

UN-SCAN-IT gel TM Version 6.3

User's Manual for

Macintosh (OS X)

Silk Scientific Inc. software and manuals are copyrighted, which means that your right to copy any part of them is limited by copyright laws. Making copies or derivatives, except in limited instances and except for making copies of software for archival purposes, without prior written authorization from Silk Scientific Inc. is prohibited by copyright law.

Silk Scientific Inc. reserves the right to make changes or improvements to its programs and instruction manuals at any time and without obligation to notify any person of, or to provide any person with, such revisions or changes.

Silk Scientific Inc. invites your comments or suggestions regarding the User's Manual and/or the UN-SCAN-IT gel software. Please feel free to contact us with any questions or technical support needs that you may have regarding the UN-SCAN-IT gel software.

Customer Support:

Silk Scientific

1060 Oakmont Lane Provo, Utah 84604 USA

Tel: 1-801-377-6978 Fax: 1-801-228-2448

E-Mail: info@silkscientific.com

System Requirements

UN-SCAN-IT gel runs on Apple Macintosh computers with:

- Macintosh OS X (10.7 or higher)
- 512 MB or more of RAM memory
- 50 MB of free hard disk space
- Scanner, digital camera, other imaging device, or standard image files

UN-SCAN-IT and ***UN-SCAN-IT gel*** are trademarks of Silk Scientific Corporation.
All other trademarks are the property of their respective owners.

Table of Contents

Introduction	i
---------------------------	---

Chapter 1: Software Installation

1.1	Overview	1
1.2	Write Privileges	1
1.3	Installing UN-SCAN-IT gel on the Hard Disk	1

Chapter 2: Obtaining the Image

2.1	Overview	3
2.2	Scanner Operation	3
2.3	Original Size	4
2.4	Scanning the Image	5
2.5	Straight Scanning.....	5
2.6	Using a Digital Camera	6
2.7	Scan.....	6
2.8	Rotating the Image	6
2.9	Saving the Image	6
2.10	Removing Grid Lines from Graphs Graphs.....	7
2.11	Maximizing the Resolution of the Image Graphs	9
2.12	Maximizing the Resolution of Digitized Data Graphs	9

Chapter 3: Running UN-SCAN-IT gel

3.1	Overview	11
3.2	Running UN-SCAN-IT gel	11
3.3	UN-SCAN-IT gel Registration Information	11

Chapter 4: File Options

4.1	Overview	13
4.2	Main Menu	13
4.3	Paste (x,y) Data from Clipboard	14
4.4	Load (x,y) Data	14
4.5	View Data Values	15
4.6	Adjust X Data Spacing	15
4.7	Copy Data to Clipboard	15
4.8	Save Data	15
4.9	Clear Data	16
4.10	Print Data	16
4.11	Restore All Default Settings	16
4.12	Scan / Modify Image	17
4.13	Exit	17

Chapter 5: Analyzing Gels

5.1	Overview	19
5.2	Main Menu	19
	Analyze Gel Image	19
5.3	Open Image File	20
5.4	Gel Analysis Mode	20
5.5	Gel Analysis Options	21
	Main Image View	22
	Color Mode	22
	Background Correction	23
	Optical Intensity Calculation	23
	Scan Direction	24
	Lane/Segment Drawing Mode	24
	Lane Width	25
	Lane/Segment Box Line Type	26
	Saturation Checking	26
	ACGT Sequence	26
	Digitizing Speed	27
5.6	Gel Analysis Screen	27
	Options	28

5.7	Drawing Lanes, Segments, and Dot Blots	33
	Lanes	33
	Segments	34
	Dot Blots	35
5.8	Begin Analysis	36
5.9	Gel Analysis Mode	36
	Lane Analysis	36
	Density Profile Toolbar	38
	Segment Analysis	40
	Dot Blot Analysis	40
5.10	Results	41
	General Results	41
	Results Screen	42
	Results Grid	43
5.11	Normalize	46
5.12	Calibrating Concentration Values	46
5.13	Editing Y Position Values	47
5.14	Calibrating Relative Mobility Values	47
5.15	Calibrating Molecular Weights Values	47
5.16	Show Concentration Curve	48
5.17	Show Molecular Weight Curve	49
5.18	Helpful Hints	49

Chapter 6: Digitizing (x,y) Graphs

6.1	Overview	51
6.2	Main Menu	51
	Digitize (x,y) Graph Image	52
	Return to Current (x,y) Graph Image	52
6.3	Open Image File	52
6.4	Graph Digitizing Mode	53
	Standard Line	53
	Intersecting Line	54
	Cyclic Graph	54
	Bar Graph/Mass Spec	55
	Dashed/Dotted Line	55
	Contour Plot	56

	Shapes/Drawings	57
	Symbols/Scatter Plot	57
	(x,y) Digitizing Tablet	58
	Polar Digitizing Tablet.....	58
6.5	Graph Digitizing Options	59
	Main Image View	59
	Color Type	59
	Scale	60
	Grid Line Filter	61
	Line Side Point Assignment.....	62
	Line Follow Direction.....	64
	Line Follow Algorithm	65
	Digitizing Speed.....	66
	Multi-Line Method.....	66
	Cyclic Line Follow Method Contour Line Start.....	67
	Cyclic Reversal Method.....	67
	Bar Graph Type.....	68
	Dashed/Dotted Line Follow Method	68
	Contour Line Follow Method	69
	Raster Scan Direction	69
	Manual Point Assignment Method.....	70
	Manual Automatic X Increments (Manual Mode).....	71
	Angle Units	71
6.6	Digitizing Screen.....	71
	Options	73
6.7	Positioning the Setup Icons	78
	Icon and Crosshair Movement.....	78
	Data Color	79
	Locating Axis Scaling Points.....	80
	Tilt Correction	83
	Locating Beginning Point	84
	Defining Vertical Limits	85
	Flat Spot / Line Thickness	86
	Locating Ending Point	87
	Variable Surface.....	88
	Pause Bars	88
6.8	Digitizing Modes	89
	Automatic Line Follow Modes.....	89
	Raster Scan Modes.....	90

	Manual Modes	91
6.9	Erasing Digitized Data Points	92
6.10	Shifting Data Vertically.....	93
6.11	Adjusting the X Spacing of the Data	93
6.12	Exporting the Digitized Data to Other Software	93

Chapter 7: Graphing / Modifying (x,y) Data

7.1	Overview	95
7.2	Main Menu	95
	Graph / Modify (x,y) Data.....	95
7.3	Cursor Movement.....	96
7.4	Graph Options	97
7.5	Graph Buttons.....	100
	Edit Point	100
	Insert Point.....	100
	Delete Point	100
	Erase Data.....	100
	Sort Data	101
	Expand	101
	Area.....	102
	Adjust X Data Spacing.....	102
	Smooth.....	103
	Derivative	103
	Least Squares.....	103
7.6	Help.....	104
7.7	Exit.....	104

Chapter 8: UN-SCAN-IT gel Help

8.1	Help Menu	105
8.2	Help.....	105
8.3	Customer Support.....	106
8.4	Show Startup Screen	106
8.5	About UN-SCAN-IT gel.....	106

Appendix A: Scanning and Modifying the Image	107
Appendix B: Troubleshooting FAQs (Gel Analysis).....	109
Appendix C: Troubleshooting FAQs (Graph Digitizing)	113
Appendix D: New Version 6.3 Features (Gel Analysis).....	119
Appendix E: New Version 6.3 Features (Graph Digitizing)	121
Glossary.....	125

INTRODUCTION

This Setup and User's Manual describes the installation and use of your UN-SCAN-IT gel – Gel Analysis Software. Chapter 1 provides information on the installation of the UN-SCAN-IT gel software. Chapter 2 describes some useful methods for scanning and saving your gel and graphical images. Chapter 3 describes how to start the UN-SCAN-IT gel program on your computer.

The remaining chapters will introduce the UN-SCAN-IT gel software step by step and familiarize you with the features of the UN-SCAN-IT gel – Gel Analysis Software. This software was written by scientists, and is intended to be very intuitive, and almost entirely menu driven. In addition, context sensitive help buttons and menus are available throughout most of the gel analysis and graph digitizing portions of the software. It is recommended that the first time user consult the manual to become familiar with all of the features of the UN-SCAN-IT gel software.

This manual contains a glossary to explain some of the analysis and digitizing terms used throughout this manual, and a troubleshooting section (Appendix B and Appendix C) to help with some of the most commonly encountered questions. Previous users of the UN-SCAN-IT gel software may also wish to refer to Appendix D and Appendix E, which list some of the newest features available with this version.

If you have difficulty obtaining or digitizing your own images, you may wish to practice on the sample images that are automatically installed into the UN-SCAN-IT gel folder. These sample images will assist you in setting up and analyzing an image for the first time. If you have further questions or need assistance, please contact customer support:

Customer Support (USA):
Tel: 1-801-377-6978
FAX: 1-801-228-2448
E-Mail: info@silkscientific.com

Gel Analysis

The UN-SCAN-IT gel software acts like a densitometer to analyze gel-type images (electrophoresis gels, TLC plates, etc.) and extract quantitative data from the images. The software requires the image to be input in a standard image format that can be obtained from a scanner, digital camera, the internet, etc.. The image should be obtained at an appropriate resolution, as the software will extract the image data at *Full Scanner Resolution*. UN-SCAN-IT gel can be used to estimate band densities, relative mobilities, concentrations, and molecular weights for each band. The data can then be exported into almost any spreadsheet, data analysis, or graphics program.

When scanning gel images, the image should generally be scanned and saved in 8-bit grayscale (256 grayscale) format (although color images are also supported). Most scanners and digital cameras include software that allow the image to be saved in an 8-bit grayscale format.

