restore the window, click on the window menu bar and select restore or double click on the double-sided arrow adjacent to the HELP menu.

Part 4: Optimizing an Image-

Throughout this tutorial we will refer to image optimization, Tool palette, and Modify View palette. These features of the Molecular Analyst/PC program makes optimization of images fast and simple. **Note that area and volume integration results are generated from the raw or filtered image data and not from enhanced, transformed, or modified data.** If not displayed, choose *Show Tools* from the TOOLS menu to display the Tool Palette as shown in Figure 2. Choose *Show Palette* from the VIEW menu to display the Modify View Palette as shown in Figure 3. These floating palettes can be moved to any location of the display screen. A detailed description of all palettes is given in Section 3.



Figure 2. The Tool Palette.



Figure 3. Modify View Palette

Magnifying the Image-

Molecular Analyst/PC

Because the example image is small, begin optimizing the image by magnifying the image to facilitate visualization. Clicking on the magnify tool icon (magnifying glass) on the tool palette, changes the cursor to a magnifying glass when placed on the image. Click on the center point in the center of the image to magnify the image by a factor of two from that point (Figure 4). A 1600% magnification is possible with the magnification tool. To undo magnification stepwise, hold the <Shift> key and click on the center point. To view sections of the magnified image use the scrolling cross. Click on the scrolling cross icon. While holding down the left mouse button, drag in any desired direction, and release the mouse button. Return to the unmagnified image by selecting Show All from the VIEW menu.

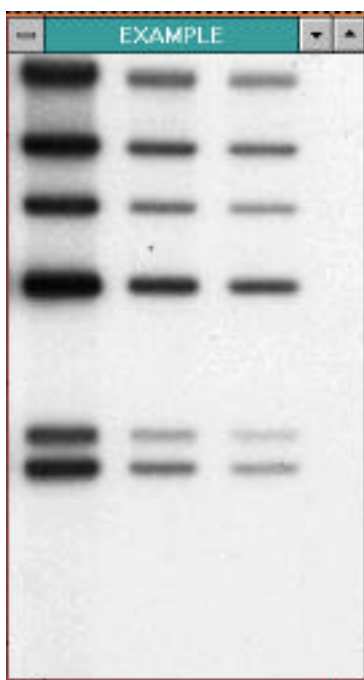


Figure 4. Magnifying an image

Filtering background-

We will now smooth the image data to reduce high frequency background noise using one of the many filters available in Molecular Analyst. Choose *View Filters* from the TRANSFORMATIONS menu. Select 3 x 3 kernel size for the Smooth (Weighted Mean) filter and click OK. The filtered image is then redrawn. View filters filter view data only and do not affect the quantitative nature of the image data; whereas raw filters filter raw data and will change the quantitative nature of the data. A detailed description of filtering is given in Section 4.

Improving visual sensitivity -

Transformation

Transformation provides a first level sensitivity adjustment to select areas of the image. Using the transformation histogram palette, it is easy to adjust gray levels or change the type of transformation to allow sensitivity adjustments to select areas of the image. Raising the minimum pixel value and lowering the maximum pixel value (a trial and error procedure) reduces the range for the distributed 256 gray levels. This can provide for greater detail to specific sections of the image. As the minimum pixel value is raised, detail to faint bands is increased. Non-linear transformation and histogram equalization are two other transformations available for optimizing images. Note that background removal is functional through transformation only over a small range. Filtering and altering brightness / contrast are recommended for background removal.

From the TRANSFORMATION menu, select *Transform*. This will display the transformation histogram palette. Next, move the right and left tab markers to the right and left ends of the histogram plot. Notice that the minimum and maximum pixel values, and the histogram plot change as the tabs are moved. Moving the tabs to the areas of the histogram plot, distribute the available gray levels to the image areas and not to background. To view changes to the image, click on the *Preview* button. To modify the histogram selection, select the *Zoom Out* button and move the tab markers to a new location. Click on the *Preview* button to view the modifications. To apply changes to the image, click on the *OK* button. The image will be redrawn according to the range selected. To return to the original histogram plot, click on the *Reset* button and then select *Preview* and *OK*. Clicking on the *OK* button will close the histogram palette. A detailed description of the TRANSFORMATION menu is given in Section 4.

Equalization scroll bar

The equalization scroll bar on the Modify View palette provides a second level sensitivity adjustment to particular areas of the image. Using the Modify View palette's equalization scroll bar, we can further enhance specific bands within the image increasing the distinction between the bands and the background. This one step sensitivity adjustment maps the 8-bit image data in a variety of different 8-bit, 256 gray level displays to allow additional sensitivity adjustment of select areas of the image. Scrolling left generates a convex equalization curve, decreasing the sensitivity of faint bands while at the same time enhancing highly visible or partially resolved bands. Scrolling right generates a concave equalization curve, increasing the sensitivity of faint bands while obscuring readily visible or partially resolved bands. Use the scroll bar to visually enhance most of the bands of lane 1 and 2 on EXAMPLE.IMG.

Altering brightness and contrast-

We will now adjust the contrast of the image using brightness and contrast tools singularly located within the gray/color scale look-up table (LUT) window. The brightness and contrast tools allow even further image enhancement and band to background distinction by compressing (Contrast) and shifting (Brightness) the 256 gray levels.

Place the mouse cursor at either the top or bottom end of the continuous gray scale window until the cursor changes to a *Circled C*. Hold the mouse button down and move this contrast tool up vertically, adjusting the contrast of the image to the point where all bands in lanes 1 and 2 are clear and distinct.

To adjust brightness, place the cursor within the continuous gray or color scale. The cursor will change to a *Circled B*. While holding down the left mouse button, slide the brightness tool vertically until the desired brightness is achieved. **Note that the brightness tool is only functional after image contrast has been modified and gray scale LUT compressed.** To undo all modifications made with the Modify View palette, use the mouse to select *Reset*. A detailed description of the Modify View palette is given in Section 3.

Part 5: Saving An Image-

Save this image by selecting *Save As* from the FILE menu. Type in EXAMPLE1.IMG and click <OK>. All of the modifications made to the image will be saved as a (*.IMG) file. This Bio-Rad Image File Format (BRIFF) is the default. Save all images to be analyzed by Molecular Analyst/PC in the BRIFF format. A detailed description on saving images as 8-bit TIFF files is provided in Section 4.

Part 6: Printing An Image-

Hard copy output of the optimized image can be obtained by selecting *Print* from the FILE menu, or by pressing *Print* on your video printers front panel. The *Page Setup* command within the FILE menu allows the size and position of the image to be changed. The type and quality of the output will depend upon the particular printer system. Molecular Analyst/PC will support most Windows 3.1 compatible printers. If you are using a film recorder to generate prints or slides, please refer to your film recorder instruction manual.

Molecular Analyst/PC

2.2 Lesson 2: Area integration

In this lesson, we will work with the optimized image from lesson 1. First we will generate a profile. Next, we will optimize the profile by subtracting background, removing high frequency noise, and direct the program to identify and integrate peaks of interest. Finally, we will save the profile and print the accompanying peak report.

Part 1: Creating A Profile-

If not already displayed, open the image "EXAMPLE1.IMG" that was optimized in lesson 1. **Note that profiles are generated from the raw or filtered data and not from visually modified data.** From the ANALYSIS menu, select *Profile Analysis*. The profile tools will appear on the right side of the tool palette (Figure 5). (Additional information on the Profile Tools are in Section 5.1). Double click on the first profile tool (single line tool) to bring up the *Profile Tool Preferences* dialog box (Figure 6). Choose the following preferences: Line Option with Unrestricted Orientation. Click on *Save Settings* box and then click on OK.



Figure 5. Tool Palette with Profile Tools.

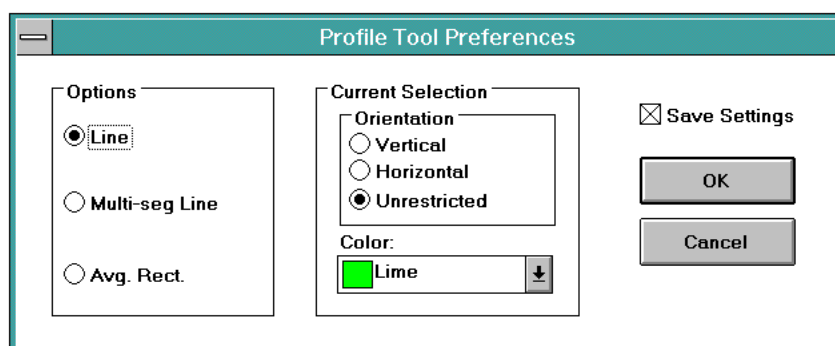


Figure 6. Profile Tool Preferences

To create a profile object for area analysis, place the line cursor above the first band over the center of the first lane of data. Click the mouse button and drag the mouse down until the line has completely passed through each band on the lane, and release the mouse button. A Fuschia colored line with squares on each end will appear (Figure 7). This is the active object and will generate the active profile. Next, go to the tool palette and click on the Text icon. Notice that the cursor has now changed to a T with a cross hair in the upper left corner when placed on the image. Position the T at the top of the lane and click the mouse button. This will display a text entry box. Backspace to remove the auto number and type in "SAMPLE1" and click on the Text button. This will display the Font Preferences dialog box. Select a 9 point font size and click OK. Click OK again to display the text.

Select *Save as...* from the FILE menu and type in EXAMPLE2.IMG in the file name box. Saving at this point will save the image, object and text. This option is convenient and prevents you from recreating and repositioning objects and text on your image.

Next, select the pointer tool from the tool palette and then double click on the line object. A *Profile Name* dialog box will appear. Name the profile SAMPLE1. To display the new profile, go to the ANALYSIS menu and select *Analyze Profile(s)*. A display screen will appear with the profile name SAMPLE1.000 and the menu commands for Profile Analyst (Figure 8).



Figure 7. Example 1 with line passing through lanes

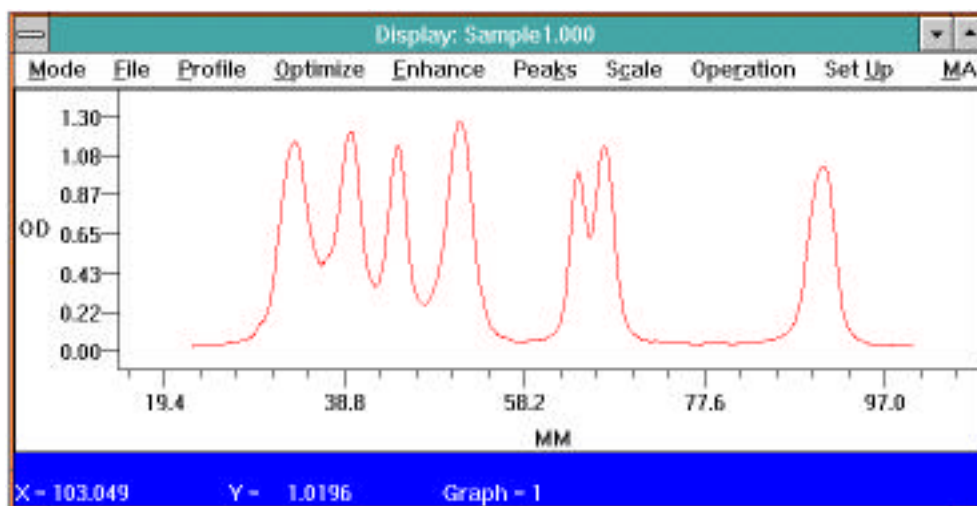


Figure 8. Profile Analyst display screen

Part 2: Optimizing The Profile-

We will begin to optimize the profile by subtracting out sample background data. First, generate a background profile that is the same length as the sample profile, by duplicating the profile object used to create the sample profile. To do this, return to Molecular Analyst by clicking on the MA menu located in the upper right corner of the Profile Analyst window. Now place the cursor on the center of the line used to create the sample profile. Notice that the cursor has changed to the curved movement arrow. Next, while pressing the <Ctrl> key, click the mouse button and drag the duplicated object to a blank area next to the sample lane. This new object is now the active object. Double click on this new line and enter the name BACKGROUND in the *Profile Name* dialog box. Display the new profile by selecting *Analyze Profile(s)* from the ANALYSIS menu. Another profile graph will appear with the new profile labeled BACKGROUND1.000 underneath the original

SAMPLE1.000 profile. Note that the background profile is the active profile and is denoted by its Fuschia color.

Before the background profile can be subtracted from the unknown profile, the profile type must be changed and then saved. With the background profile active, select the PROFILE menu and click on the *Set Type* command. Within this menu, identify the profile as background by clicking on the background radio button. Click on the *Set* command. Next, from the PROFILE menu, select *Save*.

We will now subtract the background profile from the unknown profile. First, make the unknown profile labeled SAMPLE1.000 active by double clicking on the profile bottom. Point and click on the OPTIMIZE menu and select the *Sub Background* command from the pull-down menu. A background profile information dialog box will appear containing a list box of the background profiles contained within the EXAMPLE2.SCN file. Point and click on BACKGROUND1.000-a. Notice that the "Processed" and "Background" radio buttons are highlighted signifying that the profile is a processed (ie. saved) background profile. Now point and click on the SUBTRACT command button. The program will display the resulting profile with the background removed.

Note that the *Sub Background* command is only one of three commands that eliminate background data, each of which has advantages. The two additional background removal commands are discussed in Section 5, Part 4C.

We will now trim the profile at the origin (where loaded sample meets resolving interface) and the front (location where dye front stopped) to determine the Rf value. Click on the OPTIMIZE menu and select the *Origin* command. The cursor vanishes and a pair-of-scissors cursor appears on screen. Using the mouse, move the scissors to just right of the origin at approximately $X = 24$ and click the mouse button to clip the profile at the origin. **Note the exact X coordinate position of the origin at the bottom left corner of the screen to be used later in Lesson 3: Molecular Weight Determination.** Click on the OPTIMIZE menu and select the *Front* command. Move the scissors to just left of the right last peak in the profile at and click the mouse button to clip the profile at the front line. **Note the exact X coordinate position of the front at the bottom left corner of the screen to be used later in Lesson 3.**

We will now zoom in on the section of the profile of interest using the *Zoom* command. To *Zoom*, press the **right** mouse button. A small, pull-down menu appears; drag to select the *Zoom* command from this menu and then release the right mouse button. Notice that the cursor has become a magnifying glass.

Practice executing the *Zoom* command by positioning the magnifying glass above and to the left of a portion of the profile that interests you. Press the left mouse button and drag to box the desired area; release the mouse button. The boxed area now appears at higher magnification. The zoom function is completely variable allowing you to zoom in as much as you wish.

Now, return to the previous, unmagnified profile. To do so, press the right mouse button and select the *Previous* command from the pull-down menu that appears. Again, press the right mouse button and select *Zoom*. This time we will magnify the whole of the profile so that a baseline can be readily constructed. Position the magnifying glass cursor slightly above the highest peak, and to the left of the leftmost end of the profile. Press the left mouse button and box the entire profile. Release the mouse button to execute the *Zoom*.

By zooming we have performed a "view modification". A view modification is a modification which affects only the display, or viewing of the data, but does not actually modify the data itself. When a view modification has been done, any operation performed at this point will still affect the whole profile, not just the section which is currently in view. In order to just work with the zoomed section of the profile we must perform a "data modification". A data modification is a modification which not only affects the viewing of the data, but actually transforms the data itself. All operations performed after the data modification will only affect the portion of the profile currently in view. To work with only the zoomed portion of the graph, point and click on the OPTIMIZE menu and select *Snip at View*.

Part 3: Enhancing The Profile Data

The profile has been optimized, and now we will reduce high frequency background noise in the profile using the *Smooth* command. Eliminating high frequency noise is required so that the peak detection algorithm can properly identify the peaks of interest.

Set the smooth criteria by clicking on the *ENHANCE* menu and selecting the *Criteria* command. The Enhance/Smooth Criteria dialog box appears; the cursor prompting for window size.

(In general, if you believe that your data contains a lot of noise and, therefore, needs a lot of smoothing, select a large (10-99) window size. On the other hand, if you believe that your data needs minimal smoothing, select a small (3-10) window size). Enter a window size of 9 in the window size edit field.

Next, <Tab> or move with the mouse to the Iterations Value field. The iteration value specifies how many times the smoothing will be executed. For the purposes of this tutorial, enter 3.

The enhancement boost factor (*En Boost*) is a constant which is multiplied by high spatial frequencies extracted from the data. Enter a value of 3. *En Boost* is discussed in further detail in Section 5, ENHANCE menu.

Click on the Display Each check box so that each smoothing will be displayed on screen. Set these criteria by pointing and clicking on the *Set* command button.

Finally, point and click on the *ENHANCE* menu and select the *Smooth* command. The profile will be smoothed and re drawn after each iteration.

Part 4: Identifying And Integrating Peaks

The profile has been prepared for peak identification. We have eliminated background data using the *Sub Background* command; identified the profile origin and front using the *Origin* and *Front* commands; and reduced high frequency noise using the *Smooth* command. Preliminary work being complete, we will now identify and integrate peaks.

Begin by pointing and clicking on the *PEAKS* menu and selecting the *Peak Criteria . . .* command.

The Peak Criteria dialog box appears. In it, you establish the criteria by which you define peaks. In the Amplitude edit field enter the amplitude (O.D.) you consider minimum for a peak. Using the cursor and the X and Y coordinates displayed at the bottom of the profile graph, determine the amplitude or height of the smallest peak. Enter a value below that of the smallest peak of interest. In this case enter .30 O.D.. Then, using the mouse or <Tab> key, move into the Peak Width edit field. Specify the width considered minimum for a peak. In this case, enter 0. Finally, move into the Area edit field and enter the area considered minimum for a peak; in this case, enter 0.1. To set these parameters, click on the *Set* command button. The other criteria fields are discussed in Section 5, PEAK menu.

Now, to identify and integrate the peaks allowed by your criteria, click on the *PEAK* menu and select the *Auto Identify* command. The area under the curve for the peaks which meet the specified criteria is shaded, and drop lines identify the beginning and end of each peak. The peaks are numbered from left to right starting with peak number 1. Note that if a peak at either end of the profile was not identified, the front and/or origin of the profile was probably clipped to close to the peak. If the end peaks are not identified, return to the original profile by selecting the *Original* command from the PROFILE menu and repeat the above procedures (subtract background, clip front and origin, smooth, and identify peaks).

Note: For quick information about **identified** peaks go to the *PEAK* menu, and select the *Peak Information* command. A hand-shaped-cursor appears. Move the hand to the area under any identified peak and click the mouse button. Read that peak's information, including area, from the

table that appears. To close the *Peak Information* box, click on the *OPERATION* menu and select the *Cancel* command.

Or, for quick information about peaks that have **not been identified**, click on the *PEAKS* menu. Select the *Integrate Interactive* command. The cursor vanishes, replaced by an integral-sign-cursor. Move the integral-sign to the beginning of a significant peak. Press the left mouse button and drag to the end of the peak. Note that the area of the peak appears in the lower right hand corner of the screen. These area values are volatile and will disappear as soon as another peak is integrated, or the command is quit. To quit *Integrate Interactive*, click on the *OPERATION* menu, and select the *Cancel* command.

Part 5: Saving The Profile

All of the profiles from the image, EXAMPLE2.IMG, will be saved to a file named EXAMPLE2.SCN. Profile file names have the image name and the suffix (.SCN) It is important to save profiles after peak identification or the modifications will be lost. To do so, point and click on the PROFILE menu and select the *Save* command. *Save* stores the profile, with all modifications for future use. *Save* also tags the profile name, to differentiate the processed profile from the original. For example, SAMPLE1.000 will be saved as SAMPLE1.000-a. A list of previously saved profiles may be opened within Molecular Analyst by selecting the *Open* command from the FILE menu; and selecting Profile Files(*SCN) from the *List Files Type* pull-down menu. Double clicking on the profile file name will generate a list of profiles within that file. Next, clicking on the profile name and then clicking on the Add button will generate the profile in the DISPLAY function of Profile Analyst. The same saved profiles can be similarly opened when in the Profile Analyst module by selecting the *New* command from the PROFILE menu.

Part 6: Printing Profiles and Reports-

The profiles and the results of your analysis can be printed. You can print a report that lists the profile peaks areas and/or a report that lists the molecular weights.

First, label the scan profile. Select the *Profile Label* command from the PROFILE menu. Type "Bio-Rad Tutorial Sample1" in the profile label dialog box and click on the *Set* command. Place the pointer where you want to place the text and click the mouse button.

To print the profile, press the **right mouse button** and select the *Print* command.

To print a peak report, first click on the *MODE* menu and select the *Report* function. The REPORTS window appears. Point and click on the *FUNCTIONS* menu and select the *Contents* command. A dialog box appears in which the type(s) of report to be printed may be specified. In this case, click in the Profile Data, Peak Report, and Molecular Weight Report check boxes. Click on the *Set* command button to set these choices.

Next, view the reports to be printed by clicking again on the *FUNCTIONS* menu and selecting the *View* command. A dialog box appears, prompting for a filename. Select EXAMPLE2.SCN and click on the *Print* command button under the *FUNCTIONS* menu. A second dialog box appears prompting for profile name. Select SAMPLE1.000; click once on the *Add* command button. To print the reports, select the *Print* command from the FUNCTIONS menu. Two profile reports can be viewed at one time to allow profile report comparison.

To print reports without viewing, click again on the *FUNCTIONS* menu and select the *Print* command. A dialog box appears, prompting for a filename. Select EXAMPLE2.SCN and click on the *Print* command button. A second dialog box appears prompting for profile name. Select SAMPLE1.000; click once on the *Add* command button to begin printing reports about this profile.

Save the reports using the *Save as* command from the FUNCTIONS menu. Enter in the appropriate report file name followed by the extension .rpt. Reports are saved in a ASCII tab delimited format. To return to the profile, first select *Display* from the MODE menu. Next, select *New* from the PROFILE menu. A dialog box appears, prompting for a filename. Select EXAMPLE2.SCN and click on the *Open* command button. A second dialog box appears prompting for profile name. Select SAMPLE1.000; click once on the *Add* command button. Click on the PA menu located in the upper right corner of the profile to return to the image and to Molecular Analyst.

2.3 Lesson 3: Molecular Weight Determination

In this lesson we will work, initially, with a profile containing molecular weight standards. The profile will be created, optimized, and the standard peaks identified and integrated. Next, we will associate the peaks to corresponding size standards and produce a log molecular weight vs. relative mobility standard curve. Finally, we will compare the standard profile to the profile of the unknown prepared in Lesson 2. By comparing the profiles we will determine the molecular weight and relative mobility of the unknowns.

Part 1: Displaying And Optimizing The Standard Profile-

With the image EXAMPLE2.IMG displayed, duplicate the profile object (clicking on the object with the movement arrow while holding the <Ctrl> key down) used to create the sample profile and place the new object on the second lane to the right of the sample lane. This new object is now the active object.

Double click on this new line and enter the name BRDNASTD in the *Profile Name* dialog box. Display the new profile by selecting *Analyze Profile(s)* from the ANALYSIS menu. A display screen will appear with the new profile labeled BRDNASTD.000 underneath the original SAMPLE1.000 profile, and the background profile, BACKGROUND.000. Note that the standard profile is the active profile and is denoted by its Fuschia color.

Next, prepare BRDNASTD.000 for analysis by subtracting background, identifying the front and origin, (using the same X coordinates used for the unknown scan in Lesson 2) and by smoothing the profile.

To begin, click on the *OPTIMIZE* menu and select the *Origin* command. Use the scissors-cursor to clip the profile at close to the same X coordinate (X = 24) as for the unknown profile. Click on the *OPTIMIZE* menu and select the *Front* command. Use the scissors-cursor to clip the profile at close to the same X coordinate as for the unknown profile (Section 2, Part 2).

Next, smooth the profile using the criteria and technique outlined in Part 3 of lesson 2.

Now we will subtract background data. The *Sub Background* command has been introduced. *Sub Horizontal Baseline* and *Construct Baseline*, the two additional commands for eliminating background data, are presented below.

The *Sub Horizontal Baseline* command allows you to set a horizontal baseline and subtract it from the profile. Click on the *OPTIMIZE* menu and select *Sub Horizontal Baseline*. The cursor changes to a horizontal line extending across the graph which can be adjusted in the vertical direction. Move the horizontal baseline down the graph until the Y (O.D.) value in the lower left corner of the graph reads ~0. Press the left mouse button and the horizontal line will now become the new baseline (O.D.) eliminating all area below the line.