Graph Digitizing

The UN-SCAN-IT gel software can also be used to convert hard copy graphs and graphical images to digital (x,y) data. The graph must be available in a standard image format that can be obtained from a scanner or similar image input device (such as a digital camera). The image should be obtained at an appropriate resolution, as the UN-SCAN-IT gel software will extract the underlying (x,y) data at *Full Scanner Resolution*. The data can be digitized using automatic or manual modes, and the digitized data can be exported into almost any spreadsheet, data analysis, or graphics program.

In order for UN-SCAN-IT gel to input and digitize different image formats, the Apple QuickTime application should be present on your computer. Macintosh computers generally have QuickTime already installed with the operating system. If QuickTime is not already on your computer, or you would like to download the latest version, go to www.apple.com/quicktime.

UN-SCAN-IT gel accepts a number of different image file formats from scanners and other imaging devices. These images should be saved in a standard image format as shown below...

- JPEG (.jpg)
- TIFF (.tif)
- Bitmap (.bmp)
- PICT (.pict)
- CompuServe GIF (.gif)
- GEM (.img)
- Targa (.tga)
- Any additional QuickTime Image Format

The supported image formats are shown in the Open Image File dialog box when loading an image to be digitized.

Throughout this manual we will be giving instructions that include key sequences or menu selections that should be directly input into the computer. Specific items that should be typed or selected will be indicated in **Bold**. In addition we will refer to the mouse buttons as Left and Right, where Left is referring to the primary mouse button, while Right is referring to the secondary mouse button.

Although UN-SCAN-IT gel has many graphics and other auxiliary data options, it should be noted that the digitized data can be easily exported to almost any other commercial software program that accepts (x,y) ASCII Text data. The data can be exported by saving the digitized (x,y) data in ASCII text format, or by copying the data to the Clipboard and pasting the data directly into another software package. Although this manual uses the general term ASCII to refer to the standard data format for the digitized data, this format is also often referred to as TEXT (.txt) format.

Crosshair Magnification Window

The UN-SCAN-IT analysis and digitizing screens contain a crosshair magnification window (in the upper right corner), which displays a full scanner resolution view of the region surrounding the crosshair. This view

should be used to obtain precise alignment of the crosshair when positioning interacting with the main image.

Accuracy

The UN-SCAN-IT gel software program is designed as a general tool for extracting data from images. As with any tool, the accuracy of the results can be largely dependent upon how that tool is used. The accuracy of the data is not only a function of the software, but is also dependent upon the original conditions of the experiment, the quality of the image, as well as the set-up processes and interaction performed by the user. Additional inherent limitations exist in converting image information to digital data, and thus the accuracy and precision of the data should not be assumed by the user. Silk Scientific makes no representation or warranties with respect to the absolute accuracy of the data or the fitness for any particular purpose. Please contact us if you have any questions regarding the accuracy of the results obtained using this product.

Mouse Buttons

Throughout the software, actions will require the use of the mouse buttons. The term “primary mouse button” refers to the main mouse button (usually the left mouse button), and the term “secondary mouse button” refers to the additional mouse button if present (usually the right mouse button). If your mouse only has one button, the secondary mouse button can be selected by holding the keyboard Ctrl Key while using the primary mouse button.

Primary Mouse ~ Left Mouse

Secondary Mouse ~ Right Mouse or Ctrl + Primary Mouse

License Information

This program may not be networked or otherwise installed or used on more than one computer or computer terminal at the same time (unless a site license has been obtained directly from Silk Scientific). The software is owned by Silk Scientific Corp. and is protected by the applicable United States and International copyright laws. Any printed materials accompanying the software are also copyrighted, and may not be copied. Silk Scientific's entire liability shall be repair or replacement of the software properly returned to Silk Scientific. Silk Scientific disclaims all implied

warranties of accuracy or fitness for a particular purpose, and shall not be liable for damages from loss of business profits or related income or other related damages resulting from the accuracy, use of, or inability to use this product.

Crosshair Movement

When digitizing (x,y) images, the crosshair used in the digitizing screens can be positioned using the mouse or keyboard arrow keys. Fine movements can be achieved using the keyboard **arrow keys**, while larger movements can be made by pressing the **Ctrl + arrow keys**. In addition, you may occasionally wish to restrict the crosshair movement to purely vertical or horizontal directions, which can be accomplished using the arrow keys (rather than the mouse).

Chapter 1

SOFTWARE INSTALLATION

1.1 Overview

To install the software, simply open the disk image on the CD, and drag the UN-SCAN-IT gel folder to the Applications folder on your hard disk.

Note: Do not drag the UN-SCAN-IT gel folder to your Desktop. It should be installed in the Application folder.

1.2 Write Privileges

The UN-SCAN-IT gel software installation requires the current user to have write privileges when installing the software.

1.3 Installing UN-SCAN-IT gel on the Hard Disk

- (1) Follow the on-screen instructions until UN-SCAN-IT gel has been installed. The software will notify you when the installation is complete.
- (2) Finalize Installation: With the UN-SCAN-IT gel CD in your computer's CD drive, start the UN-SCAN-IT gel software from the desktop icon or programs menu. If you do not have the UN-SCAN-IT gel CD, or your computer does not recognize the CD, select the 'Bypass CD' option and follow the instructions.

The first time the UN-SCAN-IT gel software is run on your computer, you will be prompted to enter the Serial Number and Registration Number associated with your license (these numbers are generally located on the inside cover of the User's Manual). Once the registration process is complete, remove the UN-SCAN-IT gel CD and store in a safe place.

Chapter 2

OBTAINING THE IMAGE

2.1 Overview

Although the name of this software UN-SCAN-IT gel, the image does not need to come directly from a scanner. The UN-SCAN-IT gel software can analyze almost any standard image format, and the image can come from a variety of sources (scanners, digital cameras, downloaded internet images, etc.). For simplicity, this chapter will give some guidelines for obtaining an image using a standard flat bed scanner. Virtually all scanners include their own scanning and image editing software. Before using UN-SCAN-IT gel, we recommend that you simply use the software that was included with your scanner to scan, edit, and save the image in a standard image format.

UN-SCAN-IT gel can directly import image files and digitize these images at full scanner resolution. In addition, if there is an image that can be displayed on your Macintosh computer screen, the image can be saved by using the Macintosh Grab utility or by pressing Command + Shift + 3 (this method of creating files is limited to screen resolution).

2.2 Scanner Operation

There are a large variety of affordable scanner types and models currently available. Virtually all of these scanners can easily produce images of sufficient quality for accurate digitizing.

Gel Images

We recommend that gel images generally be scanned and saved in 8-bit grayscale format (although the software does also support color images). Scanning in line art format results in a loss of information, and scanning in color format often requires additional computer memory without increasing the amount of useful information.

Caution! Images from hard copy publications (e.g. journal articles) are generally printed with dithered dots, and do not actually contain the true intensity values of the gel bands. Extreme cautions should be used exercised in analyzing dithered images.

Graphical Images

The UN-SCAN-IT gel software can digitize color, grayscale, and black & white (line art) images. Color images are often most useful for distinguishing between lines or different colors; grayscale images of often best for graphs that contain background grid lines that are a lighter shade than the data line; and black & white (line-art) images are generally best for digitizing standard black & white graphs that simply contain a dark data line on a white background.

For details regarding the actual use of your scanner and scanning software, refer to the User's Manual for your particular scanner and scanning software. Being familiar with your scanning software will help produce higher quality images and improve the analysis results obtained using the UN-SCAN-IT gel software..

2.3 Original Size

Although UN-SCAN-IT gel can digitize images of virtually any size, scanning large originals can present significant difficulties. Generally, the gel or graph should be of such size as to be easily scanned by your scanner (usually on 8½" x 11" paper or smaller). Some suggestions for dealing with larger originals include photo reducing the original, making use of a larger scanner, or using a digital camera.

Long (x,y) Graphs - To digitize long strip chart or recorder output it may be necessary to photo reduce the original, or divide the chart into scanning regions of more reasonable dimensions (e.g. 8" x 10" or smaller), as most scanners will not scan images larger than about 12" to 14". In this situation, scaling marks should be included on each portion of the image being scanned in order to insure continuity.

2.4 Scanning the Image

Many scanners have several possible 'image mode' settings. These settings are generally a selectable option contained within the scanning software.

Gels - The preferred mode for scanning most gel images in 8-bit grayscale in standard reflective mode. When scanning films or transparencies, scanners with a transparency mode can be useful in transmitting additional light through the film, thus producing a higher quality image. Although 8-bit grayscale images generally provide the best results, color images can also be digitized.

(x,y) Graphs - The preferred mode for scanning and digitizing most standard graphs is the black & white - line art mode (this mode is also referred to as 1-bit or black & white drawing). Although black & white images generally provide the sharpest images, color and grayscale images can also be useful for distinguishing colored data lines or filtering grid lines.

There are multiple resolution settings available with most scanners. Under normal circumstances UN-SCAN-IT gel analyzes the image in units of one scanner unit resolution. Therefore a scanner setting somewhere between 150 to 600 dpi is generally sufficient to obtain good results for most images. You may wish to experiment with different scanning resolutions to determine which setting is most appropriate for your needs. Please note that increasing the scanner resolution may result in significantly larger files (e.g. a 600 dpi image takes 4 times the memory of a 300 dpi image).

Graph Contrast - When scanning graphical images, the scanner contrast control should be set in such a manner as to produce an image that contains a dark continuous data line (or data points), while producing a relatively clean white (i.e. minimum stray dark pixels) background.

2.5 Straight Scanning

It is recommended that the gel or graph be scanned into the computer as straight as possible. This can generally be accomplished by simply making

sure the edges of the paper are parallel to the edge of the scanner bed.

If the image is skewed, most scanning software has a 'deskew' feature that allows the user to straighten the image (see your User's Manual for your particular scanning software). The deskew feature is only useful if the image is evenly skewed and has no unevenly skewed (waved) regions. Particular caution should be used when scanning an image from a book or technical journal, as uneven skewing may occur near the binding region of the image.

2.6 Using a Digital Camera

Consult the user's manual for your specific camera regarding instructions on how to obtain, store, and download your images. The images should be saved in a standard image format (JPG, TIF, etc.), and not in a proprietary format unique to your camera.

2.7 Scan

Now you are ready to scan/acquire the image. Consult the User's Manual for your particular scanning software for details on scanning images.

2.8 Rotating the Image

In order for UN-SCAN-IT to properly analyze the image, the image must be displayed 'right side up' on the screen before the image is saved. Virtually all scanning software has image rotation capabilities (consult your scanning software User's Manual for details on such features as *Rotate Clockwise* and *Rotate Counterclockwise*), thus the image can easily be rotated to the proper orientation.

2.9 Saving the Image

Once the image has been satisfactorily scanned (re-scan the image if it is too light, dark, waved, etc.), the image should be saved in a standard image

format so that it can be imported into UN-SCAN-IT gel. Acceptable image formats include, but are not limited to:

- JPEG (.jpg)
- TIFF (.tif)
- Bitmap (.bmp)
- PICT (.pict)
- CompuServe GIF (.gif)
- GEM (.img)
- Targa (.tga)
- Any additional QuickTime Image Format

The scanning software may also allow you to select the image file color type when saving the image (e.g. line art, 8-bit grayscale, 24-bit color, etc.). Gel images should generally be saved as 8-bit grayscale, while graphical images should generally be saved as black & white - line art (color mode can occasionally be useful for certain types of images). The image format options are often contained in the Save As or Export menu options. See the User's Manual for your particular scanner for details on saving image files.

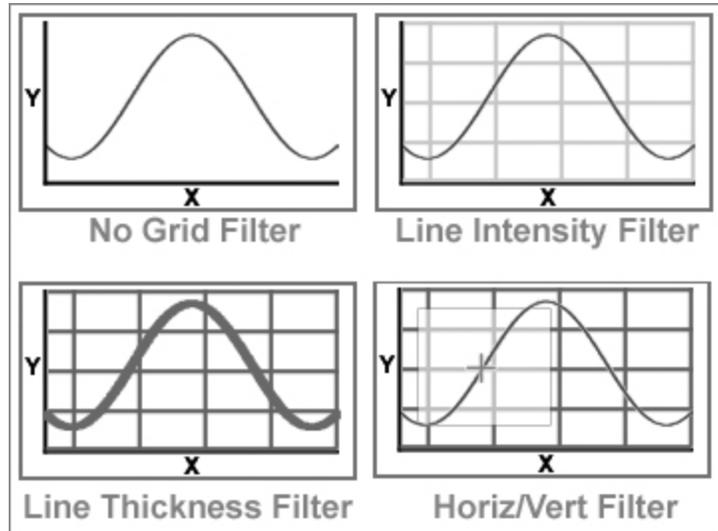
If you have images that have already been saved in image formats other than those accepted by UN-SCAN-IT gel, you may be able to read the image into your scanning software, and convert the image to a format compatible with UN-SCAN-IT gel.

2.10 Removing Grid Lines from Graphs | Graphs

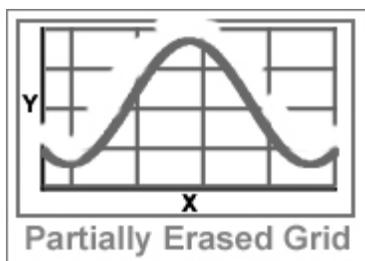
Grid lines can present a difficult problem, as they overlap the data lines in numerous locations, and can interfere with the automatic line following process. Although UN-SCAN-IT gel has features for filtering grid lines during the digitizing process, it may be easier to simply reduce or remove the grid lines during the scanning process. The scanner contrast control setting can often be used to filter out the lighter and thinner grid lines, while continuing to register the darker data lines. By experimenting with this contrast adjustment, it may be possible to completely remove the grid lines from the image. If the graphical data is currently being collected by an (x,y) recorder, it may be possible to turn over the grid paper and digitize the data

recording on the side that has no grid lines.

In addition, the UN-SCAN-IT gel software has three types of grid line filters available. One of the grid line filters uses the assumption that the grid lines are a lighter color/shade than the data line (to use this filter the image must be scanned in grayscale mode). The second filter uses the assumption that the grid lines are thinner than the data line. The third grid line filter uses the assumption that the grid lines run in a truly horizontal and vertical direction. These grid line filters are discussed later in this User's Manual.



If you are unable to remove the grid lines during the scanning process or the graph is not suitable for using the UN-SCAN-IT gel grid line filters, most scanners do have graphic editing tools (i.e. paintbrushes of various sizes), and these tools can be used to erase the grid lines where they intersect the data line. The UN-SCAN-IT gel software does have the option to simply follow along the top (or bottom) surface of the data line, therefore, you only need to erase the grid lines in the region slightly above (or below) the data line.



As an additional option for overcoming grid line difficulties can be to use the UN-SCAN-IT gel Manual Mode (similar to a digitizing tablet). In this mode, the crosshair can automatically increment along the X-axis while the user controls the Y-axis movement with the keyboard arrow keys or mouse.

2.11 Maximizing the Resolution of the Image | Graphs

When digitizing image files with the UN-SCAN-IT gel software, the resolution will generally be the same as the original resolution at which the image was scanned. Therefore, a 10-inch long graph scanned at 300 dpi will yield approximately 3,000 data points. Although some scanners operate at up to 1200 dots per inch (dpi) or higher, scanning graphs at these very high resolutions will generally not improve the quality of the data, because the resolution of the original graph is rarely above 300 dpi. In addition, scanning images at extremely high resolutions requires significantly more computer memory, disk space, and time (e.g. a 600 dpi image takes 4 times the memory of a 300 dpi image). Generally 100 to 300 dpi images are of sufficient resolution to provide excellent digitizing results, although you may wish to experiment with different scanning resolutions to determine which setting is most appropriate for your needs.

2.12 Maximizing the Resolution of Digitized Data | Graphs

Although the true optical resolution of the digitized (x,y) data from graphs is limited by the dpi resolution of the original image, the digitized data

resolution can be further enhanced. By selecting the **Adjust X Data Spacing** option available in the *Graphing* portion of the UN-SCAN-IT gel software, a cubic spline (or linear) fit of the data can be used to increase or decrease the number of data points, by adjusting the X data spacing to user defined increments.

Chapter 3

RUNNING UN-SCAN-IT GEL

3.1 Overview

This chapter describes how to start the UN-SCAN-IT gel program on your computer. Before the UN-SCAN-IT gel software can be used to analyze an image, the image file should have already been obtained from a scanner, digital camera, internet image, etc. (see previous chapter for details).

3.2 Running UN-SCAN-IT gel

Simply open the UN-SCAN-IT gel folder, and double click the UN-SCAN-IT gel program icon.

3.3 UN-SCAN-IT gel Registration Information

The first time the UN-SCAN-IT gel software is started; you should have the original UN-SCAN-IT gel CD in your computer's CD drive. If you do not have the CD, or your computer does not recognize the CD, select the 'Bypass CD' option and follow the instructions.

You will be prompted to enter Serial Number, Registration Number and User Name for your UN-SCAN-IT gel software license. The Serial Number and Registration Number are generally located on the inside front cover of the UN-SCAN-IT gel User's Manual (upgrades may contain the Serial and Registration Numbers on the front page of the User's Manual). You can enter whatever User Name you would like displayed. The UN-SCAN-IT gel software license is generally restricted to installation on one single computer terminal (unless multiple licenses or a site has been obtained).

Please return the Owner Registration card that is located in the front of the User's Manual, so that we may make you aware of any future product upgrades and assist you in your customer support needs. The

UN-SCAN-IT gel software can also be registered online at www.silkscientific.com/contact.

Chapter 4

FILE OPTIONS

4.1 Overview

This chapter will guide you through the different **File** options present in the UN-SCAN-IT gel software. Some of these file menu options can also be accessed from the toolbar located below the menus.

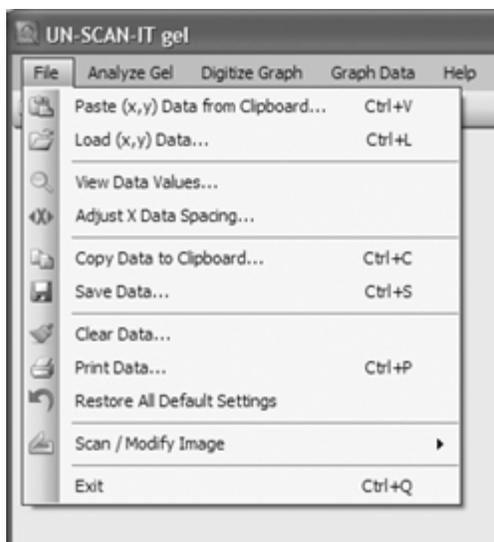
Most of the File options will only be available after your image has been digitized. See Analyzing Gels or Digitizing (x,y) Graphs chapter.

Macintosh Note: The File... Exit command is replaced by the Quit command present in the UN-SCAN-IT gel main application menu.

4.2 Main Menu

To enter the **File** portion of the UN-SCAN-IT gel program from the main menu, select:

File



File Menu

4.3 Paste (x,y) Data from Clipboard

Select **Paste (x,y) Data from Clipboard...** to paste the contents of the Macintosh clipboard into the UN-SCAN-IT gel program. The data must be in a standard (x,y) column format with no text labels in order to be pasted properly into UN-SCAN-IT gel. Using this command clears whatever (x,y) data may currently be in memory, so you may wish to save your data before using this function. . This option does not paste gel data.