Now, so that the third and final command for background elimination can be introduced, click on the *PROFILE* menu and select the *Previous* command to restore the unmodified profile.

The *Construct Baseline* command lets you draw a valley to valley baseline which is useful for profiles containing a background envelope, or a drifting baseline. To use this command, point and click on the *OPTIMIZE* menu and select *Construct Baseline*. Notice that the cursor becomes a

pointer. Use the mouse to move the pointer to a spot to the left end and **off** of the profile and click the left mouse button. Then follow the contour of the profile, moving from valley to valley pointing and clicking. See Figure 9 below for an example of baseline construction. Finally, use the mouse to click on a spot to the right and **off** of the profile. Be sure that you always extend the constructed baseline beyond the left and right ends of the profile. Once the baseline is constructed, point and click on the OPERATION menu. Select the Complete command to draw the baseline. (*Subtract Baseline* from the *Graph Setup* from the SET UP menu should be checked). Background area below the constructed line will be eliminated, and the profile redrawn.

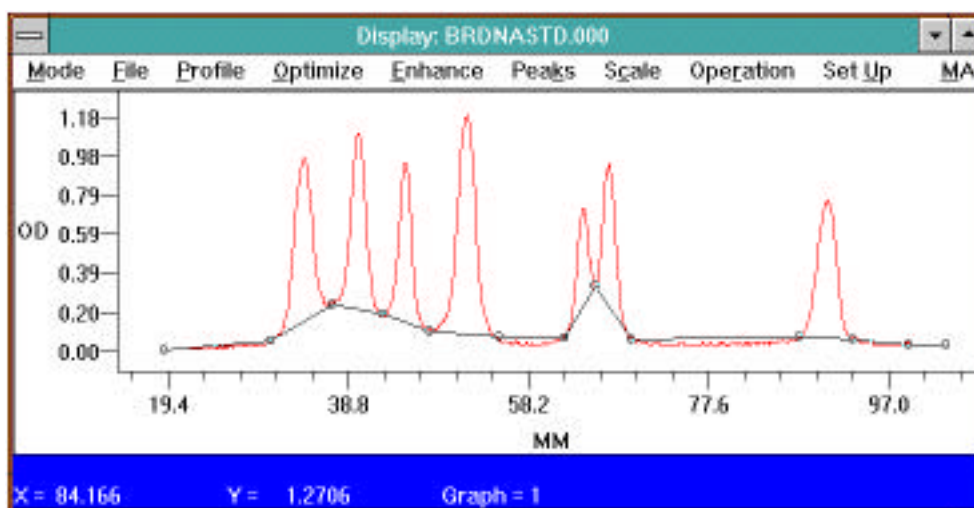


Figure 9. Construct baseline operation (sample and background profiles removed for clarity)

Part 2: Identifying Peaks On The Standard Profile-

Having prepared the standard molecular weight profile we will now integrate the peaks. We will use the same peak criteria settings previously used in Lesson 2.

From the PEAK menu, select the Auto Identify command. A total of 7 peaks should have been identified as shown below in figure 10:

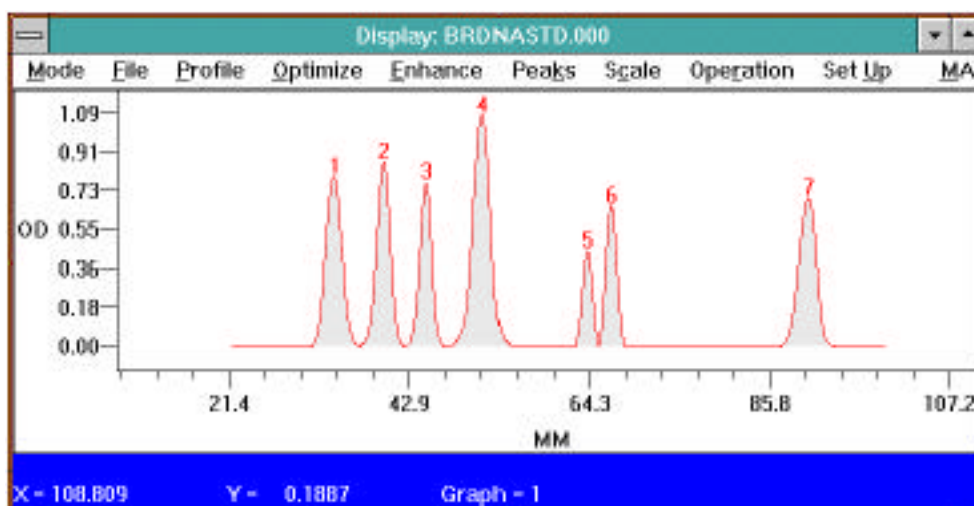


Figure 10. Automatic identification of standard peaks.

In some cases, you may choose to modify the peaks integrated by the *Auto Identify* command. The *Peak Edit* function lets you add, delete, or re-integrate the peaks on your profile. To use the *Peak Edit* function, point and click on the *PEAKS* menu and drag to select *Peak Edit*. The display window closes, and the EDIT window opens.

Select *DELETE* from the menu bar; notice that the cursor becomes a hand grenade. Position the arrow of the grenade on the filled area of peak 1 and click. The peak is deleted, and filled area removed. To restore the peak, select *CREATE* from the menu bar; notice that the cursor becomes a wand cursor. Position the wand at the beginning of the peak, click the mouse button, and drag the wand until the entire peak is shaded. Note: peaks will not be automatically renumbered until you exit the edit function.

To adjust the width of peaks, click on *ADJUST* in the menu bar. Notice that the cursor becomes a tuning-fork. Using the mouse, move the tuning fork into the area under a peak. Click and the tuning-fork-cursor vanishes, replaced by a wand cursor.

To adjust a peak, position the wand cursor at the beginning of the peak and drag. Release the wand and the width is adjusted. The wand will now change back to a tuning fork for adjusting more peaks. To undo an adjustment to a peak, delete the adjusted peak using the *DELETE* command and then use the *CREATE* command to recreate the proper peak.

Click on the *EDIT* menu and select the *Exit* command to exit the EDIT window.

Next we will save the modified standard profile. To do so, click on the *PROFILE* menu and select the *Set Type* command. Within this menu, identify the profile as a standard profile by clicking on the standard radio button. Click on the *Set* command. Next, from the *PROFILE* menu, select the *Save* command.

We will conclude this part of the lesson by printing the active profile which is currently displayed. Simply press the right mouse button and select *Print*. Profile Analyst will automatically send the graph to the Windows spooler for printing.

Part 3: Calculating Standard Curve-

The standard profile has been optimized, its peaks have been identified and integrated, and the resulting processed standard molecular weight profile has been saved. We will now associate the size standard names and molecular weights with the peaks within the standard profile. Then we will direct Profile Analyst to calculate a relative mobility vs. log molecular weight standard curve.

From within Profile Analyst, click on the *MODE* menu and selecting the *WEIGHT* function. The display window vanishes and the MOLECULAR WEIGHTS window appears. Click on the *FUNCTIONS* menu and select the *Size Standard Editor* command. A dialog box appears; in it, we will tabulate names and molecular weights for the standards. Subsequently, the table will be used to associate standard names and molecular weights with the peaks within the standard profile.

Enter the names and molecular weight values for the Bio-Rad DNA Size Standards as follows:

```
Fragment 1 <Tab> 23130 <Enter>
Fragment 2 <Tab> 9416 <Enter>
Fragment 3 <Tab> 6557 <Enter>
Fragment 4 <Tab> 4361 <Enter>
Fragment 5 <Tab> 2322 <Enter>
Fragment 6 <Tab> 2027 <Enter>
Fragment 7 <Tab> 546 <Enter>
```

Note: Do not abbreviate the molecular weights of the standards.(i.e.. kb)

Molecular Analyst/PC

Click on the *Save* command button. A dialog box appears prompting for a filename. The table will be stored in the named file. In this case, type: BRDNASTD and click on the *Done* command button.

Now, to calculate log molecular weight vs. relative mobility curve for the standard profile, return to the *FUNCTIONS* menu and select *Calculate Standard* command.

A full screen dialog box appears with interior fields: the size standard field, the peaks field, and the standard field.

First, click the *Open* command button in the size standards field. A dialog box appears, prompting for a filename. Double click on the file that holds the table of names and molecular weights for your standards. In this case, select BRDNASTD.MW. The names and molecular weights just tabulated appear in the standard field file box.

Next, click the *Open* command button in the peaks field. Again, a dialog box appears, this one prompting for the name of the file in which the standard profile is stored. Double click on EXAMPLE.SCN. A second dialog box appears prompting for the standard molecular weight profile name, select BRDNASTD.000-a. Click on the *Add* command button to open BRDNASTD.000-a. It appears on the lower part of the screen.

Now we will associate the standard names and molecular weights from the table with the peaks on the standard profile.

To do so, highlight (point and click the left mouse button) both the molecular weight size standard listed first (23130 bp) and the peak listed first (1) and click on the *Associate* command button. Continue associating standards and peaks down the tables by simply continuing to press the *Associate* command button. Note that the size standards are listed in descending order of molecular weight and that the peaks are listed in descending order of relative mobility. In this case, because there are seven standards and seven peaks, a 1:1 correspondence between molecular weight and peaks is possible.

After the 7 molecular weight standards and peaks have been associated, we are ready to calculate a standard curve.

In the standard field, click once on the *Calculate* command button. A dialog box appears; it contains check boxes in which curve regression type may be specified. In this case, click on all four check boxes, Linear, Quadratic, Cubic, and Logistic. Finally, click on the *OK* command button.

Now, click on the *Curve* command button. A scatter plot graph of relative mobility vs. log (MW) appears. So that you can evaluate the fit of each curve: click in the Linear check box to see the linear curve drawn; click in the Quadratic check box to see the quadratic curve drawn; click in the Cubic check box to see the cubic curve drawn; and click in the Logistic check box to see the logistic curve drawn. To remove any of the curves, simply click again on the appropriate check box. Due to the sigmoidal shape of the standard curve, the third or fourth order regression will often give the best fit of the curve. The user is however cautioned when using higher orders of regression (Appendix E).

After you've seen all curves, close the graph by double clicking on the small menu bar in the upper left hand corner of the box. Select *Close* from the menu that appears.

We will conclude this part of the lesson by storing the curves with the standard profile. To do so, click on the *Save* command button. Note: To see a list of the standards, relative mobilities, and molecular weights, click on the *Table* command button. To quickly close the table double click in the upper left hand corner on the menu bar.

When finished click on the *Done* command button.

Part 4: Calculating The Molecular Weight Of An Unknown-

Having prepared both the profile of the molecular weight standards (lesson 2) and the profile of the unknown (lesson 1), we will now compare the two in order to find the molecular weight of the unknowns.

First, point to the *FUNCTIONS* menu and select the *Calculate Unknown* command. A full screen dialog box appears. Click on the *Open* command button, and double click on the EXAMPLE2.SCN file. Notice that all unknown and standard profiles stored in this file are listed in the appropriate file boxes.

Next, highlight the standard profile that you would like to compare to the unknown by clicking once on it. In this case, select BRDNASTD.000-a; recall that curves of relative mobility vs. log molecular weight have been stored with this profile. Then, highlight the unknown. In this case select SAMPLE1.000-a.

Finally, click on the *Calculate* command button. The profile of the unknown appears on screen as well as four estimates of the unknown's molecular weight. Notice that the molecular weight is calculated using linear, quadratic, cubic, and logistic regressions. Move the cursor into the graph of the unknown and notice how the cursor changes to a vertical bar. As the bar is moved across the profile, the relative mobility and molecular weights values are continuously displayed on the bottom of the graph. By clicking on the regression type radio buttons below the graph, the read out will change to reflect that order of regression.

Click on the *Save* command button to save this information for later reports.

Click on the *Done* command button to exit the Calculate Unknown dialog box.

Selecting the MODE menu will allow you to return to the DISPLAY function, REPORTS function or to EXIT the Profile Analyst module. Select EXIT from the MODE menu. Next, open the FILE menu and choose EXIT. This will return you to the Molecular Analyst program.

Part 5: Exporting Profile Peak Reports and Profile Data Points To Other Applications-

The data on the profile reports can be exported to any number of spreadsheet or desktop applications which accept data from Windows clipboard.

To export data from the profile peak report to any Clipboard receiving application, first view the data to be exported. To view the reports, click on the *FUNCTIONS* menu and select the *View* command. When the dialog box appears, enter the filename and click on the *Print* command button. When the second dialog box appears enter the profile name and click once on the *Add* command button. With the profile data displayed, simply highlight the data to export (click and drag the cursor down the profile report table to highlight more than one row) and use the *Clipboard* command in the FUNCTIONS menu. This will copy the profile report values to clipboard. Next, open up the application you wish to export to and use the Paste function to transfer the data from clipboard to the new application. Using the <Alt>+<Tab> keys will allow you to rapidly move between Molecular Analyst and other open windows such as Windows Program Manager. Note that the profile report results are ASCII tab delimited values and will transfer as such.

The data points from a profile may also be exported to any number of spreadsheet or desktop applications which accept data from Windows clipboard. Using the command *Copy to Clipboard* from the FILE menu of the Profile Analyst module, data points (x-Distance and Y-O.D.) from a displayed profile can be transferred to the Microsoft Windows Clipboard to be later pasted into other clipboard receiving applications for additional analysis or publication. Please see Section 5.1, I,B for a detailed description of the *Copy to Clipboard* command.

Part 6: Moving Between Profile Analyst And Molecular Analyst-

We will finish area integration with a lesson on moving between the Molecular Analyst window and the Profile Analysis window. There are two methods to move between the Molecular Analyst window and the Profile Analyst window: Single Window and Multiple Windows methods.

Single Window-

The single window method allows you to fully display the Molecular Analyst or the Profile Analyst window at one time, and to move from one window to the other without quitting or minimizing either window.

First, return to the display window of Profile Analyst by selecting the command *Analyze Profile(s)* from the ANALYSIS menu. This will restore the profile in the display window. (Note that the command *Return to Profile Analysis* is not active at this point because the Profile Analysis module was closed using the *Exit* command in Part 4). Next, return to the Molecular Analyst window from Profile Analyst by selecting the menu command PA located in the far right corner of the profile. This command will recover the Molecular Analyst window and the image. Now, to return back to the active Profile Analyst's display window use either the command *Return to Profile Analysis* in Molecular Analyst's ANALYSIS menu, or use the *Profile Analyst II* command in the FILE menu.

Multiple Windows-

The multiple windows method allows both the Molecular Analyst and the Profile Analysis profile windows to be displayed simultaneously (Figure 11). This method is also used to move between Molecular Analyst and the Volume Analyst module.

From within Profile Analyst display window, double click on the blue *Display* title bar. This will produce a profile display window that can be moved and sized. Next, using the double sided arrows reduce the size of the profile display window. Reposition the windows so that both image and profile are visible. To move between the Profile Analyst window (profile functions) and Molecular Analyst window (image and object manipulations) use the pointer to click on the title bar of each window. The active application will have a blue title bar.

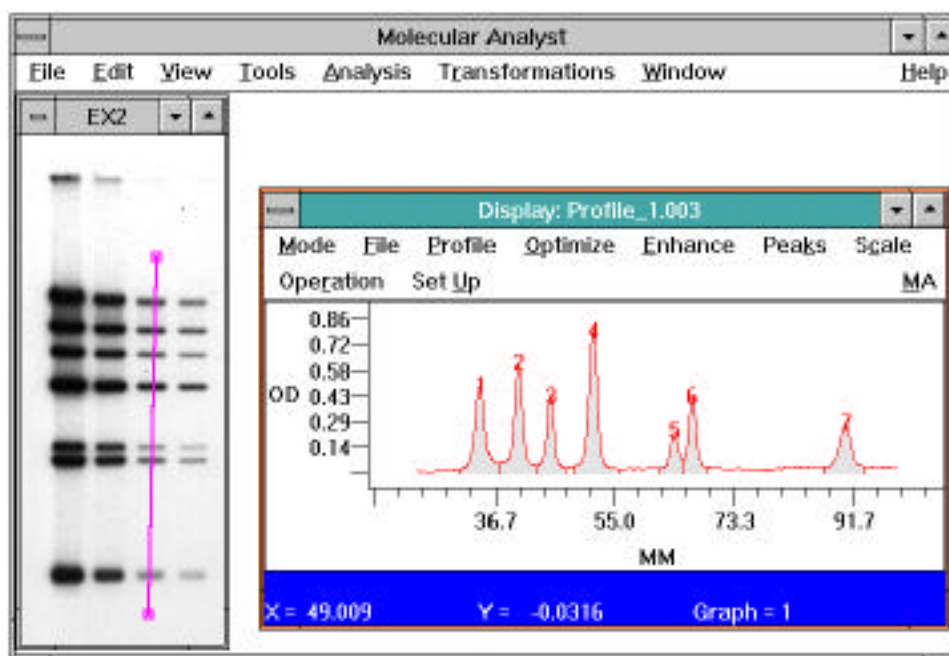


Figure 11. Displaying multiple Windows with Profile Analyst module active

When using either method, if a profile analysis object is modified or duplicated, the command *Analyze profile(s)* from the ANALYSIS menu will display a second, separate profile graph under the original profile. The new profile will be differentiated from the original by the extension .001, i.e.. Profile1.001. If another, new profile analysis object is added to the image, the command *Analyze profile(s)* from the ANALYSIS menu will also display a second, separate profile under the original profile; however, this profile will be differentiated from the original profile name by a succeeding number, i.e.. Profile2.000.

A maximum of 8 profile objects can be displayed on an image at one time. However, only a maximum of 4 profiles can be displayed at one time on the Profile Analysis window. The four profiles can be either displayed on the screen in four separate graphs as originally displayed, or added within a single graph (Refer to the *Add* command in Section 5). Additional profiles can be displayed by first closing or deleting an existing profile and then adding or creating a new profile.

2.4 Lesson 4: Volume integration

In this lesson, we will work with a displayed image to integrate two dimensional bands and generate a volume analysis report. We will first call the image up for display. Then using the volume tools we will identify the spots, and create a volume analysis report identifying total volume, % volume, area, mean, peak position, and adjusted volume. Finally, we will enter known standard values and specify a regression type in order to generate a standard curve to calculate concentrations, molecular weights or pI of unknown spots.

Part 1: Identifying Spots-

Call up the image, EXAMPLE1.IMG for display that was optimized in lesson 1. If one dimensional objects were saved from the previous lessons select *Clear All* from the EDIT menu to clear all 1D objects from the image. From the ANALYSIS menu, select Volume Analysis. The volume tools will appear on the right side of the tool palette. (Figure 12). Additional information on the volume tools and automatic spot detection are in Section 5.2. Click on the volume tool (rectangle).



Figure 12. Tool Palette with Volume tools.

Notice that when you click on the rectangle volume tool, and placed the cursor on the image window, the pointer disappears and is replaced with a rectangle with a cross-hair in the upper left corner

Using the mouse, move the cross-hair cursor to a point above and to the left of the first prominent band of the first lane of data in the image. Click the left mouse button, and drag the mouse downward and to the right. A rectangle will begin to appear on the graphics screen. Moving the mouse adjusts the size of the rectangle so that it completely encloses the band. Release the mouse button and a Fuschia rectangle will appear with a center dot enclosing the band. (Active square and rectangle objects have center dots. Active circle and ellipse objects will be surrounded by squares).

To adjust the size of the rectangle, place the cursor on a side of the rectangle you wish to adjust. Notice that the cursor now becomes a sizing square tool. Using this sizing square tool, adjust the size of the rectangle appropriately so that the band is properly enclosed. Next, place the cursor on a point inside the rectangle until the cursor changes to a curved arrow. Click and hold down the mouse button. Using this positioning arrow, move the rectangle if necessary to enclose all of the band. Now duplicate the rectangle object by placing the cursor within the rectangle (but not on the center dot) changing the cursor to the movement cursor. While holding the <CTRL> key down click the mouse button to duplicate the rectangle. With the movement cursor still present, click and drag the duplicated rectangle to the next band in the lane and release. Continue these steps until the first four bands are enclosed.

In a similar fashion, move the cross-hair of the rectangle tool on an area of background. Ideally, a background object should be placed above and below the band or spot you wish to quantitate. This

type of background subtraction is called general or global background subtraction and is useful when background is uniform. Note that when using global background subtraction, results are more accurate when objects are drawn close around image bands or spots. Click the left mouse button, and drag the mouse downward and to the right to create another rectangle to be used as background. Multiple objects on an image can be used to determine an average background value. Sizing of an object encompassing background data is unnecessary because background volume is calculated as the mean pixel density within any object regardless of shape and size. Alternatively, local background subtraction of each individual band is also available. This type of background subtraction is useful when background is non-uniform. No background object is necessary for this type of background subtraction. The command *Local Background Subtraction* is active from within the OPTIONS menu after the Volume Integration Report is generated and displayed. The *Local Background Subtraction* command subtracts the mean pixel value around the perimeter of each volume object. When using local background subtraction, results are more accurate when objects are drawn farther from image bands or spots. This command affects all volume objects displayed on the image. **Note: use either general or local background subtraction on an image.**

Next, using the text icon's auto numbering feature, number each rectangle (Object 1 through 4) as pictured in Figure 13.



Figure 13. Window with spots and background identified with rectangles and labels.

Part 2: Generating a Volume Integration Report-

We will now generate a volume report. Select *Analyze Volume Object List* from the ANALYSIS menu to display the volume analysis report (Figure 14). Position the volume report so that both the image and volume report are visible. Next, click on the OPTIONS menu and select the command *Columns...* This will display Report Column Preferences dialog box . Click on the Column Selector pull-down menu to display the available columns. Display the columns of interest. To hide columns, click on the *Hide* check box. Check the *Save Settings* box and click OK to return to the volume report. Note when calculating molecular weight or pI, the position column should be displayed.

Within the volume report are rows corresponding to Object 1 through Object 5. Click on any object in the image and notice that the corresponding row within the volume integration report becomes highlighted. Active objects in the image correspond to highlighted rows in the volume integration report. Next, double click on the background object. This will display an *Edit Object* dialog box.

The *Edit Object* dialog box features object name, standard concentration, molecular weight, and pI entry boxes, and object width and height. The *Edit Object* dialog box also allows multiple entry of standard values using the *Edit Objects* command button.

Click on the radio button labeled *Background*. Click OK to return to the volume report. Notice that the name in the *Type* column for Object 5 has been changed from *Unknown* (the default) to *Background* within the volume report. Background values will be automatically subtracted from the unknowns and standards to provide *Adjusted Volume* data. This is global background subtraction.

When using multiple background objects to average background values, first activate all of the background objects by holding down the <Shift> key and clicking on each background object. With all of the background objects selected, double click on one of the background objects to display the *Edit Object* dialog box. Clicking on the radio button labeled *Background* will change all of the objects to background.

Next, activate all of the standard objects by holding down the <CTRL> key and clicking on each object R1, R2, and R4. With all of the standard objects selected, double click on one of the standard objects to display the *Edit Object* dialog box. Click on the *Edit Objects* command button. This command will now let us enter in the standard values for each of the standard values. Click on the radio button labeled *Standard* and enter in the value .90 in the *Concentration* box. (Concentration values can not be entered unless the corresponding concentration column is visible. This also applies for molecular weight and pI determination). Click on the *Next* command button. Repeat this procedure for Object 2 and Object 4, entering concentration values of .70 and .60 respectively. Click on OK. When finished, return to the volume report. Notice that within the *Type* column of the volume report is one unknown, one background and three standards. Additionally, notice that the background volume has been subtracted from the unknown and standard volumes, generating *Adjusted Volume*.