4.4 Load (x,y) Data

Select **Load (x,y) Data...** to load a previously digitized (x,y) data file into UN-SCAN-IT gel. Do not use this option to load an image file for analysis. The standard Macintosh file dialog box will appear. Loading a file erases all digitized data presently in memory, so you may wish to save your data before loading data. This software can load (x,y) data saved by UN-SCAN-IT gel, or standard comma or tab delimited (x,y) ASCII Text data saved by other

programs. This option does not load digitized gel data.

4.5 View Data Values

Select **View Data Values...** to view the current digitized data values. Use the scroll bar to move up or down within the digitized data file.
zed data file.

4.6 Adjust X Data Spacing

The (x,y) graph digitizing process converts scanner pixel units into user defined scaled units. The resulting X data spacing may appear arbitrary as a result of this conversion. However, the X data spacing can be adjusted by fitting the digitized (x,y) data points using a cubic spline or linear fit. The X Data Spacing can be adjusted in the Graphing / Modifying (x,y) Data screen. This option does apply to gel data.

4.7 Copy Data to Clipboard

Select **Copy Data to Clipboard...** to copy all or part of the data values to the Clipboard. The data can then be pasted into other programs. Use the scroll bar to move up or down within the data file. Drag the mouse (or hold down the shift key) to highlight the data, and then select **Copy** (all of the data can be selected by clicking on the row column heading or by pressing Ctrl+A).

4.8 Save Data

Select **Save Data...** to save the data to a file. The data can be saved in tab or comma delimited ASCII Text format.

To export the data into other spreadsheet or graphics programs, save the data in ASCII Text format with the appropriate file extension if required (e.g. sample.txt, sample.dat, sample.csv, etc.). Often the File Open dialog box for these programs will only display specific file types (e.g. .xls for Excel),

however, other files can be displayed by selecting All Files *.* from the File Type drop down menu. Once the data have been imported by the other spreadsheet or programs, you can then use all of the features associated with that software to analyze, modify, or graph the data.

4.9 Clear Data

Select **Clear Data** to clear all the data values from memory. In order to prevent accidental clearing of data, you will be prompted to confirm the Clear Data command.

Graph Digitizing - The Clear Data command does not erase the graph digitizing settings, thus you may select **Return to Current (x,y) Image** from the **Digitize Graph** menu to continue digitizing the current graph image. When digitizing graphs, the Clear Data feature can be used to digitize multiple curves on a graph by digitizing each curve, saving the data, clearing the data, then returning to the current image to digitize the next curve.

4.10 Print Data

Select **Print Data...** to print the data values. If you have any difficulties printing the data, save the data in ASCII Text format (or copy to the data to the clipboard), and export the data into your word processor or spreadsheet program for printing.

4.11 Restore All Default Settings

Select **Restore All Default Settings** to restore all of the UN-SCAN-IT gel settings to their original values when the software was first installed. This feature can be useful for restoring Window sizes, default colors, and default analysis settings.

4.12 Scan / Modify Image

See **Appendix A**.

4.13 Exit

Select **Exit** to exit the UN-SCAN-IT gel software. You will be prompted to save any new data before exiting.

Chapter 5

ANALYZING GELS

5.1 Overview

This chapter will guide you through the analysis process for a gel image and explore the options available in the UN-SCAN-IT gel software. In this chapter you will be instructed to draw lane or segment boxes on the gel image, and this can generally be accomplished using the mouse and the left mouse button. Some of the options described in this chapter may not be encountered in every analysis session.

UN-SCAN-IT gel is designed as a general tool for extracting data from images. As with any tool, the accuracy of the results can be largely dependent upon how that tool is used. The accuracy of the data is not only a function of the UN-SCAN-IT gel software, but is also dependent upon the original conditions of the experiment, the quality of the image, as well as the set-up processes and interaction performed by the user. Inherent limitations exist in converting image information to digital data, and thus the accuracy and precision of the data should not be assumed by the user. Please contact Silk Scientific if you have any questions regarding the accuracy of the results obtained using this product.

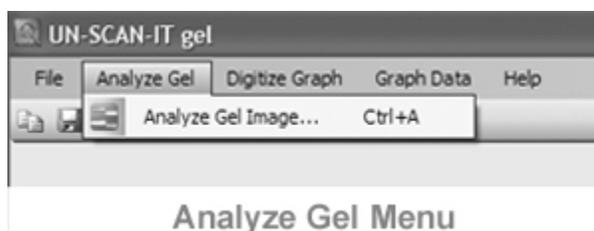
5.2 Main Menu

To enter the gel analysis portion of the UN-SCAN-IT gel program from the main menu, select:

Analyze Gel

Analyze Gel Image - main menu

Select **Analyze Gel Image...** to analyze a gel, TLC, dot blot, etc. image that was saved in a compatible image format.

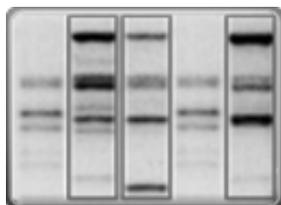


5.3 Open Image File

If **Analyze Gel Image...** was selected from the main menu, then a file dialog box will appear (only acceptable image files with the appropriate file extensions will appear in the list of files that can be selected). Select the gel image to analyze.

5.4 Gel Analysis Mode

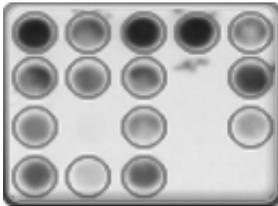
The Gel Analysis Mode allows you to select the type of analysis that you wish to perform for the image.



Lane Analysis - Select this mode to obtain a density profile and band analysis for one or more vertical gel lanes or gel sequences. Each lane can contain either a single band or multiple bands. For each lane, the software sums all of the pixel intensities in each horizontal row of the lane, and these summed values for each row represent an (x,y) value in the density profile for that lane. The software then automatically finds the peaks in that density profile that correspond to each band. Additional peaks can be added or deleted from the density profile, and the background lines can be dragged and adjusted. The pixel sums (above the background level) are then calculated for each band. When the band calculations for all the lanes have been completed, the digitized band values and parameters are displayed in a spreadsheet format below the gel image. The gel data can be exported into a spreadsheet or other data analysis software.



Segment Analysis - Select this mode to sum all of the pixel intensities within a band and perform a band analysis on one or more bands. The pixel intensities can be corrected for background by using one of several background correction methods within the software. The pixel sums (with any background corrections) are then calculated for each band. When the calculations for all the bands have been completed, the digitized band values and parameters are displayed in a spreadsheet format below the gel image. The gel data can be exported into a spreadsheet or other data analysis software.



Dot Blot Analysis - Select this mode to sum all of the pixel intensities within a circular/elliptical blot region and perform an analysis on one or more blots in a dot blot array. The pixel intensities can be corrected for background by using one of several background correction methods within the software. The pixel sums (with any background corrections) are then calculated for each blot. When the calculations for all the blots have been completed, the digitized blot values and parameters are displayed in a spreadsheet format below the dot blot image. The dot blot data can be exported into a spreadsheet or other data analysis software.

Positive/Negative - *gel analysis mode*

Select **Positive** for images where there are dark bands on a light background.
Select **Negative** for images where there are light bands on a dark background.

5.5 Gel Analysis Options

The Gel Analysis Options allows you to select from the available UN-SCAN-IT gel analysis options for the image you will be analyzing. Only

the options that are associated with the Gel Analysis Mode selected will be displayed.

Main Image View – *gel analysis options*

Maintain Aspect Ratio: This method maintains the original image aspect ratio in the main image viewing area. Although the image may not fill the entire viewing area, the image can be zoomed (1x, 2x, 4x, 8x, and 100%) to increase or adjust the size of the image within the viewing area. The zoom feature allows a closer inspection of the positioning of the lane and segment boxes. Note: The analysis may be slower while image is zoomed.

Stretch to Fit: This method stretches the image to fill the entire main viewing window, making full use of the entire image viewing area. Although this method may cause some images to appear distorted, the actual digitized data values will not be affected by the stretching.

Color Mode - *gel analysis options*

This option allows you to select the type of color differentiation UN-SCAN-IT gel will use to determine the intensity of the bands.

Grayscale Intensity - This method is for analyzing grayscale images by assigning a grayscale value between 0 (least intense) and 255 (most intense) for each pixel in the image. Most gel images should be scanned, saved, and analyzed in grayscale mode. Color information is rarely useful for distinguishing bands, and can potentially be difficult to analyze accurately.

HSB Color Intensity - This method is for analyzing color images by assigning an intensity value between 0 (least intense) and 255 (most intense) only for pixels of a similar color hue. The Single Color Hue Range value determines the color range that will be accepted and analyzed as data when using the HSB method.
(0 = Small color range : 100 = Large color range)."

Background Correction - *gel analysis options*

This option allows you to select the type of background correction that will be applied to the gel image being analyzed.

Density Profile Baseline Subtraction (Lanes Mode) - This mode uses the local minima values at the edge of each band's density profile as background. This method generally requires no additional background correction.

No Background Correction (Segments/Dot Blots Mode) - This mode accepts the intensity value of each pixel without modification. If the background is not zero (white or black), then a background correction should be used.

Subtract Single Region Background Value – This mode subtracts a single user defined background value from all pixels.

Subtract Upper and Lower Edge Interpolation – This mode subtracts an interpolated value from each pixel based upon the background value assigned at the upper and lower edge of each lane or segment box.

Subtract Four Corners Interpolation – This mode subtracts an interpolated value from each pixel based upon the background value assigned at the four corners of each lane or segment box.

Calibrate Image Background – This mode stretches the low end of the pixel spectrum to force the background to zero.

Calibrate Overall Image – This mode stretches the entire pixel spectrum to approximate multiple user defined pixel values.

Load Previous Image Calibration – This feature loads the image calibration values saved from a previous background or overall image calibration session.

Optical Intensity Calculation - *gel analysis options*

This option allows you to select the optical intensity calculation mode based

on the method that was used to obtain the image.

Linear – Standard Reflective Scan - Use this method for images that were scanned in standard reflective mode. Most scanners and digital cameras operate in reflective mode, where the light simply reflects from the surface of the sample.

Logarithmic – Film Transparency Scan – This method may be used for film images that were scanned in a transparency mode. Transparency scanners can shine light completely through the film sample to obtain the image. Caution! This method may result in large errors in the concentration values as the pixel intensities approach saturation.

Scan Direction - *gel analysis options*

This option allows you to select the direction in which UN-SCAN-IT gel analyzes the gel data and numbers the corresponding bands in each lane.

Top to Bottom - This method analyzes the image in the top to bottom direction. Use this method if the bands migrate from the top of the image to the bottom of the image.

Bottom to Top - This method analyzes the image in the bottom to top direction. Use this method if the bands migrate from the bottom of the image to the top of the image.

Lane/Segment Drawing Mode - *gel analysis options*

This option allows you to select the drawing mode that will be used to draw the lane or segment boxes on the image.

Lanes

Standard Drawing Mode - This method allows the user to draw each lane box individually by simply clicking and dragging the mouse cursor. The lane boxes can also be re-sized and moved individually.

Clone Drawing Mode - This method duplicates the single user defined lane box at specified horizontal offsets. Re-sizing or moving any lane box will re-size and move all other lanes on the image.

Lanes - This is the total number of lane boxes to be drawn on the image when using Clone Mode. The lanes value can be adjusted from the options menu when drawing.

Horizontal Spacing - This is the horizontal offset (in scanner pixel units) between lane boxes in Clone Mode. The spacing value can be adjusted from the options menu or toolbar when drawing.

Segments

Standard Drawing Mode -This method allows the user to draw each segment box individually by simply clicking and dragging the mouse cursor. The segment boxes can also be re-sized and moved individually.

Clone Drawing Mode - This method duplicates the single user defined segment box at specified horizontal and vertical offsets. Re-sizing or moving any segment will re-size and move all other segments on the image.

Columns/Rows - This is the total number of segment columns and rows to be drawn on the image when using Clone Mode. The number of cols/rows can be adjusted from the options menu when drawing.

Horizontal/Vertical Spacing - This is the horizontal and vertical offset (in scanner pixel units) between each segment in Clone Mode. These values can be adjusted from the options menu when drawing.

Lane Width - *gel analysis options*

This option allows you to select variable widths or a fixed single pixel width to each lane. Generally variable lane widths are most useful for including all pixels in the lane; however, the single pixel method can be useful in reducing problems with 'smiles' or 'tilted' lanes.

Variable - This option allows the lane boxes to be drawn at any user defined width. The lane box should generally encompass the width of the entire lane.

Single Pixel - This option only allows lane boxes to be drawn with a width of a single scanner pixel unit.

Lane/Segment Box Line Type - *gel analysis options*

This option allows you to select the line type for the lane and segment boxes drawn on the main gel image.

Solid – Solid line boxes.

Dashed – Dashed line boxes.

Dotted – Dotted line boxes.

Saturation Checking - *gel analysis options*

This option allows you to select if a warning will be displayed if the image appears to be saturated. Image saturation may result in a loss of resolution for the most intense bands.

Off - This option ignores instances when multiple adjoining pixels are at the same apparent saturation value.

On - This option provides a warning when multiple adjoining pixels are at the same apparent saturation value.

ACGT Sequence Mode - *gel analysis options*

This option provides a method for assigning ACGT values to the bands in a sequencing gel.

Off -This option assumes the image is a standard gel, and does not attempt to determine the base pair (ACGT) sequence for the gel.

On -This option determines the base pair (ACGT) sequence for a DNA type gel based on migration distance of bands in the A, C, G, and T lanes. This method only allows a maximum of 4 lanes. This option is only available in lanes mode.

Digitizing Speed - *gel analysis options*

This option allows you to select the speed used for the digitizing portion of the lane or segment analysis. The digitizing speed selected does not affect the quality of the data.

Standard – This option displays the gel digitizing process as the image is being digitized.

Turbo – This option does not display the gel digitizing process as the image is being digitized. This option can be used when maximum digitizing speed is required.

5.6 Gel Analysis Screen

After the gel analysis options are selected, the main gel analysis screen will be displayed. The main analysis screen displays the main image, a crosshair magnification window (which displays a image resolution view of the region surrounding the crosshair, box position grid that displays the coordinates of the lane or segment boxes, and a message region that guides the user through the analysis process.



As the lane and segment boxes are defined, the currently highlighted box will flash, and the coordinates will appear highlighted in the box position grid. The current pixel location and value at the center of the crosshair magnification window will appear at the bottom corner of the message region.

The main menu that appears at the top of the analysis screen contains the following features:

- Options** Use this menu to adjust the lane and segment boxes, modify the default settings, change drawing modes, save gel data, etc. These options will be discussed later in this chapter.
- Help** Select **Help** to display help topics relating to the setup, analysis, general options and results of the analysis process.
- Exit** Select **Exit** to exit the analysis screen and return to the main menu.

Options – gel analysis screen

The **Options** menu contains the following options:

- Copy Current Lane/Segment** Use this option to create an additional lane or segment box with the same width and height properties as the currently selected (highlighted) lane or segment. Lane and segment boxes can be highlighted by moving the mouse cursor over the box or by clicking on the box row in the box position grid.
- Delete Current Lane/Segment** Use this option to delete the currently selected (highlighted) lane or segment. Lane and segment boxes can be highlighted by moving the mouse cursor over the box or by clicking on the box row in the box position grid.
- Delete All Lanes/Segments** Use this option to delete all currently defined lanes or segments.
- Slide All Lanes/Segments** Use this option slide the positions of all currently defined lanes or segments. This feature can be especially useful when analyzing multiple gel images where saved lane or segment positions have been loaded, and there is a slight shift in the lane or segments positions between images.
- Align Lanes/Segments** Use this option to align all lanes or segments with respect to the currently selected (highlighted) lane or segment. Alignment can be with respect to either position or size of the selected lane or segment. Lane and segment boxes can be highlighted by moving the mouse cursor over the box or by clicking on the box row in the box position grid.

Lane/Segment Drawing Method	Use this option to switch between standard and clone drawing methods for lane or segment boxes. Details regarding the different drawing method choices can be found by selecting help during the gel analysis options setup process.
Lane/Segment Clone Settings	Use this option to modify the clone drawing settings. Lanes: The number of lanes and the horizontal spacing of the lanes can be adjusted. Segments: The number of segment columns and segment rows can be adjusted, along with the horizontal and vertical spacing of the segments.
MW Calculation Method	Use this option to switch between different methods for fitting the unknown molecular weight values to the known molecular weight values in the final gel results. Caution: When calculating Molecular Weight values based on the log of MW, small differences in Y Position can cause large differences in calculated MW values.
Lane/Segment Label Positions	Use this option to move the positions of the lane or segment labels in order to allow band features to be viewed without being covered by the segment labels. The Segment labels can be edited by double clicking on the segment label in the box positions grid, or double clicking on the label on the main gel image.
Hide/Show Segment Labels	Use this option to switch between hiding and showing the lane or segment labels.

This feature can be useful to allow band features to be viewed without being covered by the segment labels.

Set Digitizing Speed

Use this option to change the current digitizing speed.

Set Main Image View

Use this option to change the current main image view to either Maintain Aspect Ratio or Stretch to Fit. This option can also be used to set the zoom level and increase the size of the image within the viewing area. Note: Analysis may be slower while image is zoomed.

Distance Measuring Tool

Use this option to activate the distance measuring tool. This tool may be used to measure distance on the image in the horizontal (x), vertical (y), and combined (z) directions. Simply click the mouse at the point where you wish to begin the measurement, and drag the mouse cursor to any point on the image. The data can be displayed in pixels, inches, or centimeters (inches and centimeters measurements depend upon the dpi value of the original image which may not be available in some images).

Hide/Show Y Position Lines

Use this option to switch between Hiding and Showing the Y Position Lines. This feature can be useful to allow band features to be viewed without being obscured by the Y Position Lines.

Expand/Reduce Results Grid

Use this option to increase or decrease the

number of rows displayed in the results grid.

Set Screen Colors

Use this option to change the current screen colors used by the software. The crosshair color, segment box color, density profile peak colors, etc. can be adjusted. Setting these display options can be useful in making some features more visible on the analysis screen.

Current Settings and Image Info

Use this option to display the current analysis and setup options that have been selected, as well as some general information regarding the current image.

Copy Screen Image to Clipboard

Use this option to Copy the current analysis Screen Image to the Clipboard. The screen image can then be Pasted into another program (word processor, graphics, etc.).

Save Gel Data

Use this option to save the gel data values to a standard data file. The saved data file can then be read into other programs, such as spreadsheet, data analysis, or graphics software.

Load Lane/Segment Positions

Use this option to load previously saved lane or segment positions. This option can be very useful for retrieving previously saved lane or segment positions in order to re-analyze a previous image or analyze another similar image, without having to re-define the lane or segment positions.

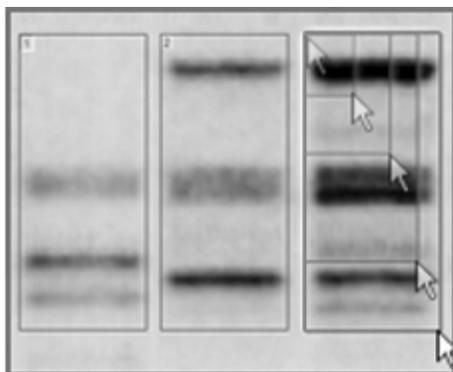
Save Intensity Calibration Data

Use this option to save the intensity

calibration data for the current gel. The saved calibration file can then be used at a future time for this gel image or another similar gel image.