EXAMPLEM : Volume Analysis										
	Label	Type	%Volume	Area mm ²	Volume OD x mm ²	Mean OD	Maximum OD	Adj Volume OD x mm ²	Concentration	
1	C1	Unknown	6.65	9.08	1.43	0.16	0.33	1.48	N/A	
2	C2	Unknown	16.90	9.08	3.75	0.41	0.83	3.75	N/A	
3	C3	Unknown	34.21	13.80	7.53	0.55	1.02	7.59	N/A	
4	C4	Unknown	9.65	5.85	2.14	0.37	0.53	2.14	N/A	
5	C5	Unknown	32.60	21.34	7.23	0.34	0.83	7.23	N/A	
h										

Figure 14. Example of the volume report with highlighted cell

Part 3: Generating a Standard Curve and Calculating Concentration-

With the volume report displayed, we will now generate a standard curve and calculate the concentration of the unknown as specified by the regression analysis. To generate the standard curve(s) and calculate the value of the unknown, click on the *OPTIONS* menu and select the command *Concentration Standard Curves*. This will display the plot of the standard curve(s) (Figure 15). Notice within the standard curve is a legend with linear, quadratic, cubic, and logistic curve icons, as well as, a legend for logarithmic or linear X and Y axis. Clicking on each icon will display or hide the curves fit. Linear Regression requires a minimum of two (2) standard data points, Quadratic Regression requires a minimum of three (3) standard data points, and Cubic Regression analysis requires a minimum of four (4) standard data points. Four Parameter Logistic Regression analysis requires a minimum of five (5) standard data points. Given 3 standard data points, both a Linear and Quadratic curve will be displayed.

Click on the curve icon and select the curve that best fits the data. Position the standard curve display and volume report so that both are visible. To calculate the concentration of the unknown for a specific regression curve, double click on the regression line. This will display the regression formula and standard error for the standard curve and calculate the unknown concentration in the volume report.

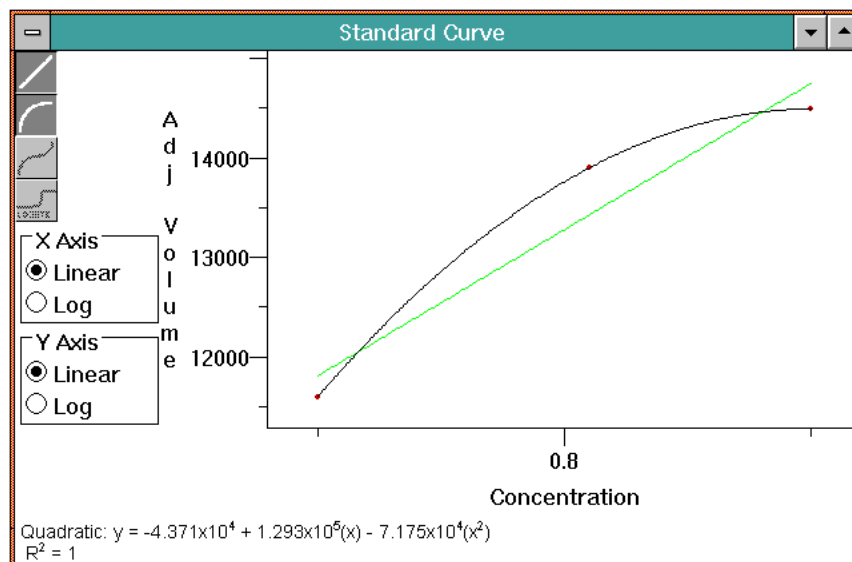


Figure 15. Linear and quadratic regression standard curves

Part 4: Exporting Volume Report Data to Other Applications-

The data on the volume report can be exported to any number of spreadsheet or desktop applications which accept data from Windows clipboard. To export data from the volume report to any Clipboard receiving application simply highlight the data to export (click and drag the cursor down the volume report table to highlight more than one row, or use the <Ctrl> key and click to highlight multiple groups of cells) and use the Copy to Clipboard command or the *Copy* command in the EDIT menu. This will copy the volume report values to clipboard. Next, open up the application you wish to export to and use the Paste or Paste Special function to transfer the volume results from clipboard to the new application. Using the <Alt>+<Tab> keys will allow you to rapidly move between Molecular Analyst and other open windows such as Windows Program Manager. Note that the volume report results are transferred in ASCII tab delimited format.

2.5 Lesson 5: Data acquisition and manipulation

In previous lessons, we have worked with example images provided with the tutorial. Now you will learn to scan your own samples the Model GS-670 Imaging Densitometer into the Molecular Analyst/PC program.

Part 1: Setting Up the Model GS-670 Imaging Densitometer

Prior to scanning an image, it is essential that the GS-670 Imaging Densitometer be warmed up. Please refer to the Model GS-670 Imaging Densitometer user's guide for detailed instructions on preparing the GS-670 Imaging Densitometer for scanning.

Part 2: Starting the Molecular Analyst/PC program-

Start the Molecular Analyst/PC program by double clicking on the Molecular Analyst icon. The program will load. After the title screen, the Molecular Analyst Window will appear.

Part 3: Setting Scan Parameters-

To set the scanning parameters, click on the FILE menu, and drag the mouse to *New*. The scan window will appear with pull-down menus, scanning area window, control buttons, and status bar. The scan window is initially blank with its dimensions representing the full size of the scanning area (Figure 16). Using the mouse, activate the first pull-down menu titled RESOLUTION. Within this menu, select 800μ from the list. This low resolution scanning will allow rapid preview of your sample for qualitative purposes. Next, select the type of edge enhancement desired. Click on the radio button titled Normal in the *Edge Enhancement* field to provide no enhancement to the image (Sharp = medium enhancement, Extra Sharp=high enhancement, Soften=softening enhancement).

Molecular Analyst/PC

Refer to Appendix F for details on the edge enhancement feature. Lastly, select the proper filter for your sample. Each filter corresponds to a wavelength range which is sensitive to specific dyes and stains

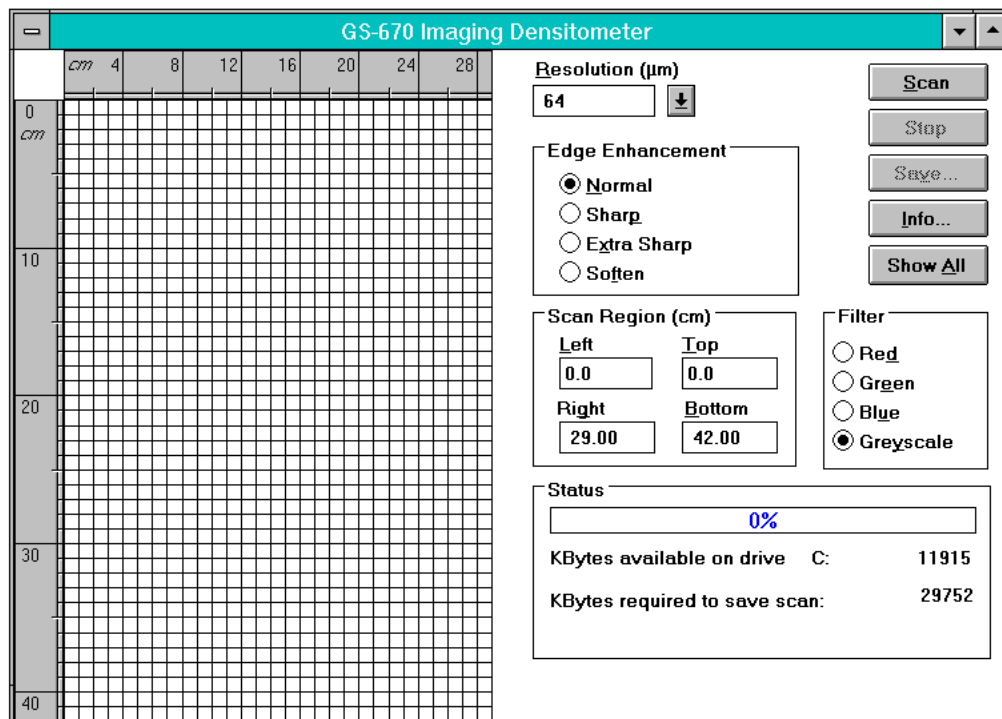


Figure 16. Scanning Window

Filter Selection:

<u>Filter Color</u>	<u>Wavelength</u>	<u>Application Examples</u>
Red	(595nm-750nm)	Coomasie G-250, BCIP/NBT, Fast Green FCF, Methylene Blue
Green	(520nm-570nm)	Coomasie R-250, Basic Fuchsin
Blue	(400nm-530nm)	Crocein Scarlet, Colloidal Gold
Gray Scale	(400nm-750nm)	Silver Stain, Copper Stain, Film, Photographs

Part 4: Identifying the Area to Scan-

Next, we will identify the scanning area. When the *Scan Window* is opened, the default scanning area is the entire scanning area (Transmittance: 29cm x 39.5cm, Reflectance: 29cm x 42cm). To identify a more specific scanning area, place the mouse onto the scanning area. Notice that the pointer is replaced with a square scanning tool when placed in the scanning region. Two metric rulers are displayed in the window to aid in selecting the scanning area. Place the square scanning tool at the upper left corner of the region to be scanned, click, and hold down the left mouse button, and drag down diagonally until the desired scanning area is selected. Release the mouse button. As the scanning region is identified the scanning coordinates (cm) are displayed within the *Scan Region Area* boxes designated *Left, Right, Top, and Down*.

Part 5: Starting The Scan

Molecular Analyst/PC

Select the *Scan* control button to begin scanning. The status bar will indicate the percent of the scan that has been completed. The scan window will update the display as the scanning process continues. Once the image has been fully scanned, it will be displayed in the scanning area at 100% of the current window size. Next, with the preview image displayed, select a higher resolution, and click on the command button *Scan*. Moreover, a more defined area of interest can also be identified in the preview image using the mouse and then rescanned. Alternatively, after displaying the preview image, selecting Show All displays the area scanned in proportion to the whole scanning area. This feature is useful to allow rapid reidentification and rescanning if the desired target area was missed. Note that at any point in the scanning process, scanning can be stopped and images either saved or rescanned at a different resolution, filter, or edge enhancement.

Part 6: Saving the Scan

Save your image as an image file with an 8 character name and a .IMG extension (TEST.IMG) and close the scan window. To view the image just captured, select *Open* from the FILE menu and click on the image name in the Graphics window's title bar, and click on the *Open* command button. The size (mb) of each file saved depends upon the total area scanned and the resolution.

Part 7: Deleting Images

We will close the tutorial by deleting an image to free computer memory. To delete an image, quit the Molecular Analyst/PC program and open File Manager in Windows. Click on the MA folder, then click on the data folder to display images and profile files. Highlight the file(s) to be deleted by clicking on them with the mouse (hold the <Ctrl> key down while clicking the mouse button for deleting multiple files) and then select *Delete* from the FILE menu or simply press the <Delete> key. Click OK at the prompt.

2.5 Tutorial Summary

To start the Molecular Analyst/PC program:

- Double click on the Molecular Analyst/PC program icon

To display an image:

- From the File menu, select Open
- Double click on the image file to be displayed

To optimize an image:

Molecular Analyst/PC

- From the View menu, select *Scale to Fit*
- Using the magnifying glass tool, magnify the image
- From the TRANSFORMATION menu, select *Raw Filter*
- From the Raw Image Filter dialog box, select *Smooth(Median)*, then click OK
- Use the Modify View palette to enhance image brightness and contrast

To generate a profile

- Select Profile Analysis from the ANALYSIS menu to display profile tools
- Highlight the *line* tool and draw a line through the image lanes
- Select *Analyze Profile(s)* from the ANALYSIS menu

To display a previously saved profile from Molecular Analyst:

- From the FILE menu, select the Open ... command
- Open the pull-down menu *List Files of Type:*, and select Profiles(*SCN)
- Double click on the File name from the Document Type Box
- Click on a profile name in the profile title bar in the Graphics window
- Click on the profile file name and click Add

To display a previously saved profile from within Profile Analyst :

- From the *MODE* menu, select the *Display* command
- From the *PROFILE* menu, select the *New* command
- In the Filename dialog box, identify the file where the profile is stored and click OK
- In the Profile name dialog box, click on the profile name; click on the *Add* command button.

To optimize a profile:

- From the *OPTIMIZE* menu, select Sub*Horizontal Baseline*, *Construct Baseline*, or *Subtract Background* command
- From the *OPTIMIZE* menu, select the *Origin* command; Clip profile at origin
- From the *OPTIMIZE* menu, select the *Front* command; Clip the profile at front
- In the DISPLAY window, press the right mouse button, select the *Zoom* command
- Box the area of interest
- From the *OPTIMIZE* menu, select the *Snip at View* command

To smooth a profile:

- From the *ENHANCE* menu, select *Criteria*
- In the Set Criteria dialog box, enter Window Size, and Iterations Value
- From the *ENHANCE* menu, select the *Smooth* command

To Identify and integrate peaks:

- From the *PEAKS* menu, select the *Peak Criteria* command
- In the Peak Criteria dialog box, enter minimum Amplitude, Width and Area.
- From the *PEAKS* menu, select *Auto Identify*
- From the *PEAKS* menu, select the *Peak Info* command
- From the *PEAKS* menu, select the *Integrate Interactive* command
- Drag the cursor from origin to front of the peak of interest

To edit peaks:

- From the *PEAKS* menu, select the *Edit. . .* command
- From the *EDIT* menu bar select *ADJUST*, *CREATE*, OR *DELETE*

To save:

- From the PROFILE menu, select the Save command

To Print a profile:

- With the mouse pointer within the graph, press the **right** mouse button and select *Print*.

To calculate a standard curve:

- From the MODE menu bar, select the Weight command
- If your standard is not a Bio-Rad standard, from the FUNCTIONS menu, select Standard Editor; enter the names and molecular weights for peaks on the standard profile
- From the FUNCTIONS menu, select Calculate Standard; in the dialog box, select the file holding the table of names and weights.
- In the dialog box, select the standard profile; associate names/molecular weights with peaks.
- In the dialog box, click on the Calculate command button; check regression type; click on the Curve command button; click on the Done command button.

To calculate the molecular weight of an unknown:

- From the MODE menu, select the Weight command.
- From the FUNCTIONS menu, select the Calculate Unknown command: open the file in which the unknown and standard profiles are stored; select standard profile for which curves have been generated, select unknown standard profile, click on the Calculate command button

To view or print reports:

- From the MODE menu, select the Report command
- From the FUNCTIONS menu select the Contents command; select the type of report desired
- From the FUNCTIONS menu, select the View command
- From the FUNCTIONS menu, select the Print command

To generate volume integration report

- From the EDIT menu, select *Clear All* to remove previous profile objects
- Select Volume Analysis from the ANALYSIS menu to display volume analysis tools
- Use the volume tools to select the desired spots and a background
- Select *Analyze Volume Object List* from the ANALYSIS menu

To create a standard curve and calculate concentration, molecular weight, or pI

- Identify objects and enter standard value in the *Edit Volume Report* command box.
- Identify a background object for global background subtraction, or select the *Local Background Subtraction* command from the OPTIONS menu for local background subtraction
- From the OPTIONS menu select the Columns command. Display the appropriate columns, choose the standard curve Y axis, and regression type
- Select the appropriate *Standard Curves* from the OPTIONS menu

To export profile or volume report data to Window's clipboard

- Highlight the data to export and select *Copy to Clipboard* or *Copy* command from the EDIT menu
- Use <Alt>+<Tab> to go to Program Manager

Molecular Analyst/PC

- Open the application and use the Paste or Paste Special function to transfer the ASCII tab delimited formatted data

To delete profiles:

- From File Manager menu, highlight profiles for deletion
- Select *Delete Profiles ...*

To print images or reports

- Select Page Setup command from the FILE menu to size and position images on Windows compatible printers
- Select Print command from the FILE menu to print reports to Windows compatible printers
- Press the Print button on the Video Printer to print images

To scan an Image

- Start the Molecular Analyst/PC program
- From the FILE menu, drag the mouse to *New*.
- Set pre-scan resolution, edge enhancement, and filter parameters
- Identify prescan area for rapid scanning
- Select the *Scan* button to start scanning
- Set the high resolution scanning parameters
- Identify the high resolution scanning area on the prescan image and select *Scan*
- Select *Save* to save the scan as an image file with .IMG ending
- Close the scan window and open the image for optimization and quantitation

Section 3 Description of Palettes

Molecular Analyst/PC provides two palettes which will aid in image enhancement and optimization. The Tool Palette provides access to all of the commonly used tools such as zooming and scrolling. The Modify View Palette provides access to all of the image display manipulation tools such as brightness, contrast, and color slider controls.

All of the palettes within the Molecular Analyst/PC are “floating” palettes. Every palette has a title bar that can be dragged to reposition the palette. Each title bar also has a close box for putting the palette away until it is needed again. Both tool palettes can be displayed automatically

3.1 The Tool Palette

Choosing *Show Tools* from the TOOLS menu displays the Tool Palette. Alternatively, selecting *Preferences Setup* under the FILE menu, and clicking on the Tool palette *Show on Entry* box, will display the Tool palette when an image is opened.

The Tool palette consists of a number of boxes, each of which displays an icon that represents a specific tool or effect. When one of the tools is selected, that specific tool icon will be highlighted and the mouse cursor changes to the tool when placed on the image.

The Tool palette consist of the following:



Pointer (Object Select)

The pointer is the default tool as it is used to select all other tools. It is also used to select windows, window sizes, and menu functions.



Block Select (Multi-object Select)

The block select tool is used to select multiple objects as an active group on an image.

- Click on the block select icon.
- Place the block select tool at one corner of the group of objects to make active.
- While holding down the left mouse button, drag diagonally until the desired objects are selected.

Deselecting objects in a group - While holding down the <Shift> key, block select on the group of objects to deselect. Alternatively, to deselect a single object from a group, place the pointer tool on the object and while holding down the <Shift> key, click the mouse button.



Magnifying Glass (Magnify)

The magnifying glass tool is used to rapidly magnify a specific point within the image by a factor of two. The maximum magnification is 1600% from the actual image size.

- Click on the magnifying glass icon.
- Magnify the image two fold from the point of clicking.
- Magnify the image another two fold from the point of clicking.

Undo magnification - Holding down the <Shift> key and clicking the mouse button de-magnifies the image two fold from the point of clicking. Alternatively, selecting *Show All* from the VIEW menu will display the original, unmodified image.

Zooming Box (Zoom)

The zooming box tool is used to quickly magnify a selected area of the image by a factor of two. The maximum zoom is 1600% from the actual image size.

- Click on the zooming box icon.
- Place the zooming box tool at one corner of the area to magnify.
- While holding down the left mouse button, drag diagonally until the desired area is selected and release the mouse button.

Undo zoom - To undo a zoom function, select *Show All* from the VIEW menu to display the original, unmodified image.

Scrolling Cross (Pan)

The scrolling cross tool is used to move an image that has been zoomed or magnified around the image window in horizontal, vertical, and diagonal directions.

- Zoom or magnify a region of interest.
- Click on the scrolling cross icon.
- While holding down the left mouse button, drag horizontally, vertically, or diagonally, and release the mouse button.





T (Text)

The text tool is used to label images or profiles. It also has an auto-numbering feature for rapid sequential numbering of objects.

- Click on the text icon.
- Move the text cursor to the location where you want the left edge of the label to appear, and click the mouse button.
- Type in the text entry box and click on the OK button.
- To alter text on an image, double click on the text. When the text entry box appears, retype the text or change text parameters. To relocate the text, first click on the text to make it active. Then, click and hold on the text and drag the text to the new location.
- To delete text, click on the text to be deleted and use the Edit Command: *Delete* or press the <Alt> + keys.

Scissors (Clip Image)

The scissors tool is used to clip and display selected areas from images.

- Click on the scissors icon.
- Place the clip image tool at the upper left corner of the region to be clipped.
- While holding down the left mouse button, drag diagonally until the desired area is selected, and release the mouse button.
- Select the yes button from the Image Clip dialog box.

Undo clip - To undo a clip function, select *Undo* from the EDIT menu or display the original, unmodified image. To close the clipped image, select *Close* from the FILE menu. At the dialog box, do not save the changes. Reopen the original image by choosing the *Open* command from the FILE menu.



3.2 The Modify View palette

Choosing Show Palette from the View menu displays and hides the Modify View palette (Figure 17). Alternatively, selecting *Preferences Setup* under the FILE menu, and clicking on the Modify View palette *Show on Entry* box, will display the Modify View palette when an image is opened.

The Modify View palette consists of vertical equalization scroll bar, brightness and contrast look-up table (LUT) window slider controls, pull-down palette menus, and control buttons. These palette features control image sensitivity, brightness and contrast, invert or negative display, and image reset. With this palette, visual sensitivity of specific sections of an image or band can be enhanced.

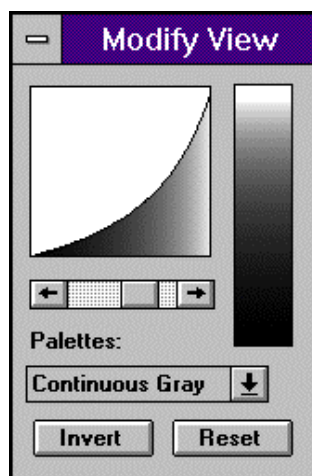


Figure 17. Modify View Palette

Pull-Down Palette Menus

- *Monochrome*: Distributes black and white throughout the 256 gray level display range.
- *8 Grays*: Distributes 8 gray levels throughout the 256 gray level display range.
- *16 Grays*: Distributes 16 gray levels throughout the 256 gray level display range.
- *32 Grays*: Distributes 32 gray levels throughout the 256 gray level display range.
- *64 Grays*: Distributes 64 gray levels throughout the 256 gray level display range.
- *8 Colors*: Distributes 8 colors throughout the 256 gray level display range.
- *16 Colors*: Distributes 16 colors throughout the 256 gray level display range.
- *32 Colors*: Distributes 32 colors throughout the 256 gray level display range.
- *Cont. Grays*: Distributes the entire gray scale throughout the 256 gray level display range.
- *Cont. R,G,B*: Distributes Red, Green, or Blue throughout the 256 gray level display range.
- *Spectrum*: Distributes entire spectrum throughout the 256 gray level display

Equalization 256 Gray Level/Color Scroll Bar

This scroll bar option controls the gray/color level, equalization curve, and sensitivity of the image as it passes through the 256 gray level or color scale. This one step sensitivity adjustment maps 8-bit raw data in a variety of different 8-bit, 256 gray level displays. Scrolling left generates a convex curve, increasing the sensitivity of faint bands and obscuring saturated bands. Scrolling right generates a concave curve, decreasing the sensitivity of faint bands and enhancing saturated bands.

Brightness and Contrast Sliding Controls

Brightness - Brightness of the image is controlled by a sliding gray/color display look-up table (LUT). Placing the pointer on a point other than black and white on the continuous gray or color scale look-up table (LUT), changes the pointer to a circled B. While holding down the left mouse button, slide the brightness tool vertically until the desired brightness is achieved. Altering the brightness shifts the equalization curve in a vertical direction. **The brightness of an image cannot be adjusted until the contrast (compressing the 256 gray levels) has been modified.**

Contrast - Contrast of the image is also controlled by a sliding gray/color display look-up table (LUT) tool. Placing the pointer at the top or bottom of the continuous gray or color scale changes the pointer to a circled C. While holding down the left mouse button, slide the contrast tool vertically until the desired contrast is achieved. Altering the contrast compresses the 256 gray levels and shifts the equalization curve toward horizontal.

Invert Button

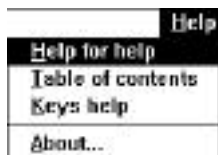
Selecting this option inverts the image and displays the negative of the image.

Reset Button

This option returns the display to its original, unmodified display.

Section 4 Description of Menus

The Molecular Analyst/PC is a menu driven software program. In this section, each of the Molecular Analyst/PC software menus and their commands will be described in detail. All of the Molecular Analyst/PC menus can be accessed from the main menu bar once an image is displayed or scanned. Maximum flexibility is achieved with the Molecular Analyst/PC program because data is easily passed from one module to the next. This easy exchange of data not only provides multitasking capabilities, but also allows data to be displayed, processed, and optimized in many different ways.



4.1 Help Menu

The Molecular Analyst/PC program has on-line, context sensitive help available. To access this feature, select Help from the menu bar. You will be provided with a list of topics regarding the use of the program. Alternatively, to access help on a particular menu function or command, highlight the desired topic and either select help or press the <F1> key.

HELP COMMAND: *H*elp for help

This command provides a table of contents and information on how to use Help.

HELP COMMAND: *T*able of Contents

This command provides a table of contents and information on the relevant topics of Molecular Analyst/PC .

HELP COMMAND: *K*ey help

This command provides information on all of the keys on the keyboard.\

HELP COMMAND: *A*bout

This command displays the name, version, and copyright date for the Molecular Analyst software program.



4.2 FILE Menu

FILE COMMAND: *New*

This command opens the scanning window for a new scanning session.

FILE COMMAND: *Open...*

Selecting this command allows you to open images or profiles from their respective directories: Molecular Image(*.IMG), or Profiles(*.SCN). The opened file will replace any current information on the display window.

FILE COMMAND: *Save*

This command allows you to save data as a file. If the data has not been saved previously, the save dialog window will appear. Select the file type and enter a file name up to 8 characters long. Files are saved in the BRIFF format (Bio-Rad Image File Format), to be used exclusively within Molecular Analyst/PC. If the file had been saved previously, the data will be saved to the previous file.

FILE COMMAND: *Save as...*

This command allows you to save data under a different name or file type. To save a file to a different disk or directory, save the file the first time using the *Save* command; the second time, use the *Save As...* command to specify an alternate destination. Different file types can be saved by selecting it from the *Save File as Type* pull-down menu. The default file type is a BRIFF format (Bio-Rad Image File Format). Alternatively, the image can also be saved in the 8-bit TIFF format (Tagged Image File Format) to be exported to many image analysis software programs. Once an image has been saved as a TIFF file, it cannot be reopened within Molecular Analyst/PC.

FILE COMMAND: *Close*

Selecting this command will close the active window displayed in the foreground and the tool palettes associated with it.

FILE COMMAND: *Print...*

This command will send the image and its associated reports to the printer.

FILE COMMAND: *Printer Setup*

This command is used to select the desired characteristics and functions for the current printer. The current printer is set using Print Manager, which is available in Window's Program Manager window.

FILE COMMAND: *Page Setup...*

This command is used to size and position images to be printed.

FILE COMMAND: *Preferences setup ...*

This command is used to determine the systems default directory, tool palette display options, fonts, rulers, filters, color selection of the profile and volume tools, and the column preferences for the volume analysis report. Within this command, the display mode may be changed from fast, intermediate, and slow. This enhancement affects image display speed and scanning resolution.

FILE COMMAND: *Profile Analyst II*

This command allows you to quickly move to the display window of the Profile Analyst module.

FILE COMMAND: *Exit*

This is used to exit from the Molecular Analyst/PC program.

4.3 EDIT Menu

Edit	
Undo View Filter	
Original	Ctrl+Z
Cut	Ctrl+X
Copy	Ctrl+C
Paste	Ctrl+V
Delete	
Clear all	Alt+Del
Select all	

EDIT COMMAND: *Undo*

This function reverses the last image operation (filter, rotate, or clip) performed.

EDIT COMMAND: *Original*

This function displays the original, unmodified image.

EDIT COMMAND: *Cut*

Selecting this command will cut any selected object and place it into the clipboard. These objects can be pasted into most clipboard compatible programs.

EDIT COMMAND: *Copy*

Copy will duplicate any selected object(s) into the clipboard and allow it to be pasted within an active image.

EDIT COMMAND: *Paste*

This function will paste any object(s) cut or copied into an active image.

EDIT COMMAND: *Delete*

This function will delete any active object(s) or text within the displayed image.

EDIT COMMAND: *Clear all*

Selecting this command will delete all objects and text within the displayed image.

EDIT COMMAND: *Select All*

This command selects all objects and text and makes them active.

EDIT COMMAND: *Copy to clipboard*

Selecting this command will copy selected rows from the results table to Windows Clipboard which can then be transferred to other applications, such as Microsoft[®] Excel[®] or Microsoft[®] Word[®]. This command is active only when result tables are displayed.

View
Hide Rulers
Show Palette
Show All
Show Actual Size
Scale To Fit
Magnify
Zoom
Pan
Redraw
Show Labels
Hide Objects

4.4 VIEW Menu

VIEW COMMAND: *Show Rulers / Hide Rulers*

Selecting this command allows you to display or hide the metric rulers. The rulers automatically change from (cm) to (mm) when the image is zoomed.

VIEW COMMAND: *Show Palette / Hide Palette*

Selecting this command allows you to display or hide the Modify View palette.

VIEW COMMAND: *Show All*

This command will reverse a magnification or zoom of the active image to show all portions of the image in the window.

VIEW COMMAND: *Show Actual Size*

Using this command displays the actual size of the image.

VIEW COMMAND: *Scale To Fit*

This command maintains the image to window aspect ratio as the display window is enlarged or reduced by a 2:1 ratio.

VIEW COMMAND: *Magnify*

When this command is selected the cursor is changed to a magnifying glass when placed on the image. This command magnifies a specific point within the image by a factor of 2 and then centers that point in the display window. Holding the <Shift> key and clicking will reduce the magnified image by a factor of 2. Alternatively, selecting *Show All* from the VIEW menu will display the original, unmodified image.

VIEW COMMAND: *Zoom*

When this command is selected the cursor is changed to a zooming box when placed on the image. This command zooms a designated area within the image and fills the current display window. To undo a zoom function, select *Show All* from the VIEW menu to display the original, unmodified image.

VIEW COMMAND: *Pan*

When this command is selected the cursor is changed to a pointed cross when placed on the image. By holding down the mouse button and dragging, this command scrolls the image across the window in the designated direction. This command is only functional after zooming or magnifying the image.

VIEW COMMAND: *Redraw*

This command redraws the image and removes any object or window artifact which may have been left behind from a previous function.

VIEW COMMAND: *Show Labels / Hide Labels*

Selecting this command allows you to show or hide the object labels on an image when the volume analysis report is present.

VIEW COMMAND: *Show Objects / Hide Objects*

This command allows you to show or hide text, profile, and volume objects. To view hidden objects, either select *Show Objects* from the VIEW menu or click on any profile or volume object icon displayed on the right side of the tool palette.

Tools
Show Tools
Image Info...
Tool Preferences...
✓ Object Select
Block Select
Line
Multi-Segment Line
Averaging Rectangle
Text
Clip Image

4.5 TOOLS Menu

TOOLS COMMAND: *Show Tools / Hide Tools*

Selecting this command allows you to display or hide the Tool Palette.

TOOLS COMMAND: *Image Info...*

This command allows you to review image information such as image creation date, image resolution, image and image file size, and to add comments, exposure times, and patient information.

TOOLS COMMAND: *Tool preferences...*

Through this command, you can select the preferences for the available tools.

TOOLS COMMAND: *Object Select*

This command allows you to use the pointer from the tool palette. It is used to select all other tools, windows, menu functions and adjusting image window size.

TOOLS COMMAND: *Block Select*

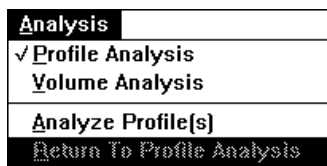
This command allows you to use the block select function from the tool palette. The block select tool is used to make multiple objects active, thereby grouping them together on an image.

TOOLS COMMAND: *Text*

Selecting this command allows you to use the text function from the tool palette. The text tool is used to rapidly annotate and number objects.

TOOLS COMMAND: *Clip Image*

This command allows you to use the clip image function from the tool palette. The clip image tool is used to clip and display selected areas from images. To undo a clip function, close the clipped image by selecting *Close* from the FILE menu. At the dialog box, do not save the changes. Reopen the original image by choosing the *Open* command from the FILE menu.



4.6 ANALYSIS Menu

ANALYSIS COMMAND: *Profile Analysis*

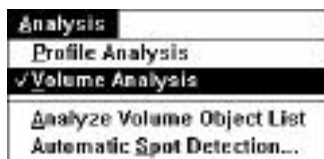
Selecting this command activates the Profile Analysis module and incorporates the profile tools onto the right side of the tool palette and into the TOOLS menu.

ANALYSIS COMMAND: *Analyze Profile(s)*

Once a profile tool has been used and an object created, this command is used to transfer the object data to the Profile Analysis module and to create a profile. Each time this command is selected, a profile is created from all active profile objects and displayed. A total of four profile graphs can be displayed at one time within the display function of the Profile Analysis module. Please refer to Section 5.1 for further information on the Profile Analysis module.

ANALYSIS COMMAND: *Return to Profile Analysis*

Selecting this command allows you to return to the Profile Analysis module. No additional profile is created.



ANALYSIS COMMAND: *Volume Analysis*

Selecting this command activates the Automatic Spot Detection and Volume Analysis module and incorporates the volume tools onto the right side of the tool palette and into the TOOLS menu. All profile objects must be removed from the image before volume tools can be used.

ANALYSIS COMMAND: *Analyze Volume Object List*

Once a volume tool has been used and an object created, selecting this command generates a volume report. Please refer to Section 5.2 for further information on the Volume Analysis Command.

ANALYSIS COMMAND: *Automatic Spot Detection*

Selecting the command *Volume Analysis* from the ANALYSIS menu activates the Automatic Spot Detection command. Selecting this command displays the spot finding criteria dialog box and allows image region, spot size, spot criteria, and artifact exclusion criteria to be entered. Please refer to Section 5.2 for further information on the *Automatic Spot Detection* command.

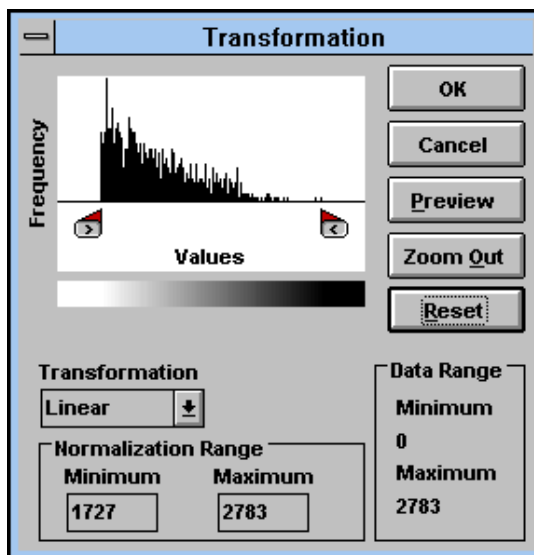
***The number of spots found is dependent upon the set criteria. All spots on an image may not conform to a single criterion, and therefore multiple analyses may be required to find all spots on some samples.**



4.7 TRANSFORMATIONS Menu

TRANSFORMATIONS COMMAND: *Transform*

Selecting this command displays the transformation histogram palette. The histogram window, which can be moved to any point on the screen, displays a distribution plot of the gray levels found within the image which is currently displayed (or the active window when two images are displayed at the same time).



You can use the Transformation Histogram Palette to examine how grays are distributed in your image. This basic information may help you determine how successfully an image can be enhanced. The histogram's horizontal axis (Grays) represents the 256 possible shades of gray that Molecular Analyst works with, from white at the far left to black at the far right. The histogram's vertical or y-axis (O.D.) shows the relative number of pixels found in the image for each possible gray shown on the horizontal axis. For example, if Molecular Analyst searches the image and finds many pixels for gray shade 200, it draws a relatively tall bar at position 200 on the histogram's horizontal axis. If Molecular Analyst finds only a few pixels for gray shade 200, it draws a relatively short bar. If no pixels are found, no bar is drawn and a gap is shown at that position. You should examine the histogram palette before optimizing that particular image.

Adjusting the Histogram Range

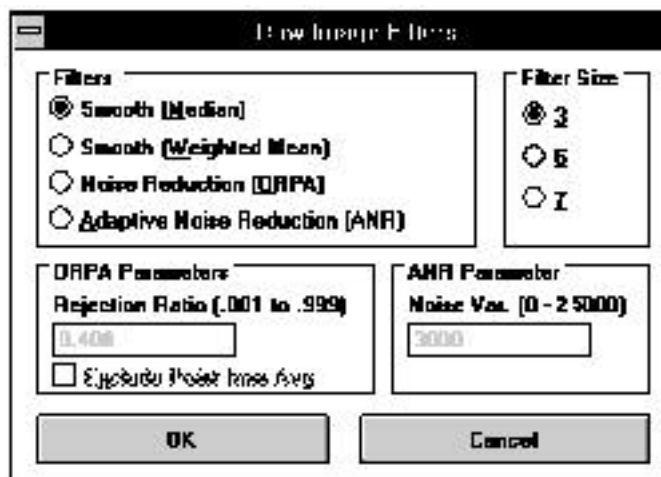
The transformation palette has the ability to view the gray level histogram, select either linear, nonlinear, or equalized transformation, adjust the minimum and maximum values for that range, and to subsequently update the image display. When an image is initially displayed, the program is using its default linear mapping mode where all 0-255 levels of the 8 bit image are mapped to the 0-255 computer display. If most of the gray level distribution in the image is concentrated in left end of the 0-255 gray level range, then low level image objects may not be very visible in the display. To "zoom" in on this section of the histogram range, click and drag the tab marker in the bottom left corner of the histogram palette to the right. To better view intense or partially resolved bands, click and drag the tab marker in the bottom right corner of the histogram palette to the left.

Dragging these tab markers changes both the histogram plot and the maximum or minimum gray level values. To view changes to the image, click on the *Preview* button. To apply changes to the image, click on the *OK* button. The image will be redrawn according to the range selected. The transformation palette also has non-linear and equalization transformations. Non-linear transformation maps the 8-bit image data in a tight distribution toward the lower or upper end of the 0-255 gray level display. Selecting "Nonlinear" from the Transformation pull-down menu activates both the non-linear slider controls and the non-linear color/gray level distribution bar. These tools allow you to preview and control the gray level distribution to each section of the image. Note that the color/gray level distribution bar aligns directly under the histogram plot. Any changes to the gray level/color distribution bar (using the slider controls) will correspond directly to the histogram underneath, and subsequently to the image when the *Preview* and *OK* button is selected. Histogram equalization is the third type of transformation available. This type of transformation takes the specified histogram area and equalizes the gray level values according to frequency. Therefore, high signal areas will be given a large number of gray levels, while background will be given fewer gray or color levels. This type of transformation can provide greater contrast and detail to some images.

To reset the histogram, click on the *Zoom Out* button. This will show you how your selected range corresponds to the entire dynamic range of the image. Clicking on the *Reset* button will move the range controls to their left and right limits. Clicking on the *Preview* or *OK* button will then apply the histogram selection to the image.

TRANSFORMATIONS COMMAND: *Raw Filters*

Selecting this command allows you to smooth high frequency noise from the **raw** data with a variety of filters. This type of filtering suppresses high frequency noise which is often the result of imperfections of the sample or image digitization and **will change the quantitative nature of the image data** . Once an image has been filtered, it can be undone using the *Undo* or *Original* command under the EDIT menu.



Smooth (Median)

This filter replaces each pixel with the median value of its neighboring pixel. This often blurs or softens the selected area.

Smooth (Weighted Median)

This filter replaces each pixel with the weighted mean value of its neighboring pixel. This filter also blurs or softens the selected area.

Out-of-Range-Pixel-Averaging (ORPA)

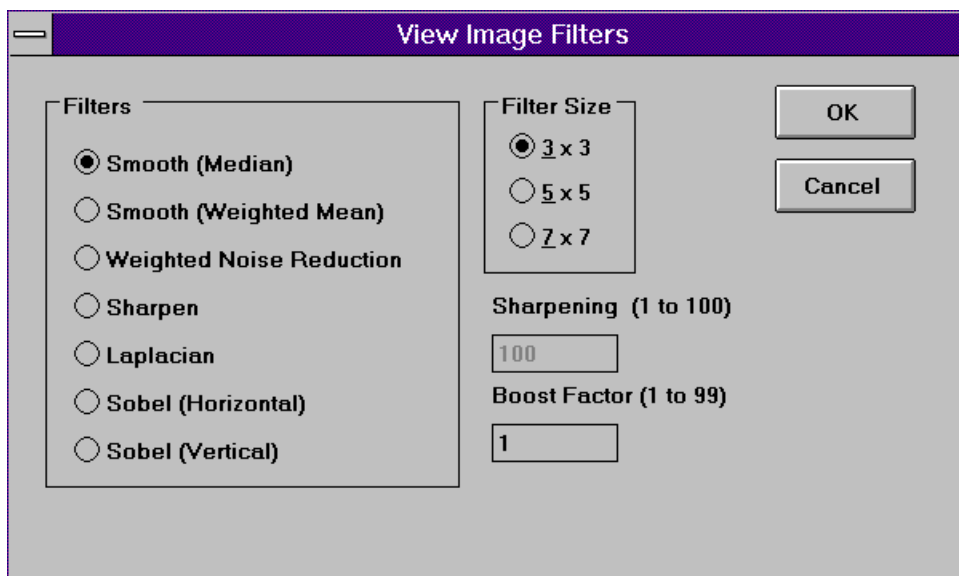
ORPA or Out-Of-Range-Pixel-Averaging reduces the overall "grain" in images.

Adaptive Noise Reduction (ANR)

This filter actively removes additive noise in images. It does not soften or blur the edges and is useful when visualizing images with poor signal-to-noise ratios.

TRANSFORMATIONS COMMAND: *View Filters*

Selecting this command allows you to smooth high frequency noise from the **view** data with a variety of filters. By passing a 3 x 3, 5 x 5, or 7 x 7 size filter grid through the data points, view filtering suppresses high frequency noise but **will not change the quantitative nature of the image data**. Once an image has been filtered, it can be undone using the *Undo* or *Original* command under the EDIT menu. Once a filtered view image has been saved, it may be undone using the transformation histogram.



Smooth (Median)

This filter replaces each pixel with the median value of its neighboring pixel. This filter also blurs or softens the selected area.

Smooth (Weighted Mean)

This filter replaces each pixel with the weighted mean value of its neighboring pixel. This often blurs or softens the selected area.

Weighted-Noise Reduction

This filter is useful for bringing out details in extremely noisy images, and can be used for good publication printing. A boost factor, selectable from .001 to .999 is used for bringing out low values and saturating high values. Each pixel is replaced with the median value of its 3x3 neighborhood. This is a time-consuming operation since each of the nine pixels in the 3x3 neighborhood must be sorted and the center pixel replaced with the median value. This filter preserves the edges of objects (bands and dots) in the images.

Sharpen

This filter increases contrast and accentuates detail in the selection. It may, however, accentuate noise.

Laplacian

This filter can be used as a preliminary for spot detections. Its effect is to enhance boundaries.

Sobel (Horizontal or Vertical)

This filter can be used to enhance electrophoretic images. The effect is to bring out edges in either horizontal or vertical directions.

TRANSFORMATIONS COMMAND: *Rotate Right 90*

This command allows you to rotate the image 90° in a clockwise direction.

TRANSFORMATIONS COMMAND: *Rotate Left 90*

This command allows you to rotate image 90° in a counter-clockwise direction.

TRANSFORMATIONS COMMAND: *Rotate 180*

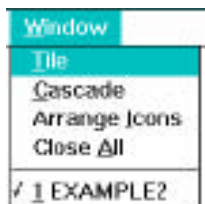
This command allows you to rotate image data points 180°.

TRANSFORMATIONS COMMAND: *Flip Horizontal*

Selecting this command allows you to flip the image relative to its vertical (left to right) center to provide a mirror image of itself.

TRANSFORMATIONS COMMAND: *Flip Vertical*

Selecting this command allows you to flip the image relative to its horizontal (top to bottom) center to provide a mirror image of itself.



4.8 WINDOWS Menu

WINDOWS COMMAND: *Tile*

This command arranges the open windows in smaller sizes to fit on the available workspace.

WINDOWS COMMAND: *Cascade*

This command resizes and layers the open windows within the workspace so that each title bar is visible

WINDOWS COMMAND: *Arrange Icons*

Using this command organizes the icons within Window's Program Manager

WINDOWS COMMAND: *Close All*

Selecting this command closes all functions and exits the application to the main file menu.

Section 5 Molecular Analyst/PC Modules: Descriptions and Use

5.1 Profile Analyst Module

Part 1: Introduction

The Profile Analyst module is a powerful analytical tool designed to analyze profiles extracted from the Molecular Analyst/PC image display module. The determination of valuable information such as sample concentration or amount of macromolecule, relative mobility and molecular weight, is easily performed with the Profile Analyst module's graphical interface, pull-down menus and dialog boxes.

The Profile Analyst module offers the following features:

- Profile tool palette for rapid object generation
- Up to four profiles can be displayed at once, either overlaid or in separate graphs
- High frequency noise may be filtered from data using a weighted moving average smoothing feature
- Any section of the profile can be zoomed as much as desired using the variable zoom tool
- Origin and front of sample can be identified for relative mobility calculations
- Area for any section under the profile curve can be rapidly determined by simply dragging the integration tool along the region(s) of interest
- All peaks of user-defined minimum size can be automatically identified
- Peaks of interest can be manually identified and/or edited
- View peak and profile information directly on screen
- Molecular weight standard curve generation with scatter plot for best fit determination
- Molecular Weight values can be determined by just moving the cursor across the unknown profile
- Profile graph can be printed at any point in the analysis
- Reports include profile information, peak data, and molecular weight data

Part 2: Profile Tool Palette

Choosing *Profile Analysis* from the ANALYSIS menu displays the profile tools on the right side of the tool palette. When displayed, the profile tools consists of three boxes, each of which represents a specific object which can be used to generate a profile from an image. When a tool is selected (clicked on) and used, the specified active object will be displayed on the image. A maximum of eight (8) objects can be displayed at one time. Conversely, profile objects can be deleted from an image using the EDIT menu command *Delete* or *Clear all*.

All of the objects (lines, multi-segments, and column averaging rectangles) can be used in vertical, horizontal, and unrestricted positions by using the profile *Tool Preferences* command which can be accessed from the FILE menu. Alternatively, double clicking on any profile tool icon in the tool palette will also display the *Tool Preferences* dialog box. Additionally, all of the profile tools may be modified by using the altering tools which are activated when the cursor is placed on different parts of the object. These cursor tools (movement arrow, activating hand, sizing square, rotating arrow, and duplication tool) are active when properly placed on parts of an object. Once a profile

tool has been used, the command *Analyze Profile(s)* from the ANALYSIS menu will send the profile to the Profile Analyst module where it will be displayed in a graph for further analysis.



Single Line Tool

The single line profile tool is used to identify lanes of data in a straight vertical, horizontal, or unrestricted line. This tool uses a single pixel cross section of the image data to display the image profile.

- Double click on the single line profile tool icon. Select either a vertical, horizontal or unrestricted line.
- Move the pointer to a location over the data, normally above the first band in the lane of interest.
- Click the mouse button and drag the mouse until the line has completely passed through the data, and release the mouse button. A single line profile object will appear in the active color.
- *Object Altering tools*- When placed on the line, these tools allow the single line object to be modified to better fit the image.

Movement arrow. Allows the line to be moved to another location. Place the cursor directly on the line, click and drag the line to the new location.

<Ctrl> +Movement arrow. Clicking the movement arrow while holding the *<Ctrl>* key down, duplicates the line.

Sizing square. Allows the line to be lengthened or shortened. Place the cursor on the square ends of the line. Notice that the cursor has changed to the sizing square. Adjust the length of a vertical or horizontal line, or the position of an unrestricted line.



Multi-Segment Tool

The multi-segment profile tool is used to identify lanes of data that are skewed or are not in a straight vertical line. This tool uses controlled line segments to identify single pixel cross sections of the data to display the image profile.

- Double click on the multi-segment profile tool icon. Select either a vertical, horizontal or unrestricted multi-segment.
- Move the pointer to a location over a skewed lane of data
- Click and hold the mouse button and move the cursor until one line segment has completely passed through one set of data. Click the mouse button. Move the mouse again and draw the another line segment through the second data point and click the mouse. Continue until a line has passed through all of the data points to analyze.
- To complete the multi-segment line, select *Done* from the tool palette or simply press the <Esc> key.
- *Object Altering tools*- When placed on the multi-segments, these tools allow the segments to be modified to better fit the data.

Movement arrow- Allows the line segments to be moved to another location. Place the cursor directly on a segment of the line (not on an anchor point). click and drag the line to a new location.

<Ctrl> +*Movement arrow*. Clicking the movement arrow while holding the <Ctrl> key down, duplicates the line segments

Sizing square- Allows each segment to be angled, lengthened, or shortened. Place the cursor on any square anchor point of the multi-segment line. Notice that the cursor has changed to the sizing square. Adjust the length or position of each segment of the line as desired.



Averaging Rectangle Tool

The averaging rectangle tool uses a user-defined rectangle to identify and average all of the data pixels in the selected area to display a profile. When used across a uniform rectangle shaped band, this tool generates the most accurate profile.

- Double click on the rectangle tool icon. Select either a vertical, horizontal or unrestricted rectangle tool. Note that using the rectangle tool in the wrong orientation will result in an improper profile calculation
- Move the pointer to a location over the data.
- Click the mouse button and drag the mouse in the orientation as selected in the tool preferences dialog box until a rectangle has completely passed through the data, and release the mouse button. A rectangle will appear. Note: Do not make the rectangle larger than the band, as this will average low background values with high sample values resulting in erroneous calculation.
- *Object Altering tools*- When placed on the rectangle, these tools allow the object to be modified to better fit the data.