5.7 Drawing Lanes, Segments, and Dot Blots

Lane, segment, and dot blot boxes can all be defined in a similar manner by clicking and dragging the mouse within the main image.



Drawing Boxes

Lanes - drawing

Lane boxes can be created by clicking and dragging the mouse within the main image to draw the box. The box will then be numbered, and the box coordinates will be displayed in the box position grid at the right side of the analysis screen. The crosshair magnification window in the upper right corner of the screen can be used for precise alignment. To obtain optimal quantification, the boxes should generally be drawn large enough to include the entire region of interest for each band.

Lane boxes can be highlighted by moving the mouse cursor over the box or by clicking on the box row in the box position grid. The highlighted box will

be the box upon which copying, deleting, aligning, clone drawing, and other modifications will center.

Lane boxes can be moved by locating the mouse cursor over the box and dragging the box to a new location. Boxes can be re-sized by locating the mouse cursor over the edge of the box and dragging to the desired size. Boxes can also be moved and re-sized by double clicking on the box position grid on the right side of the analysis screen.

Lanes should be drawn as rectangles that enclose the entire lane of interest, without including any portion of bands contained in another lane. It is generally not necessary to draw irregularly shaped lane boxes, as any background pixels included in the lane box can be ignored in the density profile screen by correctly setting the baseline. Band smiling effects can be minimized by setting the lane width to 1 pixel.

Segments - drawing

Segment boxes can be created by clicking and dragging the mouse within the main image to draw the box. The box will then be numbered, and the box coordinates will be displayed in the box position grid at the right side of the analysis screen. The crosshair magnification window in the upper right corner of the screen can be used for precise alignment of the segment. To obtain optimal quantification, the boxes should generally be drawn large enough to include the entire region of interest for each band.

Segment boxes can be highlighted by moving the mouse cursor over the box or by clicking on the box row in the box position grid. The highlighted box will be the box upon which copying, deleting, aligning, clone drawing, and other modifications will center.

Segment boxes can be moved by locating the mouse cursor over the box and dragging the box to a new location. Boxes can be re-sized by locating the mouse cursor over the edge of the box and dragging to the desired size. Boxes can also be moved and re-sized by double clicking on the box position grid on the right side of the analysis screen.

Segments should be drawn as rectangles that enclose the entire band of interest, without including any portion of other bands. It is generally not necessary to draw irregularly shaped segment boxes, as there are several background correction options available in the setup portion of the software that will reduce or eliminate any background effects. These background corrections can allow band quantification relatively independent of box size and shape. If the background is not zero and no background correction is selected, the segment boxes should all be drawn the same size to minimize background effects.

Dot Blots - drawing

Dot blot boxes/ellipses can be created by clicking and dragging the mouse within the main image to draw the box/ellipse. The box/ellipse will then be numbered, and the box/ellipse coordinates will be displayed in the grid at the right side of the analysis screen. The crosshair magnification window in the upper right corner of the screen can be used for precise alignment of the box/ellipse. To obtain optimal quantification, the boxes/ellipses should generally be drawn large enough to include the entire region of interest for each blot.

Dot blot boxes/ellipses can be highlighted by moving the mouse cursor over the box or by clicking on the box row in the box position grid. The highlighted box/ellipse will be the box/ellipse upon which copying, deleting, aligning, clone drawing, and other modifications will center.

Dot blot boxes/ellipses can be moved by locating the mouse cursor over the box and dragging the box to a new location. Boxes/ellipses can be re-sized by locating the mouse cursor over the edge of the box and dragging to the desired size. Boxes/ellipses can also be moved and re-sized by double clicking on the box position grid on the right side of the analysis screen.

Dot Blot segments should be drawn as rectangular boxes with interior circular regions (ellipses) that enclose the entire blot of interest, without including any portion of other blots. Only the circular region of the segment will be analyzed. It is generally not necessary to draw irregularly shaped dot blot boxes/ellipses, as there are several background correction options available in

the setup portion of the software that will reduce or eliminate any background effects. These background corrections can allow band quantification relatively independent of box/ellipse size and shape. If the background is not zero and no background correction is selected, the segment boxes/ellipses should all be drawn the same size to minimize background effects.

5.8 Begin Analysis

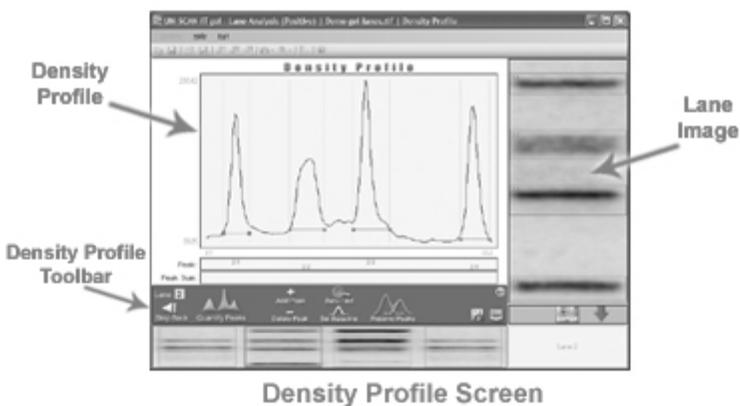
Once all of the lanes, segments, or dot blots have been defined, click the **Analyze** button at the lower right corner of the analysis screen. UN-SCAN-IT gel will then begin automatically digitizing and analyze the gel image.

5.9 Gel Analysis Mode

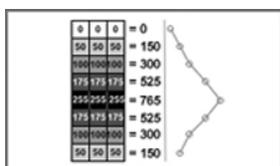
The method used for analysis the gel image varies depending upon whether you selected **Lane Analysis**, **Segment Analysis**, or **Dot Blot Analysis** during the Gel Analysis Mode setup portion of the program.

Lane Analysis – *gel analysis mode*

When the image digitizing process begins, UN-SCAN-IT gel extracts a density profile for each lane, based upon each pixel value in that lane. Each pixel value is between 0 and 255, and may have been adjusted for background if that option was selected during the setup process.



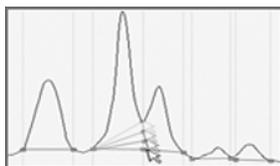
Density Profile Screen



Density Profile - General

Each (x,y) data point in the Density Profile is obtained by summing the grayscale pixel values horizontally across the lane. As a result, bands manifest themselves as peaks in the Density Profile. Generally the more intense the band...

the higher the peak, and the larger the band... the wider the peak. Therefore, integrating the area under these peaks accounts for both the intensity and size of each band. The integrated peak area can then be related to the relative concentrations for each of the bands (assuming the bands are not saturated and the optical intensity of the gel bands are relatively linear).

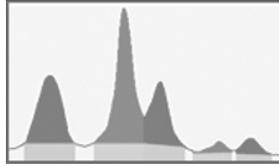


Density Profile - Peaks

The UN-SCAN-IT gel software will automatically find the peaks in the Density Profile, and set the corresponding baseline. Noise, overlapping peaks, weak signals, etc. may cause the software to miss peaks, find extra

peaks, or not set the endpoints of some peaks at their proper locations. Therefore, the software has a toolbar for adding or deleting peaks, as well as a method for adjusting the endpoints of any peak. To adjust the endpoint of any

peak, simply click the mouse on the drag box associated with that endpoint. The drag box and baseline will change color, and the endpoint can be dragged to the desired location on the Density Profile. Density Profile peaks cannot overlap.



Density Profile - Baselines

The baselines for each peak are straight lines drawn from the left endpoint to the right endpoint of each peak. These baselines should generally approximate the local minima for each peak. This baseline is used to determine the background values for each peak, and thus the integrated area of each peak will only include values above the local background. Proper placement of baselines is critical to obtaining accurate peak quantification. Proper adjustment of peak baselines can also be used to separate doublet peaks, or the Resolve Peaks feature can be used.

Density Profile Toolbar – lanes digitizing mode

The density profile screen will also display a toolbar containing a variety of buttons for use in quantifying each peak/band in the lane. The functions of these buttons are described below.



Density Profile Toolbar

Quantify Peaks / Continue - **Quantify Peaks** integrates the area of each defined peak in the Density Profile. The integrated values are displayed under each peak and carried over to a spreadsheet for later analysis. **Continue** moves on to the Density Profile for the next lane. If the final lane has been quantified, the Results screen will then be displayed.

Step Back/Undo – **Step Back** to return to the previous Density Profile. If the

current Density Profile is for the first lane, the program will return to the lane drawing portion of the software. **Undo** to remove the peak integration for the current Density Profile in order to add peaks, delete peaks, or modify peak endpoints.

Add Peak - This feature allows a peak to be manually added to the Density Profile. Click the mouse at the location on the Density Profile where you wish to add the peak, then drag the peak endpoints to their desired locations. The added peak cannot overlap any of the other currently defined peaks.

Delete Peak - This feature allows a peak to be manually deleted from the Density Profile. Click the left mouse button at the peak location on the Density Profile where you wish to delete the peak. Clicking the right mouse button on the Density Profile will delete all peaks.

Auto Find - This feature searches for peaks in the current density profile to find and assign peaks. The Peak Find feature is executed automatically when each density profile is first loaded and displayed. Re-running this feature can be useful if peaks were erroneously deleted or modified. In addition, this feature allows the Automatic Peak Finding Settings to be modified.

Set Baseline - This feature allows the baseline for all current peaks to be set to a single user defined value. Generally this function is not necessary, as peak baselines should most often be set at the natural minimum that occurs at the edge of each peak, and thus the baseline should vary accordingly for each peak. However, there may be circumstances where it is desirable to set the baseline to a common value for all peaks, therefore, the Set Baseline feature is available for this purpose.