Movement arrow- Allows the rectangle to be moved to another location. Place the cursor directly inside the rectangle (not on the center dot). click and drag the rectangle to a new location.

<Ctrl> +Movement arrow- Clicking the movement arrow while holding the *<Ctrl>* key down, duplicates the rectangle.

Sizing square- Allows the height or width of the rectangle to be lengthened or shortened. Place the cursor on any square anchor point of the multi-segment line. Notice that the cursor has changed to the sizing square. Adjust the length or position of each segment of the line as desired.

Rotating arrow- Placing the cursor on the center dot of the rectangle object, clicking and moving the mouse vertically rotates the rectangle. Note: For fine adjustments of the angle of rotation, click on the center dot, hold the mouse button down, and move the cursor farther away from the rotating point.

Part 3: Description of Profile Analysis Menus and Functions

In this section, each of the Profile Analyst module functions, menus and commands will be described in detail.

Display Function

Mode
Display
Weight
Report

A. MODE Menu

The mode menu is continuous throughout the profile analysis module, allowing the user to move rapidly from one function (Display, Weight, and Report) to another without having to go through multiple menu levels. All the Profile Analyst module functions can be accessed from the *Mode* Menu throughout the module. Also available in the *MODE* Menu is the *Exit* command, which allows the user to exit to the image module instead of to another function.

MODE COMMAND: *Display*

These commands are used to display, optimize, and enhance profile data; as well as, perform advanced algorithms for the identification of peaks. The *Display* commands appear when a profile object is used and a profile is created using the *Analyze Profile(s)* command from the image module.

MODE COMMAND: *Weight*

These commands allow for the entering of standard molecular weight values into a table, calculation of a relative mobility vs. log molecular weight standard curve, and calculation of unknown standard molecular weights.

MODE COMMAND: *Report*

These commands allow you to view or print a report of any profile contained within any file. Reports include: information about the profile itself, an area report if peaks have been identified, and a molecular weight report if molecular weights have been calculated. Note: to print the profile in a graph, the profile must be displayed from the DISPLAY window and printed by pressing the right mouse button and selecting the *Print* command.



B. FILE Menu

FILE COMMAND: *Copy Profile*

This command allows individual profiles to be copied from one file to another. This command is only functional when there are no profiles currently displayed on screen. When *Copy Profile* is selected, a dialog box appears. The left half contains Source information and the right side contains Destination information. To open the file where the profiles are currently located, the Source file, click on the *Open* command button in the Source field. Select a file name and click on *Open*. The names of the profiles in that file will appear in the source field. Click on the first profile that you wish to copy into another file. Note that the name appears in the Profile Name field. To open the destination file, click on the *Open* command button in the destination field. Select the file that you want the profile(s) to be copied into. That file name will appear in the top of the Destination field, with the profiles currently located in the file listed below. Click on the *Copy* command button. The highlighted profile in the Source field will now appear on the Destination field profile list. Repeat this procedure for as many profiles as desired. When you have finished, click on *Done*. The profile(s) will now be located in both the Source file and the Destination file. This feature is necessary for moving profiles to the same file as this is required for molecular weight analysis.

FILE COMMAND: *Copy to Clipboard*

This feature allows you to transfer data points (x-Distance and Y-O.D.) from the active profile to the Microsoft Windows Clipboard. From there the data points can be transferred to many Windows programs, such as Excel or Microsoft Word, for additional analysis or publication. If no profiles are currently displayed, selecting this function will display a dialog box prompting you for a FILE name. A second dialog box will then appear prompting for a profile name. Select one by clicking on it, and then clicking on *Add*. The data points will automatically be copied to the Windows Clipboard. If profile(s) are currently displayed on screen, the data points from the active profile will be copied to the Windows Clipboard. The Windows Clipboard accepts a maximum of 2000 data points, therefore: it may be necessary to clip the profile before copying.

FILE COMMAND: *Delete Profile . . .*

This command is only functional when there are no profiles currently displayed on screen. When this command is selected, the Windows entry box will appear which provides: a scrolling list box of the FILE names within the current drive and directory, and an entry box for entering the FILE name to open for deleting profiles. Notice that the entry box will have *"*.SCN"* highlighted, indicating that .SCN must follow a FILE name. Move the mouse pointer to the FILE name in the list box containing the profile(s) that you wish to delete, and double click with the left mouse button.

Locating FILES in other drives and directories

Changing directories within current drive:

To change directories within the current drive, using the scroll bars if necessary, double click on [..]. The list box will now list the directories available on the current drive. Double clicking on the desired directory will open that directory and list the existing FILES within that directory which have the FILE extension (.SCN).

After opening a profile file for deletion of profile(s), a new dialog box will appear listing all the profiles within the FILE. To delete a single profile, point and click once on the profile name to highlight it, then click on the *DELETE* Command Button. To delete more than one profile at a time, press and hold <SHIFT> while pointing and clicking on all the profiles you wish to delete. When you have finished deleting profiles, point and click on the *CANCEL* or *EXIT* Command Buttons. It is important to note that at this point the profile names have been deleted from the FILE, however, disk space occupied by the deleted profiles will not be freed until the *Compact FILE* command is executed as outlined below.

FILE COMMAND: *Compact FILE*

This command is also only functional when there are no profiles currently displayed on screen. Disk space occupied by profiles deleted using the *Delete Profile . . .* command is not freed up until either the Profile Analyst program is exited, or the *Compact FILE* command is used. By using the *Compact FILE* command, it is possible to free up the disk space occupied by the deleted profiles without exiting the Profile Analyst software program.

Profile
New...
Add...
Delete
Close Graph
Original
Previous
Save
Save As...
Set Type
Profile Label
Profile Info

C. PROFILE Menu

PROFILE COMMAND: *New . . .*

The *New . . .* command will display a new graph containing a selected profile. A maximum of four different graphs can be displayed at once, with each graph containing only one profile. To display a new graph containing a profile from within the profile list box, simply double click on the desired profile name, or enter the profile name complete with extensions and appendixes and then click on the *ADD* Command Button.

The profile-information dialog box contains a list box of all of the profiles contained within the file which has been selected. After you point and click on one of the profile names within the list box, the Type and Operations boxes will now show all the identifying characteristics of the profile. The Type box provides the following information:

Type Box

Raw - This identifies the highlighted profile as being an unprocessed profile. This type of profile is always the original profile Raw profiles will never have a character appendix.

Processed - This identifies the highlighted profile as being processed in some manner. Processed profiles will always have at least one of the operations in the operations box highlighted, identifying the manner in which it has been processed. Processed profile names will always be followed by a character appendix, identifying it as being a processed profile.

Standard - This identifies the highlighted profile as having been tagged as a standard profile. The processed standard profile is used in the generation of a standard curve for molecular weight determination of unknown samples.

Background - This identifies the highlighted profile as having been tagged as a background profile. The background profile is data associated with the background of the electrophoretic sample, but not the sample itself. A background profile can be subtracted from a standard or unknown profile to remove all area which is background data. A background profile can be displayed, but the Profile Analyst II software will not allow it to be processed in any manner.

Unknowns- This identifies the highlighted profile as having been tagged as an unknown profile. An Unknown profile is any one-dimensional data which contains sample data, and is not a standard profile.

PROFILE COMMAND: *Add . . .*

The *Add . . .* command will add a profile to an active graph. This command can be used until a maximum of four profiles are displayed, in one or more graphs.

PROFILE COMMAND: *Delete*

The *Delete* command will delete only the active profile (denoted by its color).

PROFILE COMMAND: *Close Graph*

This command closes the active graph with all its contents. To close a graph which does not contain an active profile, double click on one of the profiles within that graph making it active and then select the *Close Graph* command.

PROFILE COMMAND: *Original*

This command removes the active profile and replaces it with the original version of the profile. The *Original* command is necessary to restore a profile to original form once any "data modification" has been performed upon it.

PROFILE COMMAND: *Previous*

When this command is selected, the program will toggle back and forth between the current and the previous profiles which has had a modification performed upon it.

PROFILE COMMAND: *Save*

When this command is selected, the active profile will be saved with any modifications which have been performed. When a raw profile is received, the data is stored in binary in the following format: FILE.SCN. As individual raw unprocessed profiles are created and stored into the files, they are sequentially given a three digit extension to their root name, starting with .000, in the format: root name.XXX, i.e., Profile.000. Using the *Save* command will not write over the original profile data, instead it will save processed profiles (background subtracted, peaks identified, etc..) using the same profile name along with a character appendix, i.e., Profile001-a. Molecular weight standards data table files are stored in the following format: standardfile.MW.

PROFILE COMMAND: *Save As . . .*

When this command is selected, the active profile can be saved using any root name desired up to fifteen characters in length. The program will automatically add the appendix "a" identifying the profile as a processed profile. Note: no extension is necessary when enter the profile name, as the program will automatically add the following extension and appendix ".000-a".

PROFILE COMMAND: *Set Type*

This command allows you to change the profile type: Standard, Background, and Unknown. The name of the active profile will appear at the top of the box, and its currently set type will be selected. Click on the radio button corresponding to the type you wish to change the active profile to. After making the designation, click on *Set*. Select *Save* under the *PROFILE* menu to implement this change. Once changed, the profile will be saved both with its original type and the new type designation. This command is useful for identifying a profile as a background profile to be subtracted from other profiles; or as a standard profile for calculating a molecular weight standard curve. The default profile type is unknown.

PROFILE COMMAND: *Label Profile*

This command adds a label to your profile. Type in the text (up to 80 characters) and click on the *Set* button. You will now see the cursor in the shape of a pointing hand. Move the cursor to the location within the graph where you want the left edge of the label to appear and click the mouse button. To relocate or change the profile label, simply repeat the above procedure. To add a profile label to any other profile which is currently displayed on screen, simply double click on that profile with the pointer to make it active, then repeat the above procedure.

PROFILE COMMAND: *Profile Info*

When this command is selected, a "hand pointer" icon will appear for pointing and clicking on any profile within the active graph. By placing the "finger" of the icon on one of the profiles in the graph, and clicking with the left mouse button, a table of information regarding that profile will appear on screen. Pointing and clicking with the icon on another profile within the active graph will update the profile information table to reflect that profile. When finished with the *Profile Info* command, click on the *OPERATION* menu, then click on either *Complete* or *Cancel* to remove the profile information box.

Optimize
Snip at <u>V</u> iew
Construct Baseline
Sub Constructed Baseline
Sub <u>H</u> orizontal Baseline
Sub <u>B</u> ackground
<u>D</u> ifference Profile
<u>A</u> verage Profiles...
<u>O</u> rigin
Front
Flip
Align <u>2</u> pts
Align <u>4</u> pts
<u>D</u> ragXY
Drag <u>X</u>
Drag <u>Y</u>

C. OPTIMIZE Menu

OPTIMIZE COMMAND: *Snip at View*

When this command is selected, any view modifications performed with the *Zoom* function (accessed by pressing the right mouse button when the cursor is placed in a profile graph) will be transformed into a data modification. The user can therefore zoom in on any section of the displayed profile and analyze that section of the profile exclusively by pointing and clicking on the *Snip at View* command. Once the *Snip at View* command has been used, either the *Original* or *Previous* command must be used to restore the original or previous data.

OPTIMIZE COMMAND: *Construct Baseline*

The command lets you draw a valley to valley baseline which is useful for profiles containing a drifting baseline. To use this command, point and click on the *OPTIMIZE* menu and select *Construct Baseline*. Notice that the cursor becomes a pointer. Use the mouse to move the pointer to a spot to the left end and off of the profile and click. Then follow the contour of the profile, moving from valley to valley pointing and clicking. Finally point and click on a point to the right end and off of the profile. Be sure that you always extend the constructed baseline beyond the left and right ends of the profile. Once the baseline is constructed, point and click on the *OPERATION* menu. Select the *Complete* command to draw the baseline. Next, select *Sub Constructed Baseline* to subtract the baseline.

OPTIMIZE COMMAND: *Sub Construct Baseline*

The *Sub Construct Baseline* command is only operational if the *Subtract Baseline* Check Box is deactivated under the *SET UP* Menu, and if a baseline has been constructed using the *Construct Baseline* command. The constructed baseline will then be filled in and remain visible after the *Complete* command, under the *OPERATION* Menu has been selected. This allows the constructed baseline to be displayed in the same manner as a profile for comparison. To perform the subtraction of the constructed baseline from the active profile, simply point and click on the *Sub Construct Baseline* command.

OPTIMIZE COMMAND: *Sub Horizontal Baseline*

When this command is selected, a horizontal baseline can be selected and automatically subtracted from the active profile. This command is useful for subtracting background from profiles containing a uniform background level. To subtract a horizontal baseline from the active profile, simply point and click on *Sub Horizontal Baseline*, the mouse pointer will change to a horizontal line which is adjustable in the vertical direction. Move the horizontal line cursor up or down until the desired baseline position is located, then click on the left mouse button to automatically subtract the horizontal baseline.

OPTIMIZE COMMAND: *Subtract Background*

When this command is selected, actual background profiles from the image can be subtracted from the active profile. This method of background subtraction is usually the most accurate and should be the method of choice when available. To perform the subtraction of a background profile from an active profile, click on the *OPTIMIZE* Menu, then click on the *Subtract Background* command. A list box will display all background profiles in the active profile file. Only profiles designated as background profiles (using the *Set Type* command from the *PROFILE* menu and then subsequently saved) will be available for subtraction. Once a background profile is selected it is immediately subtracted from the active profile, and the resulting profile is then displayed.

OPTIMIZE COMMAND: *Difference Profile*

The *Difference Profile* command allows the subtraction of any profile (except a background profile) from the active profile, creating a difference profile. This difference profile can be subsequently analyzed in the same manner as an original profile, however, it cannot be saved. The *Difference Profile* command is useful for the determination of the difference between two profiles such as a control and an experimental sample. To subtract one profile from another both profiles must be displayed in the same graph. When two profiles are displayed in the same graph, the program will automatically subtract the non-active profile from the active profile. If more than two profiles are displayed in the same graph, when the *Difference Profile* command is selected a hand will appear. Point the hand to the profile which you wish to subtract the active scan from, and click the left mouse button. Once the difference profile has been executed, the resulting difference profile will be displayed as the active profile.

OPTIMIZE COMMAND: *Average Profiles*

The *Average Profiles* command allows you to average any number of different profiles. Selecting this command when there are no profiles currently displayed on screen will display an entry box prompting for a filename. Click on the filename containing the profiles to be averaged. Note: If the profiles are not within the same file, the *Copy Profile* command must be used to move profiles to a common file for averaging. Click on all the profile names which you wish to be averaged and then click on the *Avg* button. Only the highlighted profiles will be averaged. The resulting average profile will then be displayed on screen.

If two to three profiles are currently displayed on screen in the same graph, or in separate graphs, selecting this command will average all of the profiles displayed on screen. The resulting average profile will be displayed as the active profile in the active graph, along with all of the original profiles in their respective graphs. Note: To average more than three profiles, select the *Average Profiles* command without any graph displayed in the Display Window, as described above.

OPTIMIZE COMMAND: *Origin*

The *Origin* command allows the identification of the gel lane origin within the profile. The *Origin* command is used in conjunction with the *Front* command for the determination of peak relative mobility, and subsequent molecular weight analysis. To specify the origin of the displayed active profile, click on the *OPTIMIZE* Menu, then click on *Origin*. The mouse pointer will change to a scissors icon which should be placed at the origin resolving interface, near the left edge of the profile (X min.). Press the left mouse button and the portion of the profile left of the origin will be deleted with the point of "cut" now being the new origin.

OPTIMIZE COMMAND: *Front*

The *Front* command allows the identification of the sample lane front within the profile. The *Front* command is used in conjunction with the *Origin* command for the determination of peak relative mobility, and subsequent molecular weight analysis. To specify the front of the displayed active profile, click on the *OPTIMIZE* Menu, then click on *Front*. The mouse pointer will change to a scissors icon which should be placed at the dye front, near the right edge of the profile (X max.) on or above the front location. Press the left mouse button and the portion of the profile right of the front will be deleted with the point of "cut" now being the profile front.

OPTIMIZE COMMAND: *Flip*

The *Flip* command is used to flip a profile which was drawn in the incorrect orientation, with the dye front near the left edge of the profile. To flip a profile simply click on the *OPTIMIZE* Menu, then click on *Flip*. The data point sequence of the displayed active profile will then be reversed, with the origin of the profile now located near the X minimum location.

OPTIMIZE COMMAND: *Align 2 pts*

The *Align 2 pts* command allows two overlaid profiles to be aligned along the X-axis in relation to two corresponding points on each profile. By aligning the overlaid profiles, the differences in profile object drawing position or sample shape can be compensated. Thus comparison of peak mobilities among different profiles is facilitated with the use *Align 2 pts* command. To align any two profiles which are overlaid in the same graph, click on the OPTIMIZE Menu, then click on *Align 2 pts*. The mouse pointer will be replaced by a downward pointing hand. Move the “finger” of the hand to a point on one of the profiles, such as the top of a peak, which you wish to align with a point on the other profile, and click the left mouse button. A small circle will remain identifying the first point location. Now move the hand to a desired or corresponding point along the second profile, and click or press and release the left mouse button. Once a point on the second profile has been identified, the program will automatically align the two profile along the X-axis.

OPTIMIZE COMMAND: *Align 4 pts*

The *Align 4 pts* command allows two profiles to be aligned not only along the X-axis, but along the Y-axis so that the profiles can be precisely overlaid on top of each other. By providing two “landmark” points along each profile, the *Align 4 pts* command uses a stretching routine, allowing for slight mobility differences between different gel lanes. To align and stretch any two profiles which are overlaid in the same graph, click on the OPTIMIZE Menu, then click on *Align 4 pts*. The mouse pointer will be replaced by a downward pointing hand. Move the “finger” of the hand to a point on one of the profiles, such as the top of a peak, which you wish to align with a point on the other profile, and click the left mouse button. A small circle will remain identifying the first point location. Now move the hand to a desired or corresponding point along the second profile, and click or press and release the left mouse button. Repeat this procedure with the remaining two points. Note that the order of identification of the four points on the profiles is not important, i.e., two points can be identified on the first profile before any point is identified on the second profile. Once all four points have been identified, the program will automatically align the two profiles stretching the second profile as necessary.

OPTIMIZE COMMAND: *DragXY*

The *DragXY* command allows an active overlaid profile to be dragged in any direction around the graph. To drag an active profile in a XY direction, click on the OPTIMIZE Menu, then click on *DragXY*. The mouse cross hair will be replaced by a vertical fish hook icon. Move the fish hook icon to a location within the graph where you wish to move the active profile, and click the left mouse button. The active profile will now appear at the new location. By pressing the left mouse button instead of clicking, a connecting line will be drawn from the profile to the new location and the profile will not move until the mouse button is released.

OPTIMIZE COMMAND: *DragX*

The *DragX* command allows an active overlaid profile to be dragged only in the X direction along the graph. This can be useful for quickly aligning or organizing many profiles within the graph to facilitate interpretation while maintaining the profiles original Y position. To drag an active profile in the X direction, click on the OPTIMIZE Menu, then click on *DragX*. The mouse cross hair will be replaced by a horizontal fish hook icon. Move the fish hook icon to any location on the active profile, and click the mouse button. Next, move the fish hook to the new X location, and hold down the mouse button. Notice that a connecting line will form between the two points. The profile will move when the mouse button is released.

OPTIMIZE COMMAND: *DragY*

The *DragY* command allows an active overlaid profile to be dragged only in the Y direction along the graph. This can be useful for quickly aligning or organizing many profiles within the graph to facilitate interpretation while maintaining the profiles original X (distance) position. To drag an active profile in the Y direction, click on the *OPTIMIZE* Menu, then click on *DragY*. The mouse cross hair will be replaced by a vertical fish hook icon. Move the fish hook icon to any location on the active profile, and click the mouse button. Next, move the fish hook to the new Y location, and hold down the mouse button. Notice that a connecting line will form between the two points. The profile will move when the mouse button is released.



E. ENHANCE Menu

ENHANCE COMMAND: *Criteria*

The *Criteria* command allows you to specify the level of smoothing or enhancement which you desire for your particular sample data. When finished setting the desired parameters, click on the *Set* command button.

Window Size- This entry field allows you to enter the filtering window size value used by the *SMOOTH* command. Enter a value from 3 (minimum smoothing), up to 99 (maximum smoothing). This value determines the number of data points which the moving weighted algorithm will use to smooth the data.

Iterations- This entry field allows you to enter the number of iterations (passes) you wish to have the *SMOOTH* command perform using the specified window size. Enter a value from 1 to 99 smoothing passes.

En Boost. The enhancement boost factor is a constant which is multiplied by high spatial frequencies extracted from the data. The result is then added back into the original data. Enter a boost value between 1 and 10 as desired.

Display Each- When this check box is activated, the program will successively display each smoothed result profile when an iteration is specified.

ENHANCE COMMAND: *Smooth*

When this command is selected, the program will smooth or suppress high frequency noise within the active profile according to the window size and number of iterations assigned under *Criteria*. The algorithm used by the *Smooth* command is simply a moving weighted average of nearby data points. The larger the window size the more the data will be suppressed down to a lower frequency. The application of a moving weighted average filter to smooth the original data will change the shape of the curve, however, it will not change the area under the curve. That is, for every peak reduced an equivalent amount of area is distributed to the surrounding valleys.

ENHANCE COMMAND: *Enhance*

When this command is selected, the program will enhance the active profile according to the enhancement boost factor (En Boost) assigned under *Criteria*. The enhancement feature is designed to boost high spatial frequencies of partially resolved peaks. This is done by extracting the high spatial frequencies, multiplying them by a constant (the boost factor) and adding them back into the original data. In the enhancement process, we have extracted rather than suppressed components above the enhancement frequency. Note: Do not use boost factors so high that the results go off scale. If this rule is followed, the measured areas of peaks will be accurate.

Peaks
Peak Criteria...
Edit...
Auto Identify
Gaussian
Integrate Interactive
Peak Info

F. PEAKS Menu

PEAKS COMMAND: *Peak Criteria . . .*

This command allows you to enter specific peak criteria parameters for use with the automatic peak identification routine. The criteria which you set are exclusion values which will determine which peaks within the profile will be identified and integrated. Any or all of the peak identification parameters can be used to eliminate unwanted peaks when using the automatic peak identification routine.

Amplitude- This entry field allows you to select a minimum peak amplitude, whereby all peaks with a maximum peak height **below** this value will not be identified.

Width- This entry field allows you to select a minimum peak width (mm) "cut-off" value, whereby all peaks with a width less than this minimum value will not be identified.

Area- This entry field allows you to select a minimum peak area "cut-off" value, whereby all peaks with an area less than this minimum value will not be identified.

Sm Win- The Smoothing Window entry field allows you to select a smoothing window value for use in the program's automatic peak identification routine. Using a smoothing window has the same effect as the *Enhance* Menu's *Smooth* command. Entering increasing smoothing window values along with at least one iteration, identifies fewer unwanted peaks **Note:** for the smoothing window to be operational a smoothing iteration value of at least one must be entered.

Sm Iter- The smoothing iterations entry field allows you to select the number of passes which the smoothing window will make over the profile. The more iterations that are performed, the more accurate the smoothing and subsequent peak identification will be. If the automatic peak identification routine is still identifying unwanted noise, either increase the smoothing window size, enter peak amplitude, width, or area criteria's, or perform an actual smoothing of the data points using the *Smooth* command.

Calc'd Win- This is only a message box displaying the smoothing window value calculated by the program during the last automatic peak identification. This value will be zero when the program is first started. The calculated smoothing window value serves a guide to the user when setting the smoothing window value under *Sm Win*. Increase the smoothing window to identify fewer peaks and decrease the smoothing window to identify more peaks.

PEAKS COMMAND: Edit . . .

The Edit . . . command allows you to manually create, adjust, or delete any peak within the active profile. When the Edit . . . command is selected, the PEAK EDIT window will replace the DISPLAY window, providing a new menu for peak editing. The following menu items are available within the PEAK EDIT window:

Edit- The Edit Menu contains the Cancel and Exit commands. Both commands will exit the PEAK EDIT window and return to the DISPLAY window. When the Exit command is used, all changes made in the PEAK EDIT window will be reflected in the DISPLAY window. To cancel all editing done within the PEAK EDIT window and return to the DISPLAY window simply press the Cancel command.

Create- The Create command allows you to quickly manually identify and number peaks within the active profile. When selected, the pointer will change to a "magic wand" icon. Simply move the point of the "magic wand" to the origin of the peak you wish to identify, and press the left mouse button. Continue pressing the left mouse button while dragging the "magic wand" to the right until the end (front) of the peak is reached. The area under the curve will fill in as the "magic wand" is dragged across the profile. If the boundaries of the peak you are creating overlap a previously created peak, the area of overlap will not be filled in. When finished creating peaks, either click on the Cancel or the Exit command under the Edit menu to return to the DISPLAY window.

Ajust- The Ajust command allows you to quickly adjust the boundaries of a previously identified or created peak. When this menu/command is selected, the pointer will change to a "tuning fork" icon. Simply place the arrow head of the icon over the peak which you wish to adjust and press the left mouse button. The "tuning fork" icon will now change to a "magic wand" icon allowing you to redraw the area for that peak as outlined above under Create. When the mouse button is released after the adjustment, the icon will change back to the "tuning fork" allowing you to select another peak for adjustment. Click on either the Cancel command or on the Exit command under the Edit menu to return to the DISPLAY window.

Delate- The Delate command allows you to rapidly delete any peak within the active profile. When this command is selected, the pointer will change to a "hand grenade" icon. Simply place the arrow head of the icon over the peak which you wish to delete and press the left mouse button. The peak will immediately disappear as the graph is updated. Click on either the Cancel command or the Exit command under the Edit menu to return to the DISPLAY window.

Cancel- This command allows you to cancel the current edit operation.

PEAKS COMMAND: *Auto Identify*

The *Auto Identify* command performs an automatic identification of all peaks within the active profile, which meet the criteria set under *Peak Criteria* . . . The integration algorithm used in the automatic peak identification program is a trapezoidal integration algorithm. To use the *Auto Identify* command, simply point and click on **PEAKS** and select *Auto Identify*. The area values for each identified peak can be found using the *Peak Info* command or by saving the profile and printing a report.

PEAKS COMMAND: *Gaussian*

The *Gaussian* curve fitting command is used for the integration of overlapping peaks. Because overlapping peaks are not "baseline" resolved, using the integration values determined with *Auto Identify* command would not be precisely accurate. The *Gaussian* curve fitting command calculates what the normal progression of the peak would be if it were not smeared (completely resolved), providing for more accurate quantitation values. The *Gaussian* curve fitting command can only be used once the peaks of interest have been identified using the *Auto Identify*, or *Edit* . . . commands. Once all the desired peaks within the profile have been identified, simply point and click on **PEAKS**, and select *Gaussian*. Instead of filling in the area under the curve, the *Gaussian* curve fitting command will overlay a different colored trace for the area boundary which was integrated for each peak. The area values for each identified peak can be found using the *Peak Info* command or by saving the profile and printing a report. All identified peaks within a particular profile may not be identified by the Gaussian function if the peak and /or the profile do not meet certain criteria required by the function.

PEAKS COMMAND: *Integrate Interactive*

The *Integrate Interactive* command allows you to quickly get area values for any peak or segment of the active profile. These values are not retained anywhere within the program and must be written down if they are to be used further. To use the *Integrate Interactive* command, simply click on **PEAKS**, and select *Integrate Interactive*. The pointer will change to an "integral" icon. Place the icon at the beginning of the area you wish to integrate, and press the left mouse button. The area box in the lower right corner will now display an area value of zero. Drag the icon to the end of the area you wish to integrate and release the mouse button. The area box will now display the integral value for the area just identified. Every time the left mouse button is pressed the area value will zero, and a new area can be integrated by dragging the icon. Please note that the icon must be dragged from left to right for proper integration. To exit the *Integrate Interactive* command, click on **OPERATION** and select *Cancel*.

PEAKS COMMAND: *Peak Info*

The *Peak Info* command provides information regarding any peak which has been identified within the active profile. To use the *Peak Info* command, point and click on **PEAKS** and select *Peak Info*. The pointer will change to a "hand" icon. Using the icon, point to any peak area, and click in the left mouse button.

The following is the information provided in the Peak Information dialog box:

Number- This identifies which peak the peak information box is currently listing information for.

Name- If the peak is a standard from a standard profile which has had a molecular weight standard curve calculated, then name belonging to the peak would be displayed here.

Start- This identifies the x location (mm) where the peak starts (origin).

End- This identifies the x location (mm) where the peak ends (front).

Amplitude- This identifies the maximum peak height (O.D.).

Amp X Loc- This identifies the x location (mm) of the peak amplitude.

Width- This identifies the width of the peak (End minus Start).

Area- This identifies the area (O.D./ mm) associated with the peak. In terms of absolute quantitation, this value would be equivalent to concentration.

Percent Total- This identifies the percentage of the total area under the profile curve which is associated with that peak.

Percent Selected. This identifies the percentage of the total area selected which is associated with that peak. The total selected area is the area comprised of only the peaks which have been identified.

Distance- This identifies the distance (mm) the peak amplitude is from profile origin.

RF- This is the relative mobility value calculated for the peak. This is the ratio of the distances traveled by the spot and the dye front. This value is always less than one.

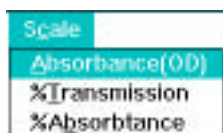
MW- This is the molecular weight value for the peak which was determined using the molecular weight function. A value will only be displayed if the molecular weight of the peak has been previously calculated.

Amount- The amount in peak will only be calculated and displayed here if a profile track loading amount was entered during the capture process. This amount in peak value is calculated from the percent total profile area attributed to this peak.

Mean- The mean value will only be displayed if Gaussian curve fitting was performed. This is the mean x value (mm) for the curve.

Std Dev- The standard deviation value is one standard deviation from the calculated mean. This value will only be displayed if Gaussian curve fitting was performed.

Amp- The Gaussian curve amplitude (max. curve height) will only be displayed here if Gaussian curve fitting was performed.



G. SCALE Menu

SCALE COMMAND: Absorbance (OD)

If the data units of the currently active scan are % transmission/reflectance, or %absorbance, selecting this command will convert the data units to absorbance (OD), and the Y axis of the graph will reflect this change. The OD scale is used for concentration quantities and represents $-\log_{10}$ (data/calibration).

SCALE COMMAND: % Transmission

If the data units of the currently active scan are absorbance, or % absorbance, selecting this command will convert the data units to % transmission, and the Y axis of the graph will reflect this change. This scale is useful for analyzing data which is in the % transmission/reflectance mode. Such data can be from photographs of ethidium bromide gels, whereby the bands in the photograph are white, and the background is black.

SCALE COMMAND: % Absorbance

If the data units of the currently active scan are absorbance, or % transmission / reflectance, selecting this command will convert the data units to % absorbance, and the Y axis of the graph will reflect this change.



H. OPERATION Menu

OPERATION COMMAND: Complete

The Complete command will finish in any operation which is currently in progress. This command is used after manually constructing a baseline. Complete instructs the program to fill in the constructed baseline or to go ahead and subtract the baseline from the active profile, depending upon whether the *Baseline Subtraction* box is checked under the *Graph Set Up* menu. Additionally, the Complete command will terminate the *Peak Info* and *Graph Info* commands.

OPERATION COMMAND: Cancel

The Cancel command will cancel any operation currently in progress. The Cancel command will be highlighted when available for canceling a operation in progress. Simply click on the OPERATIONS Menu and select Cancel and the operation will be canceled, bringing the program back to its previous status.

OPERATION COMMAND: Redraw

The Redraw command will redraw all profiles displayed and removes any profile or window artifact which may have been left behind from a previous function. Simply click on the OPERATIONS Menu and select Redraw and all profiles will be re drawn.



I. SETUP Menu

SETUP COMMAND: *Graph Header*

The *Graph Header* command allows you display text at the top of the graph containing the active profile. When selected, a dialog box appears where you can enter text and set horizontal justification. Click on the *Set* button when finished. This is a convenient way to identify a graph for printing, and while it is on the screen. The header however is not saved once the graph has been closed. This command is only functional when a profile graph is displayed.

SETUP COMMAND: *Graph Footer*

This command is identical to *Graph Header*, but the text appears at the bottom of the graph. This command is only functional when a profile graph is displayed.

SETUP COMMAND: *Graph Setup . . .*

This command allows you to have complete control of the graph display parameters. When this command is selected the following dialog box will appear:

Axis Calculations Field-

Range of Active Profile- Graph axis scaling will adjust according to only the active profile, and will not take into account the range of other profiles within the same graph.

Range of All Profiles- Graph axis scaling will adjust according to the range of all profiles within the graph.

Axis Padding (%)- This entry field allows you to enter the percentage of the graph which you want to have as padding around the profiles. If no padding is selected, the zero values for the profile(s) will lay directly on the X-axis. The default value is 10% padding.

Axis Labels Field-

Labels On All Graphs- Axis labels will be drawn on all graphs when multiple graphs are displayed. This is the default setting.

Labels On Single Graph- Axis labels are drawn when a single graph is displayed, however, when a new graph is added no axes will be displayed on any of the graphs.

Labels On No Graph- No axis labels will be drawn on any of the graphs.

Graph Operations-

Fill Peaks On Identify- Each peak on the graph will be filled in once it is identified using either the automatic or manual peak identification methods. If this box is not checked, peaks will not be filled in, and the only way to identify the peaks in the graph will be if the labels and perpendiculars are displayed.

Display Peak Labels- Peak labels, numbers, and molecular weight standard names, will be displayed on the graph above the corresponding peak.

Display Perpendiculars- When this box is checked, perpendiculars will be displayed identifying the X (mm) boundaries for each peak.

Other Options-

Subtract Baseline- When this box is checked, the subtract constructed baseline function is changed from a three step operation to a two step operation. The default, two step method is as follows: 1) A baseline is constructed using the *Construct Baseline* command from the *Optimize* Menu. 2) The *Complete* command is selected from the *Operation* Menu, and the constructed baseline is automatically subtracted from the active profile.

When this check box is not checked, the subtract constructed baseline function becomes a three step operation: 1) A baseline is constructed using the *Construct Baseline* command from the *Optimize* Menu. 2) The *Complete* command is selected from the *Operation* Menu, the constructed baseline becomes filled in for viewing and is not subtracted from the active profile until; 3) the *Sub Constructed Baseline* command from the *Optimize* Menu is selected.

Cross Hair Cursor- When this box is checked, the mouse cursor is changed from an arrow to a cross hair cursor. This is the default option.

SETUP COMMAND: *Colors*

The *Colors* command allows you to adjust the colors of all profiles and the graph background. This enables you to easily differentiate the profiles. The default colors for the profiles and graph background are: Profile 0= white. Profile 1= red. Profile 2= yellow. Profile 3= blue. Active profile= Fuschia. Graph background=black.

To change the color from its default value perform the following steps:

- Select the *Colors* command from the *SET UP* Menu to display the Colors dialog box.
- Click on the background or appropriate profile command button. The color window will now display the color currently assigned to that profile or background.
- Adjust any or all of the 3 color wheels by pressing either the up or down arrow until the desired color shade is displayed in shade window.
- Set the color by clicking on the *Fix* command button. Continue this procedure for any or all of the graph options as desired. When finished simply click on the *Exit* command button.

To reset the color or gray shades to their default values, simply click on the *Default* command button, then the *Exit* command button.

J. Right Mouse Button Commands

When a profile is displayed in the DISPLAY function, the **right** mouse button can be pressed to activate an additional command menu. This small menu box contains commands which act upon the active profile, and will only be displayed when the cursor is in the active graph. If more than one graph is displayed, the menu box will only be active in the graph containing the active profile. The following are the commands available in this menu box:

Zoom-

When the *Zoom* command is activated, the cursor will change to a magnifying glass. To zoom a specific section of the active profile, place the magnifying glass above and to the left

of the area you want to box for zooming. Press the left mouse button and drag the mouse down and to the right, until the desired area is boxed. Release the left mouse button. To work only with the zoomed section, click on the *Optimize* Menu and select the *Snip at View* command. This performs a data modification, eliminating all sections of the profile not currently in view.

Scroll-

When the *Scroll* command is selected, the cursor will change to a scroll icon, allowing you to drag the profile(s) about the graph. Using *Scroll* command will drag all profiles within the graph, and maintain their exact coordinates. This command differs from the drag commands in that the coordinates of the profiles are always maintained. When finished scrolling, press the right mouse button and select the *End* command.

End-

The *End* command will terminate either the *Zoom* or *Scroll* commands.

Previous-

The *Previous* command will toggle between the current display and the previous view modification which was performed. If you wish to go back to the zoomed section simply select *Previous* again. This command will not be operational if a data modification has been performed using the *Snip at View* command.

Restore- If a series of view modifications have been performed, e.g., multiple levels of zoom, and you wish to restore the original profile or the last data modification, simply select the *Restore* command.

Print- The *Print* command allows you to print the active graph and its contents. This is the only way to print the actual profiles. When this command is selected, a dialog box will appear stating that the graph is being sent to the printer. To cancel the *Print* command, click on the *Cancel* command button in the dialog box.

Molecular Weight Function

These commands allow for the entering of standard molecular weight values into a table, calculation of a relative mobility vs. log molecular weight standard curve, and calculation of unknown standard molecular weights.

A. MODE Menu

The mode menu is continuous throughout the Profile Analyst software program, allowing the user to move rapidly from one function (Display, Weight, and Report) to another without having to go through multiple menu levels. Also available in the *MODE* Menu is the *Exit* command, which allows the user to exit to the main menu or to another function.



B. FUNCTIONS Menu

FUNCTIONS COMMAND: *Size Standard Editor*

When this command is selected, the Standard Editor dialog box will appear allowing you either create a new standard molecular weight file (table) or edit an existing one. Each standard name and corresponding molecular weight in a standard molecular weight file is subsequently associated with a peak in a standard profile, using the *Calculate Standard* function.



Figure 18. Size Standard Editor dialog box.

To create a new standard molecular weight file:

Click on the *FUNCTIONS* menu and select the *Size Standard Editor* command. A dialog box appears. Enter the names and molecular weight values for your molecular weight standards by simply typing in the standard name, pressing <Tab>, and entering the corresponding weight. **Note: The DNA size standards must be entered in terms of bases and not kilobases.** Finally click on the <Enter> command button or press <Return>. Continue this procedure until all the standard names and molecular weights have been entered. Note that the order of entry is not important as they will automatically be placed in order of decreasing molecular weight. When finished, click on the *Save* command button. A dialog box appears prompting for a filename. Enter a filename and click on the *OK* command button. The filename will automatically be appended with a .MW extension.

To edit an existing standard molecular weight file:

Click on the **F**UNCTIONS menu and select the **S**ize **S**tandard **E**ditor command. A dialog box appears. Click on the **O**pen command button. A standard Windows entry box appears listing only the existing standard molecular weight files within the current drive and directory. Double click on the standard file that you wish to open. Once opened, the name of the file will be in the title of the dialog box, and the first standard and corresponding molecular weight of the file will be displayed in the entry boxes.

To add a standard to the weight table, type over the currently highlighted name and molecular weight and then click on **E**nter. Note: the standard and molecular weight you've typed over is not deleted from the table.

To delete, or change any of the values within the table use the **N**ext or **P**revious commands to step through the table until the desired standard and molecular weight are displayed. To delete that table entry press Delete and both the standard and corresponding molecular weight will be deleted from the table. To change any standard or molecular weight value for an entry, highlight that value and then enter the new value. Click on **E**nter and the table will be updated to reflect the changes.

To start a new standard molecular weight file:

Click on the **N**ew command button and a dialog box will appear asking if you want to save changes to the current file. Answer as appropriate. The edit fields will now clear allowing entry of data for a new standard molecular weight file.

FUNCTIONS **C**OMMAND: **C**alculate **S**tandard **C**ommand

The **C**alculate **S**tandard command is used to calculate a relative mobility vs. log molecular weight standard curve from standard profile data. When this command is selected, the following full screen dialog box appears with interior fields: the size standards field, the peak field, and the standard field.

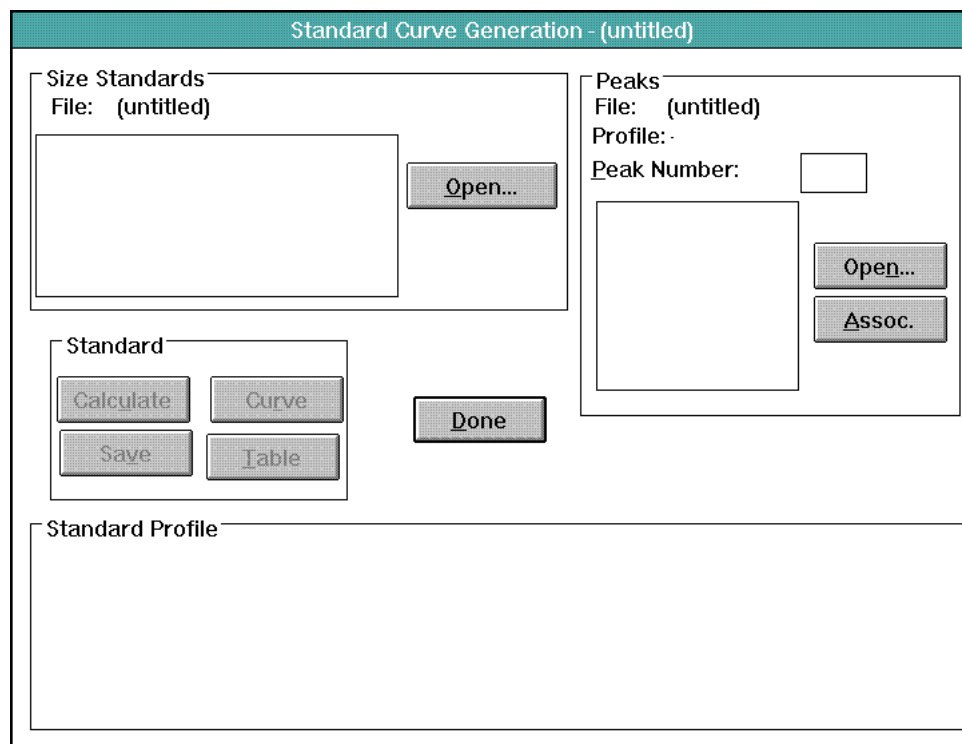


Figure 19. Standard Curve Generation dialog box.

Size Standards Field

The size standard field contains a scrolling list of standards and their corresponding molecular weights which can be associated with peaks within a profile file. The *Open* command button in the standard field opens an entry box which prompts for a standard molecular weight filename. This entry box will only list filenames which have the .MW extension. Double click on a standard molecular weight file to open it. To view the complete standard and molecular weight table it may be necessary to use the scroll bar.

Peak Field

The peak field displays a scrolling list of the peaks contained within a standard FILE which is opened. Clicking on the *Open* command button in the peaks field will display a dialog box which prompts for the name of the file in which the molecular weight standards profile is stored. Select a standard profile file by double clicking on it. A second dialog box will then appear prompting for the standard profile name. Double click on the desired standard profile name. The table will now list all the peaks which have been previously identified in the profile. The profile itself will also be displayed in the graph in the lower part of the screen. The *Assoc.* command button is used to associate a highlighted standard listed in the Size Standards field, to a appropriate highlighted Peak number in the Peak field. To continue associating size standards and peaks down the tables, simply continue to press the *Associate* command button. Note that the size standards are listed in descending order of molecular weight and that the peaks are listed in descending order of relative mobility.

Standard Field

The standard field contains the command buttons associated with calculation of the standard curve. Once the peaks have been associated with the correct size standard name and weight, the *Calculate* command button will become highlighted. Click on the *Calculate* command button to specify any or all the Regression types which you would like to have calculated. After clicking on the desired Regression type click on the *OK* command button. The regressions will then be quickly calculated, the Regression type dialog box will disappear, and the Calculate button will be grayed. To see the relative mobility vs. log (MW) scatter plot and the different regression curves calculated, click on the *Curve* command button. A scatter plot graph of relative mobility vs. log (MW) will then appear. To display the different regression curves on the graph, simply click on any or all of the check boxes along the bottom. To remove any of the curves, simply click again on the appropriate check box. The graph will update showing the selected regression curves in different colors. This feature is useful for determining which regression type provides the best fit of the data points. Due to the sigmoidal shape of many standard curves, the third or fourth order regression will often give the best fit of the curve. The user is however cautioned when using higher orders of regression (Appendix E). To close the scatter plot double click on the box in the upper left corner. Once the standard curves have been calculated, the *Save* command button must be selected in order to use this data for calculation of an unknown. The *Table* command button will display a list of the associations. To quickly close the table double click in the upper left hand corner on the menu bar.

Standard Profile Graph

The standard profile which is selected for generation of the standard curve is displayed in this field. The peaks which have been identified in this profile are shaded and have a peak number located above it. If the number of peaks which have been identified in the profile is greater than the number of standards listed in the molecular weight file, then either the molecular weight file or the profile will have to be edited.

When the cursor is moved into the profile graph the cursor becomes a vertical line which when moved left and right in the graph will correspond to the X (mm) readout value on the bottom of the graph.

FUNCTIONS COMMAND: *Calculate Unknown Command*

The *Calculate Unknown* command allows for the calculation of an unknown sample's molecular weights using a standard curve generated with the *Calculate Standard* command. Both the standard profile(s) and unknown profile(s) must be contained within the same file to be available for use in the *Calculate Unknown* command. When this command is selected, the following full screen dialog box appears with interior fields: standards, unknowns, unknown profile, and estimated molecular weight.

The dialog box is titled "Calculate Unknown Molecular Weight". It features a "File:" label at the top. The main area is divided into several sections: a "Standards" list box on the left, a set of checkboxes for "Linear", "Quadratic", "Cubic", and "Logistic" in the center, and an "Unknowns" list box on the right. Below the "Standards" list box are buttons for "Open", "Table", and "Calculate". Below the "Unknown Profile" list box are buttons for "Save", "Est. MW", and "Done". At the bottom of the dialog are radio buttons for "Linear", "Quadratic", "Cubic", and "Logistic". At the very bottom is an "Estimated MW" list box.

Figure 20. Calculate Unknown Molecular Weight dialog box.

Standards Field

The standards field will display all the standard profile names within the opened file, which are available for use in calculation of the unknown molecular weights. Only standard profiles which have had peaks identified and a standard curve calculated will be listed in this box. When a standard profile is highlighted in the list box, the regression radio button(s) on the right will be highlighted identifying the types of regressions which have been calculated using this profile. Use the scroll bar if necessary to highlight a desired standard profile.

Unknowns Field

The unknowns field will display all the unknown profile names within the opened file, which are available for use in calculation of unknown molecular weights. All profile names within this list box will have an appendix, e.g. sample.000-a, as only profiles which have had peaks identified will be available for molecular weight calculation. Use the scroll bar if necessary to highlight a desired unknown profile. Note that the unknown profile(s) must be from the same file as the standard profile(s).

Unknown Profile Field

The unknown profile field displays a graph of the unknown profile and provides an on-screen readout of the X (mm) location, relative mobility (Rf), and the calculated molecular weights, at the position of the cursor. When the cursor is moved into the graph it changes to a vertical line, enabling you to precisely position the mouse. The default on-screen readout values are for the cubic regression curve type. To change the on-screen readout values to reflect the specific regression curves, simply click on the appropriate radio button below the graph.

Estimated MW Field

The estimated MW field displays a table of the relative mobility (Rf), calculated molecular weights (linear, quadratic, cubic, and logistic), for each peak identified within the unknown profile. When the *Table* command button is pressed, Rf, MW, and standard names for each peak of the standard profile are displayed in this field. To view the estimated molecular weights again, click on the *Est. MW* command button to the right of the field. Use the scroll bar if necessary to view the full list.

Calculating Unknown Molecular Weight-

To calculate the molecular weight of an unknown, click on the *Open* command button. The Open file entry box will be displayed, listing all files within the current drive and directory. Double click on the file containing the standard and unknown profile(s) you wish to use in the calculation. Note: the standard and unknown profile(s) must be contained within the same file. Once opened, the file name will be displayed at the top of the dialog box, and the standard and unknown profiles available for calculation will be displayed in their respective fields. Scrolling if necessary, highlight both the standard profile and the unknown profile, which you wish to use for the molecular weight calculation. Click on the *Calculate* command button and the unknown profile graph will be displayed, along with the estimated molecular weights.