Resolve Peaks - The software may miss peaks, find extra peaks, or not resolve overlapping peaks. The Resolve Peaks feature can be used to overlay Gaussian peaks on the density profile to estimate where the baseline should be set if the peaks were represented by a Normal distribution. The three Gaussian peaks in the upper left corner of the density profile can be used to overlay the peaks you wish to fit. You can use one, two, or all three of the Gaussian peaks to fit single, double, or triple peaks. The Gaussian peaks should be dragged and resized to overlay the density profile peaks you wish to fit. To obtain the best fit, you may wish to Maximize the

Window to assist in visually overlaying the peaks. After the Gaussian peaks have been overlaid on the density profile, press Enter to automatically estimate the locations of the baselines that best fit the density profile for those peaks. Confirm visually that the new baseline locations are acceptable. Additional sessions using the Resolve Peaks feature can allow additional peaks to be resolved for that lane.

Save Density Profile as (x,y) Data - This feature allows the (x,y) data values that make up the Density Profile to be saved to a file. The x values represent the row distances from the edge of the gel, and the y values represent the pixel sums across each row of the lane. The data can be saved in (x,y) ASCII format, that can be exported into graphing or spreadsheet programs for further analysis or presentation.

Copy Screen Image to Clipboard - Use this option to Copy the current density profile Screen Image to the Clipboard. The screen image can then be Pasted into another program (word processor, graphics, etc.).

Segment Analysis - *gel analysis mode*

When the image digitizing process begins, UN-SCAN-IT gel sums the pixel values for all pixels within each rectangular segment region. Each pixel value is between 0 and 255, and may have been adjusted for background if that option was selected during the setup process.

When the digitizing process is complete, UN-SCAN-IT gel overlays the band segment boxes on the main image, and a results grid is displayed at the bottom of the gel analysis screen.

Dot Blot Analysis - *gel analysis mode*

When the image digitizing process begins, UN-SCAN-IT gel sums the pixel values for all pixels within each elliptical segment region. Each pixel value is between 0 and 255, and may have been adjusted for background if that option was selected during the setup process.

When the digitizing process is complete, UN-SCAN-IT gel overlays the band segment boxes/ellipses on the main image, and a results grid is displayed at the bottom of the gel analysis screen.

5.10 Results

General Results - *results*

Simply because the software obtains a digitized value for a gel segment... that does not assure the accuracy of that data value. Scientific expertise, visual approximation, and common sense should be used to verify the gel data. Obtaining consistent and accurate gel analysis results requires consideration of all processes involved in quantifying a gel, including...

Experimental - The experimental portion is the first and most important step to accurately quantifying a gel. Accurately quantifying a gel may be difficult or impossible if one or more of these conditions are prevalent...

- Non-Linear dyes (or other indicator methods)
- Band saturation
- Overlapping bands or 'smiling' bands
- Tilted lanes or bands
- Smudged bands
- etc.

Obtaining the Image - Obtaining a good image is the second most important step to accurately quantifying a gel. Whether the image is obtained with a scanner, a digital camera, or other image input device, the image should be...

- Scanned straight with no significant tilt
- Right side up with gel bands migrating up or down
- Not saturated
- Near the middle of the dynamic range of the scanner
- Reasonably high resolution (generally 150 - 600 dpi)
- Saved in a compatible image format

Software Analysis - The software analysis is the third most important step to accurately quantifying a gel. If the experimental or obtaining the image steps were not performed properly, then no software analysis will be able to obtain accurate results. Although this software is designed to be powerful and intuitive, please let us know if you have any questions or need assistance quantifying your gel images.

Results Screen - *results*

After the digitizing process is complete for the gel image, a results screen will be shown, and a variety of quantified gel parameters displayed.



Results Screen

Results Grid - results

The results grid is located at the bottom of the gel analysis screen. The gel results displayed in this grid can be used to estimate concentrations, relative mobility, and molecular weight values for each segment. The size of the grid can be expanded or reduced by selecting the Expand/Reduce Grid option. The gel parameter definitions are described below, or can be displayed by clicking on the grid column headings at the top of the results grid.

Segment This column displays the Segment labels that correspond to each analyzed Segment. The Segment labels can be edited by double clicking on the segment label in the results grid, or double clicking on the segment label on the main gel image.

Pixel Total This column displays the Pixel Total that corresponds to each analyzed Segment. The Pixel Total reflects any corrections that were made to adjust for background. The Pixel Total values are generally what is used to calculate the relative Concentrations of gel bands. The Pixel Total values are calculated by the software, and cannot be

directly modified.

- Concentration** This column displays the Concentration that correspond to each analyzed Segment. These values will be displayed as zero until a Concentration calibration has been performed for the gel. The Concentration can be calibrated by double clicking on the Concentration value for the Segment of known Concentration, and entering that Concentration value. Entered values will be indicated by an asterisk. Once two or more Concentration values have been entered, a least squares equation will be used to fit the Concentrations of the remaining Segments. To erase a calibration value for a Segment that has been entered, double click that Segment's Concentration value and enter zero (0). The Concentration calibration curve can be displayed by clicking the Show Concentration button on the toolbar.
- Y Position** This column displays the Y Position that corresponds to each analyzed Segment. This position is generally chosen at the most intense vertical portion of the Segment. The Y Positions can be modified by double clicking on the Y Position value in the grid, or by using the mouse to drag the dashed Y Position line on the main gel image.
- Rel Mobility** This column displays the Relative Mobility for each analyzed Segment. This value is calculated using the Y Position values, and is relative to a user defined starting point and ending point (tracking dye). These values will be displayed as zero until the starting and ending point have been defined.
- Mol Weight** This column displays the Molecular Weight values that correspond to each analyzed Segment. These values will be displayed as zero until a Molecular Weight calibration has been performed for the gel. The Molecular Weight values can be calibrated by double clicking on the Molecular Weight value for the Segment of known Molecular Weight, and entering that Molecular Weight value. Entered values

will be indicated by an asterisk. Once two or more Molecular Weight values have been entered, a least squares equation will be used to fit the Log MW relative to the Y position of the remaining Segments. To erase a calibration value for a Segment that has been entered, double click that Segment's Molecular Weight value and enter zero (0). In order to obtain the best fit, several Molecular Weight calculation methods are available from the Options Menu. The Molecular Weight calibration curve can be displayed by clicking the Show MW button on the toolbar.

Pixel Total % This column displays the Pixel Total % that corresponds to each analyzed Segment. In Lanes mode, the Pixel Total % will correspond to the relative percentage of that Segment's Pixel Total to the Pixel Total for all of the defined Segments in that Lane. In Segments mode, the Pixel Total % will correspond to the relative percentage of that Segment's Pixel Total to the Pixel Total for all of the defined Segments in the entire gel. The Pixel Total % values are calculated by the software, and cannot be directly modified.

Segment Size This column displays the Segment Size that correspond to each analyzed Segment. The Segment Size is determined by simply multiplying the Segment width by the Segment height (in pixel units) for rectangular segments, or is simply the total number of pixels (i.e. area) digitized within the elliptical Segment region for dot blots. The Segment Size values are calculated by the software, and cannot be directly modified.

Segment Size % This column displays the Segment Size % that corresponds to each analyzed Segment. In Lanes mode, the Segment Size % will correspond to the relative percentage of that Segment's Size to the Segment Size total for all of the defined Segments in that Lane. In Segments mode, the Segment Size % will correspond to the relative percentage of that Segment's Size to the Segment Size total for all of

the defined Segments in the entire gel. The Segment Size % values are calculated by the software, and cannot be directly modified.

Maximum Pixel This column displays the Maximum Pixel value measured within that segment (value can range from 0 to 255). The Maximum Pixel value reflects any corrections that were made to adjust for background. The Maximum Pixel values are calculated by the software, and cannot be directly modified.

Average Pixel This column displays the Average Pixel value measured within that segment (value can range from 0 to 255). This value is calculated by taking the Pixel Total and dividing by the Segment Size. The Average Pixel value reflects any corrections that were made to adjust for background. The Average Pixel values are calculated by the software, and cannot be directly modified.

5.11 Normalize

The Pixel Total values for all segments can be Normalized to a user defined segment by clicking the Normalize button above the results grid. Normalization can be undone.

5.12 Calibrating Concentration Values

The Concentration values can be calibrated by double clicking on the Concentration value for the Segment of known Concentration, and entering that Concentration value. Entered values will be indicated by an asterisk. Once two or more Concentration values have been entered, a least squares equation will be used to fit the Concentrations of the remaining Segments. To erase a calibration value for a Segment that has been entered, double click that Segment's Concentration value and enter zero (0).

5.13 Editing Y Position Values

The Y Positions can be modified by double clicking on the Y Position value in the grid, or by using the mouse to drag the dashed Y Position line on the main gel image.

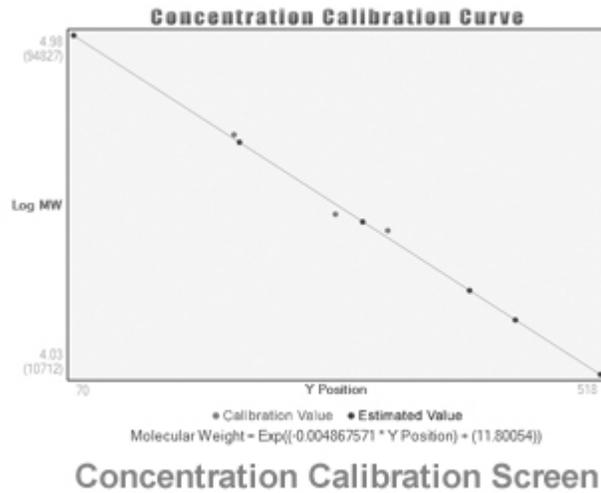
5.14 Calibrating Relative Mobility Values

By double clicking on the Rel Mobility column, the start Y Position and tracking dye Y Position can be defined.