Move the cursor into the graph, and read the relative mobility and molecular weight values for any position along the profile. Click on the regression radio buttons so that the readout values reflect those regression curves. To save the calculated molecular weights for the unknown profile click on the *Save* command button before exiting using the *Done* command button. To calculate the molecular weight of another unknown profile in the opened file, using the same standard profile, highlight the new unknown profile name then press *Calculate*. The new unknown profile graph will be displayed and the estimated molecular weight table will be updated to reflect the new profile. If calculation of unknown using another standard profile is desired, simply highlight the unknown profile and the new standard profile then press *Calculate*.

Reports Function

The Reports commands allow you to print, view, or export a report on a profile contained within any file. The reports include: capture settings, information about the profile itself, an area report if peaks have been identified, and a molecular weight report if molecular weights have been

calculated. Note: to print the profile in a graph, the profile must be displayed using the DISPLAY function and printed by pressing the right mouse button and selecting the *Print* command.

A. MODE Menu

The mode menu is continuous throughout the Profile Analysis software program, allowing the user to move rapidly from one function to another without having to go through multiple menu levels. Also available in the *MODE* Menu is the *Exit* command, which allows the user to exit to another function or if selected again, to exit to the image module.



B. FUNCTIONS Menu

FUNCTIONS COMMAND: *C*ontents

When this command is selected, a dialog box appears containing check boxes for each section of a report which can be viewed or printed. Each report will always contain the profile name, profile type, the file name where it exists, and the date when it was printed. The following is a description of the report sections which can be printed:

Capture Settings- When this check box is checked, a list of the capture settings will be printed.

Profile Data - When this box is checked, a section of general profile information will be printed. This section includes origin and front X (mm) location, number of peaks identified in the profile, and total area under the profile curve.

Peak report- When this box is checked, information about each identified peak will be printed. This information includes: identification of whether Gaussian modeling has been performed, peak number, position within the profile, distance from the origin, peak relative mobility, maximum peak height, peak width, percentage of total area peak comprises, and amount of material in peak.

Molecular Weight Report- When this check box is checked, the molecular weight report section will be printed. If the profile being printed is a standard profile then the report will be a standard report consisting of: the peak number, peak name, relative mobility (Rf), and known molecular weight. Additionally, information regarding the standard curves will be provided. This includes: the equation, the coefficient values, R squared, mean Rf, mean Rf standard error, for each order of regression. This information is used to determine the regression order which provides the best fit, or most useful information. Normally, the regression order providing the highest R squared value, and the lowest standard error would provide the most accurate molecular weight values. However the user is cautioned when using higher orders of regression (see Appendix E).

If the profile is an unknown profile then the report will consist of: the unknown peak number, its relative mobility, and the molecular weights as calculated using the first, second, and third order regressions.

Top Printer Margin- This command adjusts the left printer margin. Simply enter a value between 0 and 8.

After selecting the desired options to be printed, click on the *Set* command button.

FUNCTIONS COMMAND: *V*iew

When this command is selected, a dialog box appears, prompting for a filename. Select the file name and click on the *P*rint command button. A second dialog box appears prompting for profile name. Select the profile name and click on the *A*dd command button. The report sections selected with the *C*ontents command will be displayed. Two full reports can be viewed at one time to allow profile report comparison. To print active reports at this point, select the *P*rint command from the FUNCTIONS menu. To make either of the viewed reports active, click on the title bar of the report you wish to print.

FUNCTIONS COMMAND: *P*rint

When this command is selected, the open file entry box will appear. Double click on the file which contains the profile report you wish to print. A new dialog box will appear containing a list box of the profiles contained within the file you have opened. Click on a profile name to highlight it. The two fields to the right contain radio buttons which will highlight, identifying the type of profile and any operations which have been performed on it. Click on the *A*dd command button to send this profile report to the printer.

FUNCTIONS COMMAND: *C*lipboard

Selecting this command will copy only selected sections from a viewed profile report to Windows Clipboard which can then be transferred to other applications, such as Microsoft Excel or Word. Report data will be transferred in a ASCII tab delimited format.

FUNCTIONS COMMAND: *S*ave as

Selecting this command allows you to save the reports. Enter in the appropriate report file name followed by the extension .rpt and click on OK. Reports are saved in a ASCII tab delimited format.

5.2 Volume Analyst Module

Part 1: Introduction

The Volume Analysis module provides a volume report of two-dimensional images scanned by the GS-670 Imaging Densitometer. Utilizing the Volume Tool Palette or the Automatic Spot Detection Molecular Analyst/PC

features, the determination of valuable information such as concentration, molecular weight, and pI; as well as, integrated sample volume and percent volume is easily performed.

The Image Analyst module offers the following features:

- Volume Tool Palette and automatic spot detection for rapid volume analysis
- Volume report can be produced to display volume, adjusted volume, and area
- Volume report data can be sorted by area, intensity, and position
- Standard curve can be generated using multiple regression analyses
- Concentration, molecular weight, and pI of unknowns can be calculated

Part 2: Volume Tools

Choosing *Volume Analysis* from the ANALYSIS menu displays the Volume Tools on the right side of the Tool Palette. When displayed, the Volume tools consists of five boxes, each of which represents a specific object used to identify spots on an image and generate a volume integration report. Double clicking on the tool icon displays the preferences for that specific tool. When a tool is selected and used, the specified object will be displayed on the image. All of the objects (circle, ellipse, rectangle, square, and grid) can be modified by using the object tool which is activated when the cursor is placed on an active object. A maximum of 200 volume objects can be created and displayed on an image. Conversely, volume objects can be deleted from an image using the EDIT menu command *Delete* or *Clear all*. Selecting *Analyze Volume Profile(s)* from the ANALYSIS menu to displays the volume integration report for all objects within the image.



Circle Tool

- Click on the circle tool icon.
- Using the mouse, move the cross-hair cursor to a point above and to the left of the spot in the image you want to quantitate.
- Click the left mouse button, and drag the mouse downward and to the right. A square will appear on the graphics screen. Moving the mouse adjusts the size of the square so that it completely encloses the spot. A circle will appear surrounding the spot when the mouse button is released.
- *Object Altering tools*- When placed on the circle, these tools allow the object to be modified to better fit the data.

Movement arrow- Allows the circle to be moved to another location

<Ctrl> +Movement arrow- Clicking the movement arrow while holding the *<Ctrl>* key down, duplicates the circle

Sizing square- When placed on the square surrounding the circle, this option allows the circle to be reduced or enlarged



Ellipse Tool

- Click on the ellipse tool icon.
- Using the mouse, move the cross-hair cursor to a point above and to the left of the spot in the image you want to quantitate.
- Click the left mouse button, and drag the mouse downward and to the right. A square will appear on the graphics screen. Moving the mouse adjusts the size of the square so that it completely encloses the spot. An ellipse will appear surrounding the spot when the mouse button is released.
- *Object Altering tools*- When placed on the ellipse, these tools allow the object to be modified to better fit the data.

Movement arrow- Allows the ellipse to be moved to another location

<Ctrl> +*Movement arrow*- Clicking the movement arrow while holding the <Ctrl> key down, duplicates the ellipse

Sizing square- When placed on the square surrounding the ellipse, this option allows the ellipse to be reduced or enlarged



Rectangle Tool

- Click on the rectangle tool icon.
- Using the mouse, move the cross-hair cursor to a point above and to the left of the spot in the image you want to quantitate.
- Click the left mouse button, and drag the mouse downward and to the right. A square will appear on the graphics screen. Moving the mouse adjusts the size of the square so that it completely encloses the spot. A rectangle will appear surrounding the spot when the mouse button is released.
- *Object Altering tools*- When placed on the rectangle, these tools allow the object to be modified to better fit the data.

Movement arrow- Allows the rectangle to be moved to another location

<Ctrl> +*Movement arrow*- Clicking the movement arrow while holding the <Ctrl> key down, duplicates the rectangle

Sizing square- When placed on the rectangle, this option allows the rectangle to be reduced or enlarged

Rotating arrow- Clicking the mouse on the center dot and moving the mouse vertically rotates the rectangle. Note: For fine adjustments of the angle of rotation, click on the center dot, hold the mouse button down, and move the cursor farther away from the rotating point.



Square Tool

- Click on the square tool icon.
- Using the mouse, move the cross-hair cursor to a point above and to the left of the spot in the image you want to quantitate.
- Click the left mouse button, and drag the mouse downward and to the right. A square will appear on the graphics screen. Moving the mouse adjusts the size of the square so that it completely encloses the spot. A square will appear surrounding the spot when the mouse button is released.
- *Object Altering tools*- When placed on the square, these tools allow the object to be modified to better fit the data.

Movement arrow- Allows the square to be moved to another location

<Ctrl> +*Movement arrow*- Clicking the movement arrow while holding the <Ctrl> key down, duplicates the square

Sizing square- When placed on the rectangle, this option allows the square to be reduced or enlarged

Rotating arrow- Clicking the mouse on the center dot and moving the mouse vertically rotates the square. Note: For fine adjustments of the angle of rotation, click on the center dot, hold the mouse button down, and move the cursor farther away from the rotating point.



Grid Tool

- Double click on the Grid icon to display *Grid Preferences*.

Options Field-

- Select either a 8 x 12 (96 well Bio-Dot apparatus format), 8 x 6 (48 well Bio-Dot SF Slot Blot format), or custom grid configuration. For custom configuration, designate the number of columns and rows from the *Grid* field. Click on the radio button.

Grid Field-

- Custom configuration allows you to choose the shape of the cells in a grid. If using a custom configuration, select the shape (circle, ellipse, square, or rectangle) of the cell of the grid. Click on the radio button. The 96 well Bio-Dot configuration always use circle cells, and the 48 well Bio-Dot SF configuration always use rectangle cells.
- Using the pull down menus, select the color of the grid, and the color of the sizing cell.
- Custom configuration also allows you to group or ungroup the cells in a grid. Check the *Grouped* box if you wish to move the grid components as a group. Leave the box unchecked if you want to move each cell independently. This may be preferred if spots vary significantly in location. Ungrouped cells can be later grouped and moved as one using the *Block Select* tool from the Tool Palette.
- Check the *Save Settings* box and Click OK.

Making the Grid-

- Using the mouse, move the cross-hair cursor to a point above and to the left of the rows and columns of spots in the image.
- Click the left mouse button and drag the mouse downward and to the right. A square will appear on the graphics screen. Moving the mouse adjusts the size of the square so that it completely encloses all of the spots. A grid with the specified cells will appear when the mouse button is released.

Moving the Grid-

- Ungrouped - Each individual cell can be moved by first activating the grid cell of interest. Once the cell is active, the cursor becomes the moving arrow tool. Hold down the left mouse button and move the cell to another location.
- Grouped - A grouped grid can only be moved if the entire grid is active. This is accomplished by placing the cursor on a point of the grid other than the sizing cell and clicking the mouse. Once the grid is active, the cursor becomes the moving arrow tool. Hold down the left mouse button and move the cell to another location. More than one grid cell can be moved by using the block select tool to activate multiple cells.
- Cut and Paste - Activate the grid by clicking on it with the mouse and select *Cut* from the EDIT menu. The grid will disappear from the screen. Next, select Paste from the EDIT menu. Place the cursor, now a Capital P, to the new grid location, and click the left mouse button.

Sizing the Grid-

- Ungrouped - Ungrouped cells **cannot** be sized as a complete grid.
- Grouped - With an active grid, place the cursor at the edge of the grid. Notice that the cursor has changed to the sizing square. Click the mouse button and drag the sizing square until the grid is the proper length and width. **Note:** The grid can only be reduced to the size in which all of the cells are contained in the grid. To further reduce the size of the grid, reduce the size of the cells.

Sizing Cells in a Grid-

- Ungrouped - Click the hand pointer on any cell of the ungrouped grid to activate the cell. Place the cursor on edge of the cell (the dotted square if the cell is a circle or ellipse). Notice that the cursor has changed to the sizing square. Click the mouse button and drag the sizing square until the cell is the appropriate size, and release the mouse button. All of the cells within the ungrouped grid will change to the same size as the modified cell.
- Grouped - Click the hand pointer on the sizing cell (upper left cell) of the grouped grid. Place the cursor on edge of the sizing cell (the dotted square if the cell is a circle or ellipse). Notice that the cursor has changed to the sizing square. Click the mouse button and drag the sizing square until the cell is the appropriate size, and release the mouse button. All of the cells within the grid will change to the same size as the modified cell.

Rotating a Grid-

- **Grouped** - Place the mouse on the center dot of the grid. Notice that the icon has changed to a rotating arrow. Click and hold the mouse button on the center dot. Moving the mouse vertically while holding down the left mouse button, rotates the column.

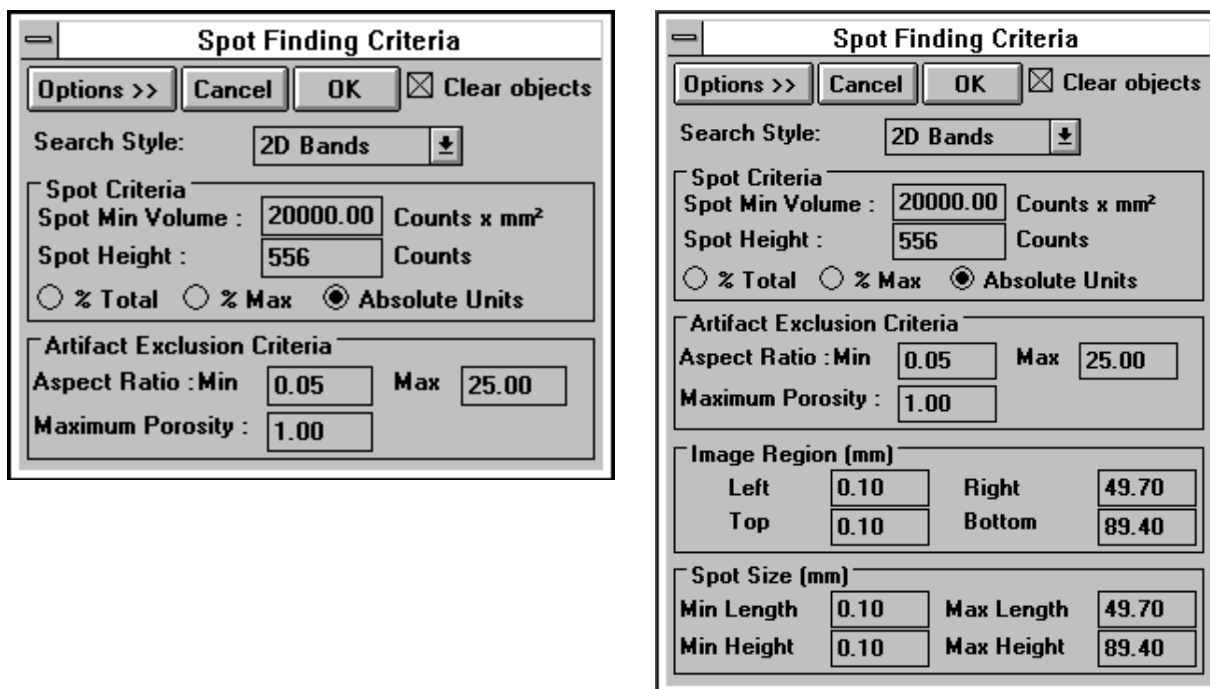
Duplicating a Grid

- **Copy and Paste** - Activate the grid by clicking on it with the mouse and select *Copy* from the EDIT menu. Next, select *Paste* from the EDIT menu. Place the cursor, now a Capital P, to the new grid location, and click the left mouse button. Grids can be copied and pasted within the same image, or between two different images.

- **<Ctrl> + Movement arrow**- Clicking the movement arrow while holding the <Ctrl> key down.

Part 3: Automatic Spot Detection

Selecting *Volume Analysis* from the ANALYSIS menu also activates the *Automatic Spot Detection* command. Selecting this command displays a spot finding criteria dialog box. Within this box, image region, spot size, spot criteria, and artifact exclusion criteria can be set (Figure 21a, b).



a. b.
Figure 21. Spot finding criteria dialog box, (a) unexpanded, (b) expanded with *Options>>* button.

Search Style - This pull-down menu allows rapid selection of 2D bands or spots.

Spot criteria - Minimum peak density and peak height of the spots to be found. Spot minimum volume is the smallest peak density in O.D.x mm². Spot height is the smallest peak height or
Molecular Analyst/PC

amplitude in the z direction in O.D. Spot criteria can be defined as % total, % maximum, or absolute units. Spot criteria (spot minimum volume and spot height) is critical in detecting variations in the number of spots detected.

Artifact exclusion criteria - Consists of aspect ratio and porosity criteria and controls the identification of narrow, elongated spots which may be smears or artifacts.

Aspect Ratio:

Aspect ratio limits peak identification of smears in the x or y direction. Aspect ratio is calculated from the dimensions of the object by the formula (Y size)/(X size). Tall thin objects will have high aspect ratios and low flat objects will have small ratios. A round peak will have a ratio of 1.0. The aspect ratio is useful in rejecting artifacts since most artifacts have either very small or very large aspect ratios, whereas most peaks will have an aspect ratio close to 1.0. A range from 0.25 to 4.0 would allow a 4:1 elongation in either direction and is a reasonable starting range.

Porosity:

Porosity limits peak identification of smears in the diagonal direction. Porosity is useful in distinguishing spots from smears, streaks, and scratches. It is determined by drawing a rectangle around each object. If the object is a spot, most of the rectangle is filled. If the object is a smear, little of the rectangle is filled. The fraction of the rectangle not filled is the porosity.

$$\text{Porosity} = 1 - [(\text{area of the rectangle}) / (\text{X size}) \times (\text{Y size})]$$

A spot has a porosity close to 0. A smear has a porosity close to 1.0 (Most of the area of the rectangle is not filled by the object. As a rule of thumb, an object with a porosity over 0.7 is most likely an artifact. However, each sample should be tested experimentally to determine the porosity limits.

Image region criteria - Visible when the *Options>>* key is selected, this criteria limits the specific area of an image for spot detection. Using the image rulers, left, right, top, and bottom millimeter dimensions may be entered. This criteria will default to the entire image area.

Spot size criteria - Visible when the *Options>>* key is selected, this criteria limits the minimum size of the spots to be found.

Note: All volume objects displayed through the use of the automatic spot detection feature cannot be moved, copied, or duplicated. Only volume objects drawn manually can be moved, copied, or duplicated.

***The number of spots found is dependent upon the set criteria. All spots on an image may not conform to a single criterion, and therefore multiple analyses may be required to find all spots on some samples.**

Part 4: Description of Volume report

The volume report provides quantitative information about each object on the image. Within the report, values can be sorted; column values can be hidden; standard values can be entered; and a standard curve can be generated. The volume report results include:

Label - Identifies the object's label as it is displayed on the screen (R=Rectangle, C=Circle, E=Eclipse, S=Square, G=Grid).

Name - Double clicking on the active cell opens the Edit Volume Report dialog box and allows you to change the name of the object in the report. The Label, not the Name, is displayed on the image. To display a name on the image, use the Text tool.

Type- Sample types include *Unknown*, *Standard*, or *Background*. Double clicking on the active cell opens the *Edit Volume Report* dialog box and allows you to change sample type.

% Vol- The percent volume of the object in relation to the total volume of all objects identified

Area- Area of the identified object is in units of mm^2 .

Pixels- Total number of pixels within the object. This is dependant of the resolution selected.

Volume- The integrated volume of the object is $(\text{O.D.} * \text{Area})$.

Mean- Average O.D. of the pixels within the object, where $\text{O.D.} = \text{Volume} / \text{Area}$.

Density- Average O.D. over the object's area in units of $\text{O.D.} / \text{mm}^2$.

Maximum- Maximum O.D. value within the object.

Minimum- Minimum O.D. value within the object.

Standard Deviation - Standard deviation from the mean.

Position - X and Y coordinates for the maximum value on the object.

Adj Volume - The integrated volume of the object adjusted for background removal.

Concentration - Concentration of unknowns is calculated from the standard curve, created by specifying standard concentrations and regression analysis.

Molecular Weight - Molecular weight of unknowns is calculated from the standard curve, created by specifying standard Y position and regression analysis

pI - The iso-electric point of unknowns is calculated from the standard curve, created by specifying standard X position and regression analysis.

Note that the column display default settings within the volume report do not include *Position*, *Molecular Weight*, or *pI* columns. These columns must be displayed before calculating molecular weight or pI. To display hidden columns, select either the *Columns* command under the OPTIONS menu or *Preferences setup* under the File menu. Uncheck the *Hide* box and click OK. The volume report may need to be expanded to display the new columns; or alternatively, the columns can be viewed using the scroll bar on the volume report.

Part 5: Editing the Volume Report

Double clicking on an active object(s) within the image or on a cell of the volume integration report will open up an *Edit Volume Report* dialog box. The *Edit Volume Report* dialog box displays the object name and type parameters, and standard value entry boxes (Figure 22).

Object Type - Using the radio buttons, specify whether the object is either an Unknown, Background or Standard. If the object is a standard, enter the standard concentration, molecular weight, or pI in the appropriate box. Note that standard values can be entered only if the standard column is displayed.

Object Name- Enter in the name of the object to be displayed in the volume report.

Figure 22. Edit Volume Report dialog box for entering a single value.

The *Edit Volume Report* dialog box also allows an active group of objects to be associated with one object type, background, or standard value. This feature is useful to average the background values of many background objects or to give many standard objects the same standard value. When the *Edit Volume Report* dialog box is opened when many objects are active, *Group* will be displayed in the Row # heading. This indicates that any object type or standard value change will affect all of the active objects (Figure 23).

Figure 23. Edit Volume Report dialog box for entering group values

Additionally, the *Edit Volume Report* dialog box allows rapid entry of different standard values using the *Edit Objects* command button. Clicking on the *Edit Objects* command button, entering a standard value, and then clicking on the *Next* control button, allows each of the objects to rapidly be given a different standard value (Figure 24). This function is only active when multiple standard objects are active.

Row 1
Label: R1

Type

Unknown
 Background
 Standard

Concentration

Molecular Weight

pI

OK
Cancel
Edit Group
Next
Previous

Name

Figure 24. Edit Volume Report dialog box for rapidly entering multiple standard values

Part 6: Description of Menus

Options	Window
Sort by <u>A</u> rea	
✓ Sort by Adj <u>V</u> olume	
Sort by <u>X</u> Position	
Sort by <u>Y</u> Position	
Sort by <u>T</u> ype	
Sort by <u>N</u> ame	
Sort by <u>C</u> reation Order	
<u>C</u> olumns...	
✓ <u>G</u> rid lines	
<u>C</u> oncentration Standard Curves...	
<u>M</u> olecular Weight Standard Curves...	
<u>p</u> I Standard Curves...	
✓ <u>A</u> utomatic Recalculation	
Recalculate <u>N</u> ow	F9
Recalculate <u>L</u> abels	
<u>L</u> ocal Background Subtraction	

OPTIONS Menu

The options menu allows functions to be performed upon the volume report data and the standard curve.

OPTIONS COMMAND: *Sort by Area*

When this command is selected, objects in the Volume report will be sorted and displayed in order of increasing Area

OPTIONS COMMAND: *Sort by AdjVolume*

When this command is selected, objects in the Adjusted Volume report will be sorted and displayed in order of increasing Volume

OPTIONS COMMAND: *Sort by X Position*

When this command is selected, objects in the Volume report will be sorted and displayed in order of increasing X Position

OPTIONS COMMAND: *Sort by Y Position*

This command allows objects in the Volume report to be sorted and displayed in order of increasing Y Position

OPTIONS COMMAND: *Sort by Type*

This command allows objects in the Volume report to be sorted by type

OPTIONS COMMAND: *Sort by Name*

This command allows objects in the Volume report to be sorted by name

OPTIONS COMMAND: *Sort by Creation Order*

This command allows objects in the Volume report to be sorted and displayed in creation order

OPTIONS COMMAND: *Columns...*

This option allows specific columns to be hidden from view and column widths to be modified to provide a more concise volume report. Selecting this command also allows the standard curve Y axis and regression type to be set for Concentration, Molecular Weight, and pI.

OPTIONS COMMAND: *Grid Lines*

Selecting this option displays or hides the Volume Report grid lines

OPTIONS COMMAND: *Concentration Standard Curves*

This option creates the concentration standard curve based on adjusted volume.

OPTIONS COMMAND: *Molecular Weight Standard Curves*

This option creates the molecular weight standard curve based on the object's Y position.

Options	Window
Sort by <u>A</u> rea	
✓ Sort by Adj <u>V</u> olume	
Sort by <u>X</u> Position	
Sort by <u>Y</u> Position	
Sort by <u>T</u> ype	
Sort by <u>N</u> ame	
Sort by <u>C</u> reation Order	
<u>C</u> olumns...	
✓ <u>G</u> rid lines	
<u>C</u> oncentration Standard Curves...	
<u>M</u> olecular Weight Standard Curves...	
<u>pI</u> Standard Curves...	
✓ <u>A</u> utomatic Recalculation	
Recalculate Now	F9
Recalculate Labels	
<u>L</u> ocal Background Subtraction	

OPTIONS COMMAND: *pI Standard Curves*

This option creates the pI standard curve based on the object's X position.

OPTIONS COMMAND: *Automatic Recalc*

Selecting this option allows the values in the Volume Report to be automatically recalculated. This option is convenient when values are modified.

OPTIONS COMMAND: *Recalc Now*

This option allows you to recalculate values at any time.

OPTIONS COMMAND: *Recalculate Labels*

This option allows you to renumber volume object labels.

OPTIONS COMMAND: *Local Background Subtraction*

This option subtracts the mean pixel value concentrically outside each individual object perimeter.

Part 7: Description of the Standard Curve

The volume integration module of Molecular Analyst/PC permits sophisticated graphing of the standard curves using a variety of graph types, regression analyses, and graph axes. From the standard curve graphs, concentration, molecular weight, and isoelectric point can be calculated.

With the volume analysis report displayed and objects identified as either background, standards, or unknowns, selecting the command *Columns...* from the OPTIONS menu displays the *Report Column Preferences* dialog box. Within this dialog box, the *Standard Curve Y Axis*, *Regression Type*, and *X and Y Axes* can be identified. Values for the *Standard Curve Y Axis* and *Regression Type* can only be entered into the (Concentration), (Molecular Weight), and (pI) columns. Concentration is calculated by entering *Adjusted Volume* as the standard curve Y axis. Molecular weight and pI are calculated by entering *Y Position* and *X Position* respectively as the standard curve Y axis.

Regression type is also set within the *Report Column Preferences* dialog box. The possible regression analyses include: Linear Regression, Quadratic Regression, Cubic Regression, and 4-Parameter Logistic Regression. Linear Regression requires a minimum of two (2) standard data points, Quadratic Regression requires a minimum of three (3) standard data points, and Cubic Regression requires a minimum of four (4) standard data points. 4-Parameter Logistic Regression requires a minimum of five (5) standard data points. The type of regression curve can also be displayed or hidden on the graph by using the regression curve icons located within the standard curve. Moreover, multiple regression analysis curves will be displayed given that the minimum number of standard data points for that curve are available (i.e., given 3 standard data points, both linear and quadratic curves will be displayed). See Appendix E for a detailed description of the regression analyses.

Lastly, the volume integration module utilizes 2 different axes to produce the standard curve: *Linear*, and *Log*. To view each type of graph axis, simply click on the radio button. Start with the simplest regression method, *Linear*, and examine the fit of the data in each set of axes: *Linear-Linear*, *Linear-Log*, *Log-Linear*, and *Log-Log*. The goal is to find the set of curve fitting conditions that best matches the data. Move to the next order of regression, Quadratic, Cubic, and finally Logistic. To calculate concentration of the unknowns for a specific regression analysis, simply double click on the standard curve. This will display the regression formula and standard error for that curve, and calculate the unknown concentrations in the volume report. To view a different curve, simply select the appropriate icon and double click on the standard curve. The unknown values will automatically recalculate using the selected regression analysis.

Appendices

APPENDIX A: Computer System and Configuration

Appendix A

Section I ATI 8514 Ultra 256 gray level graphics engine (Only with graphics CARD)

Within the [8514UTIL] directory, type in INSTALL. This will then display the configuration for the ATI 8514 Ultra 256 gray level graphics engine. Set the Power-UP Configuration to the following:

Monitor type: Interlaced 1024 x 768

Appendix A

Section II Necessary DOS directories

[DOS] - Contains files necessary for DOS.

[8514UTIL] - Contains files and driver for ATI 8514 Ultra 256 gray level graphics engine.

[GPIB-PCW] - Displayed after GPIB installation and contains the driver, test, and configuration software.

[AUTOEXEC.BAT] - Contains files that start memory resident utilities and sets up environment variables.

[CONFIG.SYS] - Contains files that define device drivers and specifies MS-DOS configuration.

[MA]- This directory contains the files necessary to run Molecular Analyst and corresponding modules.

[DATA] - This sub-directory resides in the [MA] directory and contains the profile and image files.

[TEMP] - Stores files temporarily.

[TMP] - Stores files temporarily.

[WIN31] - Contains files necessary to run Windows 3.1. Also contains GPIB.dll and GPIB.ini files.

Appendix A

Section III Autoexec.Bat File

```
VER
C:\WINDOWS\SMARTDRV.EXE /L C+ 4096 4096
PATH=C:\WINDOWS;C:\DOS
PROMPT=$P$G
SET TEMP=C:\TEMP
SET TMP=C:\TEMP
WIN
```

Appendix A

Section IV Configure Sys File

```
DEVICE=C:\WINDOWS\HIMEM.SYS
DOS=HIGH
FILES=30
BUFFERS=20
STACKS=9,256
```

Appendix A

Molecular Analyst/PC

Section V Windows Setup

Display: 8514/a
Keyboard: Enhanced 101
Mouse: Logitech

Appendix A

Section VI GPIB Jumper Configuration and GPIB Software Installation

<u>Dip Switches</u>	0 = DEPRESSION	OFF	ON	
	A3	0		I0
	A4	0		I1
	A5	0		I2
	A6		0	A14
	A7		0	A13
	A8		0	
	A9		0	
	7210	0		9914
	PCII		0	PC IIA

GPIB Software Settings

DMA 1
Interrupt 7
IO Address 2e1H

Jumper Positions

IRQ7
DRQ1
DACK1
W1

Quick Software Installation

- 1: Insert the diskette labeled "National Instruments: GPIB software" in the **A:** drive.
- 2: Select "Run" from the "File" menu in the Application Manager.
- 3: Type **a:install** and press the OK button. The GPIB software installation screen is displayed.
- 4: Use the Down Arrow to move the check mark next to the "Windows GPIB Installation" and press Enter.
- 5: Use the Down Arrow to move the check mark next to the "Partial Installation" option and press Enter. The full Installation won't hurt, but will fill disk space with unnecessary software.
- 6: Use the Down Arrow to move the check mark next to the "Microsoft C language interface" option and press the space bar to remove the check mark and then press Enter. The extra software installed by including the "Microsoft C language interface" option won't hurt, but will fill disk space with unnecessary software.
- 7: Destination directories will be displayed allowing user editing. The default values should be OK for most installations. Press Enter when you are satisfied with the directory information.
- 8: When the message "Transfer Complete" appears, press the Enter key.
- 9: Press the Enter key again to select the "Win 3.1+" option.
- 10: Press the ESC key to exit the installation program.

The installation accomplishes two main tasks: (1) It copies two files into the windows directory - gpib.dll and gpib.ini. (2) It creates a directory called **c:\gpib-pcw** containing diagnostic software.

The diagnostic program "**wibtest.exe**" may be run to confirm correct installation of the board. From File Manager, double click on wibtest.exe. Next select the command Test GPIB0 from the Software Diagnostics menu. (Test GPIB1,2, ect. refer to other GPIB boards, if present). If the wibtest was successful, exit, and instal Molecular Analyst/PC software. If the wibtest was not successful, either the GPIB board was not present, not configured properly, or there exists a conflict with another board or device driver.

To check or to change the configuration of the GPIB board and driver, double click on wibconf.exe from the File Manager. Click OK on the parameters dialog box. (If the Windows directory is located somewhere other than C:\ drive, enter in the location and click OK.

At the GPIB Device Map, select F8. This will display the GPIB0 configuration screen. Next, press the <End> key on your keyboard. This will bring the cursor directly to the Interrupt Level, DMA Channel, and Base I/O Address configurations. These software configurations reflect jumper and dip switch positions on the GPIB board. Change the Interrupt level, DMA channel and Base I/O address using the right and left arrow keys to the following:

Base I/O address	02E1H (change dip switches on the board as displayed on the screen)
DMA	None (change jumpers and dip switches on the board as displayed on the screen)
Interrupt	None (remove jumpers from the board as displayed on the screen)

Push the <esc> key to exit to the GPIB0 map, and then again to exit to File Manager. Wibtest.exe may be run again to confirm correct installation of the board. If installation is correct, load Molecular Analyst/PC software. Remember to connect all cables, start the densitometer, and then start the computer.

APPENDIX B: Transferring Image Files and Data File Management

Transferring Image Files-

Because Molecular Analyst/PC can produce images of significant size (up to 30 megabytes), it may be necessary to transfer image files in the Bio-Rad Image File Format (BRIFF) to free up computer hard disk space. There are a variety of ways to transfer and store image files including optical disk drives, tapedrives, transportable-removable media disk drives, and harddrive backup software applications (ie, DOS command Backup and Restore, or FastBack). Each of these methods accept the images as 8-bit TIFF files. These stored image files can then be transferred to desktop, image analysis, or publication software applications. Molecular Analyst/PC will not accept 8-bit or 16-bit TIFF files.

Managing the Data Base:

Periodically it will be necessary to delete unwanted files, created by the Profile Analyst module, from your hard disk in order to free-up hard disk space. The files can be archived on floppy disks for future use. Deletion of files is accomplished using Windows File Manager, while deletion of the

profiles contained within the files is accomplished using the *Delete Profile ...* function from the DISPLAY window.

To delete files from a hard disk use the following procedure:

(1) Bring up File Manager by double clicking on the icon. (2) Specify which disk drive you wish to delete files from by clicking on the appropriate icon. (3) Point and click on the file which you wish to delete. (4) Click on FILE in the menu bar and then click on the DELETE command. The file will then be deleted from the disk drive. Continue this process until all of the unwanted files are deleted.

To delete unwanted profiles from within files, use the following procedure:

(1) After initiating the Profile Analyst II program, click on the *MODE* menu and select *Display* from the menu. (2) Click on the *FILE* menu and select *Delete Profile ...* from the menu. (3) Double click on the file name which contains the profile(s) which you wish to delete. A dialog box showing the profiles contained within the select file will appear. (4) To delete one profile, double click on the single profile to be deleted. To delete many profiles from the selected FILE, press and hold the <Shift> key and click the left mouse button on all profiles which you wish to delete. Click on the *Delete* command button to delete the selected profiles. The "Open File Name:" dialog box will then reappear enabling you to delete profiles from another FILE. If you are finished deleting profiles, click on the *Exit* command button to go back to the *Display* window.

APPENDIX C: Use of Estimated Molecular Weights

1. The user is cautioned when using the estimated molecular weights calculated with higher order regressions. Higher orders of regression often result in a "better fit" because they make more concessions to particular measurement errors in the data used to derive the standard curve. While this can increase the amount of useful information, it can also reduce the likelihood that the standard curve represents the sample data. This is because the precision with which the standards data are modeled is not always matched by the sample data.

2. The user is also cautioned when using sample data where the x and y values do not fall within the x and y range used to calculate the standard curve. Using these values outside of the standard curve x and y range may result in undefined or absurd values (the Profile Analyst will display 0.000 for values outside of the range). If higher orders of regression of standard data produce several undefined sample values, then it may not be the best model to choose for the standard curve.

APPENDIX D: File Size and Resolution when Scanning a 29.5 cm x 42 cm Screen

<u>Resolution</u>	<u>Scanning Only</u>	<u>Saved Image File</u>	<u>Scanning and Saving</u>
800 μ	0.4 mb	0.8 mb	1.2 mb

400μ	1.4 mb	2.8 mb	4.2 mb
200μ	6.0 mb	12.0 mb	18.0 mb
100μ	24.0 mb	48.0 mb	72.0 mb
64μ	30.0 mb	60.0 mb	90.0 mb

The file size of an image is dependent upon both the scanning resolution and scanning area specified. The resolution settings and corresponding file sizes are displayed in the above table. However, there are two additional factors to consider when relating image size to available disk space: **the size of the stored image, and the size required to scan and to save an image.**

The stored image file is comprised of raw data, copied from the temporary file, and view data. The raw and view data are of equal size, with 1 byte of raw data corresponding to 1 byte of view data. Therefore, a 29.5 cm x 42 cm area scanned at 64μ, generates an image file which requires approximately 30 mb of disk space for raw data and 30 mb of disk space for view data, for a total image file size of 60 mb.

However, the hard disk size requirement is quite different when scanning and when scanning **and** saving an image. When a sample is scanned, the raw data is stored in a temporary file. The size of this temporary file corresponds to the size of the raw data (ie. 30 mb of raw data corresponds to 30 mb of disk space within the temporary file). Consequently, a 29.5 cm x 42 cm area can be scanned at 64μ and an image generated with only 30 mb of available disk space. Moreover, when the image is then saved, both raw data contained within the temporary file and raw data and view data, which will comprise the image file, are also saved to the hard disk. The total disk space now required for both scanning a sample and saving the image file and the raw data from the temporary file is 90 mb. Thus, to save an image once a sample has been scanned, it requires available disk space for raw data, view data, and raw data from the temporary file. Please note that the temporary file is emptied when the image is saved and is then used for image scanning and manipulation (rotation, filtering, clipping, transformation, etc.). Due to the large potential file sizes generated by this imaging system, it is recommended that images be saved to an external storage device for optimum system performance.

APPENDIX E: Regression Analyses

Linear Regression:

When analyzing data, always start from the simplest set of curve fitting conditions. Begin with the linear regression. Examine the fit of the data in each of the four sets of axes: Linear-Linear, Linear-Log, Log-Linear, and Log-Log. The goal is to find the set of curve fitting conditions that best matches the data. The data appears in the graphs as circles, the regression appears as a bold, smooth line or curve. The correlation coefficient (r) gives an indication of how well the regression model fits the data. In effect, the correlation coefficient is the square root of the proportion of total variation that can be explained by the regression.

$$r = (\text{explained variation} / \text{total variation})^{1/2}$$

The amount of explained variation increases as the goodness of fit increases. In a perfect fit, all of the variation is explained, the explained variation equals the total variation, and the ratio of the explained variation to total variation becomes 1.000. Or, more simply, the closer the correlation coefficient is to 1.000, the better the fit of the regression. A perfect fit with a correlation coefficient of 1.000 would indicate 100% certainty of the fit. The correlation coefficient of 0.9995 in the graph above indicates 99.95% certainty of fit.

Quadratic Regression:

The next level of sophistication of curve fitting is the quadratic regression. Select *Quadratic* and examine the fit of the regression in all four sets of axes. Again, the goal is to find the set of regression conditions in which the regression most closely matches the data. The goodness of fit in this case is measured by the standard error of the regression. The lower the standard error, the better the fit. Visual comparison alone indicates that the Log-Log fit is superior to the Log-Linear fit. The corresponding standard errors confirm this visual evaluation; the Log-Log fit has the lower standard error.

Removing outliers or tails from the standard curve may improve your fit significantly, and allow you to use a simpler level of curve fitting. Outliers are removed from the volume report.

Recall that a quadratic expression $Y = a + bX + cX^2$ may be solved by setting the equation equal to zero and solving for X. The root is unsolvable if the expression under the square root sign is a negative value.

The quadratic formula describes a parabola, which appears as a bowl when plotted. The portion of the quadratic curve near the inflection point has a very shallow slope. A small difference in Y value in this portion of the curve translates into a large difference in the corresponding calculation of X. Reliability in this portion of the curve depends on the steepness of the parabola.

Cubic Regression:

Cubic regression is the next highest order of regression and is a second order quadratic regression analyses.

Logit-Log or Four Parameter Regression:

The four-parameter logistic model is the highest level of curve fitting available and is used to describe the smooth, symmetrical, sigmoidal relationship between electrophoretic mobility (distance migrated) and log of molecular size (kb): $\text{distance} = a - \frac{d}{1 + (\text{size}/c)^b} + d$. The four parameters (a,b,c,d) are estimated by nonlinear least-squares curve fitting using the Marquardt-Levenberg algorithm, where a represents an upper plateau, b a slope factor, c the midpoint, and d the lower plateau. Estimates of size for unknown species are accompanied by estimates of the standard error (SE)

Mathematical Equations

For Linear Regression analysis:

Where x_i is the X value of "i", \bar{X} is mean X value, y_i is the Y value of "i", and \bar{Y} is the mean Y value, we define:

$$\text{The slope, } \beta = \frac{\sum(x_i - \bar{X})(y_i - \bar{Y})}{\sum(x_i - \bar{X})^2}$$

$$\text{The intercept, } a = \bar{y} - \beta \bar{x}$$

$$\text{Then: } y = a + \beta x$$

For a Quadratic Regression analysis:

Where x_i is the X value of "i", \bar{X} is the mean X value, y_i is the Y value of "i", \bar{Y} is the mean Y value, a is the zero order coefficient of the regression, β is the first order coefficient of the regression, and N is the number of sample pairs, we define:

$$S_{xx} = 1/N \sum (x_i - \bar{X})^2$$

$$S_{xy} = 1/N \sum (x_i - \bar{X})(y_i - \bar{Y})$$

$$S_{yy} = 1/N \sum (y_i - \bar{Y})^2$$

$$S_{xx^2} = 1/N \sum (x_i - \bar{X})(x_i^2 - \bar{X}^2)$$

$$S_{x^2x} = 1/N \sum (x_i^2 - \bar{X}^2)^2$$

$$S_{yx^2} = 1/N \sum (y_i - \bar{Y})(x_i^2 - \bar{X}^2)$$

Then: g is the second order coefficient of the quadratic equation.

$$g = (S_{xx}S_{yx^2} - S_{xx^2}S_{xy}) / (S_{xx}S_{x^2x} - (S_{xx^2})^2)$$

β is the first order coefficient of the quadratic equation.

$$\beta = (S_{xy}S_{x^2x} - S_{x^2y}S_{xx}) / (S_{xx}S_{x^2x} - (S_{xx^2})^2)$$

a is the zero order coefficient of the quadratic equation.

$$a = \bar{y} - \beta \bar{x} - g \bar{x}^2$$

Standard error of the estimate:

$$S_D = \{ \sum (y_i - a - \beta x_i - g x_i^2)^2 / (N - 3) \}^{1/2}$$

For a Cubic Regression analysis:

Cubic Regression has a third order coefficient of the of a quadratic equation.

For a Four Parameter Regression analysis:

$$Y = \{ (a-d) / (1 + (x/b)^c) \} + d$$

Where Y is the absorbance value, x is the size value, a is the upper plateau, d is the lower plateau, c is the midpoint, and b is the slope of the linear portion of the curve.

Oerter KE; Munson PJ; McBride WO; Rodbard D.

Computerized estimation of size of nucleic acid fragments using the four-parameter logistic model. Analytical Biochemistry, 1990 Sep, 189(2):235-43.

Appendix F: Edge Enhancement

The GS-670 has a built-in image enhancement feature. This feature allows the edges of an image to be softwen of sharpened. In normal mode, data is passed from the CCD array to the computer with no modification other than look up table scaling. In the soften mode, a pixel is averaged with the pixels in the next adjacent rows and columns. The average is weighted so that the overall intensity of the image does not change. Soften mode will minimize moire patterns. In sharp mode, a fraction of the value of pixels in the adjacent rows and columns is subtracted from each pixel. The coefficients are selected so that the overall intesity of the image does not change. This enhances any pixel which is different from its neighbors. The effect of this average is to sharpen edges. If a scan is noisy or the sample quality poor, it will also enhance defects. Extra sharp mode is the same as sharp except that a larger portion of the neighboring pixels are subtracted.

APPENDIX G: Troubleshooting

Image Display

Condition

Image does not look like what was expected

Possible Cause/Solution

- The Modify View palette is not set properly.
- Calibration strips not used properly
- Masking template not used properly
- Diffussing plate not used
- Scan window not clean
- Improper filter selected

Cannot Acquire Scanner

- GPIB interface not attached in proper order
- Densitometer turned on after computer

No data transfer at all

- The GPIB interface cable was not used
- The GPIB card not used
- GPIB software not installed properly

Background Subtraction

The Sub Background command is not functional

- The background profile which was imported was not set as background using the PROFILE command Set Type

- The background profile was not saved

The Point-to-Point baseline is not functional

- The baseline endpoints do not extend beyond the far left and right edges of the profile

Peak Identification Problems

No peaks were identified after using the Auto Identify command.

- The Peak Criteria is excluding all peaks within the profile

Molecular Analyst/PC

-The profile was not selected as “Active”

The first and last peak of the profile is not identified The profile was clipped too short using the OPTIMIZE commands Origin and *Front*.

APPENDIX H: Technical Support

Bio-Rad provides complete technical support on all features of the Profile Analyst II software. If you have any problems, or any questions on the use of the features, please call Bio-Rad Technical Service at:

1-800-4-BIO-RAD (1-800-424-6723)

Please report any reproducible software problems to Bio-Rad Technical Service so we can eliminate them from future versions.

Information in this manual is subject to change without notice.

Molecular Analyst software is a registered trademark of Bio-Rad Laboratories.

IBM is a registered trademark of International Business Machines. MS-DOS, Windows, Excel, and Word are registered trademarks of Microsoft Corporation.

Adjusting the Histogram Range 48
 Altering brightness and contrast- 16
 ANALYSIS Menu 47
 Automatic Spot Detection 85
 Averaging Rectangle Tool 55
 Block Select (Multi-object Select) 38
 Brightness and Contrast Sliding Controls 41
 Calculating Standard Curve- 25
 Calculating The Molecular Weight Of An Unknown- 26
 Circle Tool 81
 Creating A Profile 17
 Description of the Standard Curve 91
 Description of Volume report 86
 Display Function 56
 Displaying an Image 13
 Displaying And Optimizing The Standard Profile 22
 EDIT Menu 44
 Editing the Volume Report 87
 Ellipse Tool 82
 ENHANCE Menu 64
 Enhancing The Profile Data 20
 Equalization 256 Gray Level/Color Scroll Bar 41
 Equalization scroll bar 16
 Exporting Profile Peak Reports and Profile Data Points To Other Applications- 27
 Exporting Volume Report Data to Other Applications 32
 FILE Menu 57
 FILE Menu 43
 Filtering background- 15
 FUNCTIONS Menu 79
 FUNCTIONS Menu 73
 Generating a Standard Curve and Calculating Concentration- 31
 Generating a Volume Integration Report- 30
 Grid Tool 83
 Help Menu 42
 Identifying And Integrating Peaks 20
 Identifying Peaks On The Standard Profile 23
 Identifying Spots 28
 Improving visual sensitivity 15
 Invert Button 41
 Magnifying Glass (Magnify) 39
 Magnifying the Image- 15
 MODE Menu 72, 79
 MODE Menu 56
 Molecular Weight Function 72
 Moving Between Profile Analyst And Molecular Analyst- 27
 Multi-Segment Tool 54
 OPERATION Menu 69
 OPTIMIZE Menu 60
 Optimizing an Image 14
 Optimizing The Profile 19
 OPTIONS Menu 90
 PEAKS Menu 65
 Pointer (Object Select) 38
 Printing An Image 17
 Printing Profiles and Reports 21
 PROFILE Menu 58
 profile information box. 60
 Profile Tool Palette 52
 Pull-Down Palette Menus 41
 Rectangle Tool 82
 Reports Function 79
 Reset Button 41
 Right Mouse Button Commands 71
 Saving An Image 16

Saving The Profile 21
SCALE COMMAND
 % Absorbance 69
 % Transmission 69
 Absorbance (OD) 69
SCALE Menu 69
Scissors (Clip Image) 40
Scrolling Cross (Pan) 39
SETUP Menu 70
Single Line Tool 53
Square Tool 83
Standard profile used for molecular weight determination of unknown samples. 58
T (Text) 40
The Modify View palette 40
The Tool Palette 38
TOOLS Menu 46
Transformation 15
TRANSFORMATIONS Menu 48
VIEW Menu 45
Volume Analyst Module 81
Volume Tools 81
Windows Manipulation 13
WINDOWS Menu 51
Zooming Box (Zoom) 39