5.15 Calibrating Molecular Weight Values

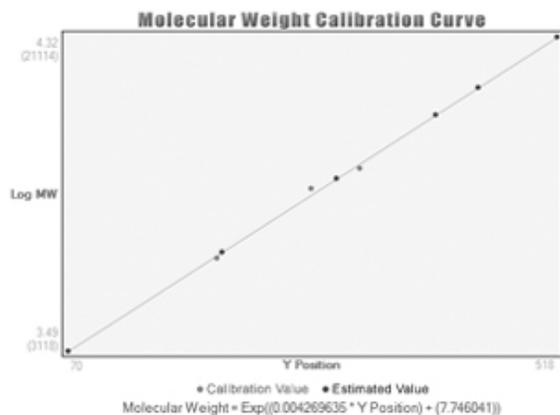
The Molecular Weight values can be calibrated by double clicking on the Mol Weight value for the Segment of known MW, and entering the MW value. Entered values will be indicated by an asterisk. Once two or more MW values have been entered, a least squares equation will be used to fit the Log MW relative to the Y position of the remaining Segments. To erase a calibration value for a Segment that has been entered, double click that Segment's MW value and enter zero (0). **Caution:** MW values as a function of the Log(MW) may potentially contain large errors, as small differences in position can equate to large differences in MW due to the Log function.

5.16 Show Concentration Curve



Clicking the Show Concentration Curve button will display a least squares fit of the Concentrations for all segments as calculated relative to any standard Concentrations that have been entered for known segments. Two or more segments of known Concentrations must be entered before the least squares fit can be applied and the results displayed. The least squares equation will be displayed at the top of the graph.

5.17 Show Molecular Weight Curve



Molecular Weight Calibration Screen

Clicking the Show Mol Weight Curve button will display a least squares fit of the Log MW (or Linear MW) values for all segments as calculated relative to any standard Molecular Weight Values that have been entered for known segments. Two or more segments of known Molecular Weight must be entered before the least squares fit can be applied and the results displayed. The least squares equation will be displayed at the top of the graph.

5.17 Helpful Hints

- When drawing lanes, draw lane limits well above and below the end of the lane.
- When analyzing lanes, manually adjust any peak baselines that are not near the local minima in the density profile screen.
- When drawing lanes or segments, draw the box wide enough to encompass all of the pixels to be included with that band.
- Use background corrections for segments or dot blots.

Chapter 6

DIGITIZING (X,Y) GRAPHS

6.1 Overview

This chapter will guide you through the digitizing process for graphical images, and explore the options available in the UN-SCAN-IT gel software. In this chapter you will be instructed to drag the setup icons and move the digitizing crosshair to different locations on the image. These movements can be accomplished with the mouse, the keyboard arrow keys for fine movement, or the **Ctrl** + arrow keys for coarse movements. Some of the options described in this chapter may not be encountered in every digitizing session.

Many of the UN-SCAN-IT gel digitizing menu options can also be accessed from the toolbar located below the menus. Help menus and context sensitive help buttons can be used to provide helpful information regarding the digitizing controls and options.

UN-SCAN-IT gel is designed as a general tool for extracting data from images. As with any tool, the accuracy of the results can be largely dependent upon how that tool is used. The accuracy of the digitized data is not only a function of the UN-SCAN-IT gel software, but is also dependent upon the original conditions of the experiment, the quality of the image, as well as the set-up processes and interaction performed by the user. Inherent limitations exist in converting image information to digital data, and thus the accuracy and precision of the data should not be assumed by the user. Please contact Silk Scientific if you have any questions regarding the accuracy of the results obtained using this product.

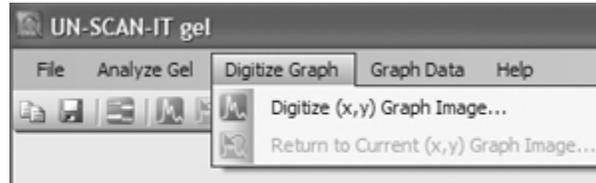
6.2 Main Menu

To enter the (x,y) graph digitizing portion of the UN-SCAN-IT gel program from the main menu, select:

Digitize Graph

Digitize (x,y) Graph Image - main menu

Select **Digitize New (x,y) Graph Image...** to digitize a graph that was saved in a compatible image format.



Digitize Graph Menu

Return to Current (x,y) Graph Image - main menu

Select **Return to Current (x,y) Graph Image...** to return to a currently selected graph and allow additional points on the graph to be digitized without requiring the re-entry of the graph's scaling points. All digitized data points currently in memory will be drawn to the screen, or the data points can be cleared by selecting **Clear Current (x,y) Data** from the **Options** menu in the digitizing screen.

6.3 Open Image File

If **Digitize New (x,y) Graph Image...** was selected from the main menu, a Macintosh file dialog box will appear (only acceptable image files with the appropriate file extensions will appear in the list of files that can be selected). Select the image you wish to digitize.

Sample Images

Several sample images are also included in the UN-SCAN-IT gel folder. You may find it useful to load and digitize one of these sample images before trying one of your own images. These sample images assist you in identifying the axis setup parameters, and are relatively straightforward to digitize.

6.4 Graph Digitizing Mode

All UN-SCAN-IT gel digitizing modes (except Polar Digitizing Tablet) assume an **(x,y) Cartesian Coordinate** system where the x axis and y axis are **orthogonal** (right angles). UN-SCAN-IT gel does not digitize circular graphs or graphs that are recorded in a format other than standard (x,y) Cartesian Coordinates

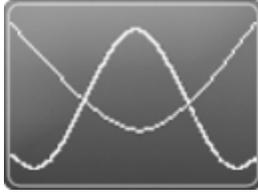
The Graph Digitizing Mode allows you to select the type of digitizing to be performed on your image.



Standard Line

(Automatic Line Follow for Standard Curves)

In this mode, UN-SCAN-IT gel follows a line on the graphical image. The crosshair automatically searches up and down (as it moves along the line) until it finds the line and records the single valued (x,y) point. Then the crosshair automatically increments 1 resolution unit in the X direction, and repeats the point assignment process until digitizing is completed. The default *line follow direction* is left to right, and the default resolution is 1 scanner unit. These default settings can be modified in the **Options** portion of the Digitizing Screen. This mode is most useful for digitizing single line (x,y) graphs or multiple curves that do not intersect. Extraneous points, marks and labels in the digitizing region generally do not present a problem unless they touch or overlap the curve of interest. The automatic digitizing process can be paused at any point by pressing the **Enter** key or selecting the Pause button.



Intersecting Line

(Automatic Line Follow for Intersecting Curves)

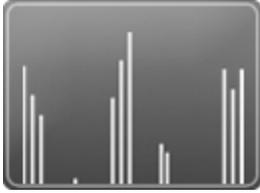
This mode is the same as Standard Line, except that this mode includes features for dealing with intersecting curves. Pause Bars can be manually inserted along the curve, which cause the software to pause at these intersection points. Additionally, an overlap search can be used to automatically estimate the location of intersection points during the digitizing process. This mode is most useful for digitizing (x,y) graphs with multiple curves that intersect. The automatic digitizing process can be paused at any point by pressing the **Enter** key or selecting the Pause button.



Cyclic Graph

(Automatic Line Follow with Auto Turn Around)

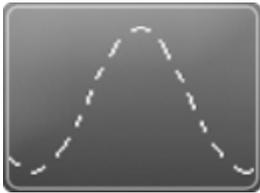
This mode is the same as Standard Line, except that the user is prompted to select a turn around point where the software automatically changes *Line Follow Direction* and *Line Follow Side* when the turn around point is encountered. This mode is most useful for digitizing cyclic graphs such as cyclic voltamograms. The automatic digitizing process can be paused at any point by pressing the **Enter** key or selecting the Pause button.



Bar Graph/Mass Spec

(Automatic Line Follow w/ Bar Graph Spacing)

This mode is the same as Standard Line, except that upon completion of the digitizing process for the curve/bar graph, the data can be converted to bar graph type values. This mode allows the data to be converted to discrete, evenly spaced-bar type values by finding the nearest X (and associated Y) value for each step along the X-axis. This mode is most useful for bar graphs such as mass specs. The automatic digitizing process can be paused at any point by pressing the **Enter** key or selecting the Pause button.



Dashed/Dotted Line

(Automatic Line Follow with Interactive Line Limits)

This mode is similar to Standard Line, except the user will select a limited vertical search region that will be guided along the curve with the mouse. While the mouse button is depressed, the crosshair will automatically follow along the data line closest to the current position of the mouse cursor, and will not search beyond the vertical limits shown above and below the mouse cursor. Additionally, the crosshair will not proceed horizontally beyond the current position of the mouse cursor. This process allows broken lines to be digitized without the crosshair jumping to another curve. This mode is most useful for graphs that contain one or more curves that are not continuous (such as dashed line, dotted lines, etc.). Extraneous points, marks, and labels in the digitizing region where the line is not continuous may present a

problem and should be cleaned up during the scanning process. The digitizing process can be paused at any point by simply releasing the mouse button during digitizing.



Contour Plot

(Automatic Line Follow w/ Interactive Line Limits and Auto Turn Around)

This mode is the same as Dashed/Dotted Line, except when the crosshair encounters a location where no data line is present; the software automatically changes Line Follow Direction and Line Follow Side to follow the contour of the curve. This mode is most useful for digitizing non single-valued curves such as contour plots. The digitizing process can be paused at any point by simply releasing the mouse button during digitizing..



Shapes/Drawings

(Automatic Raster Scan w/ All Points Accepted as Data)

In this mode, UN-SCAN-IT gel searches an entire user-selected rectangular area in both the X and Y directions, and records the position of all of the data points in the specified region. This mode is most useful for digitizing shapes, figures, and drawings. Extraneous points, marks and labels in the digitizing region present a problem, and should be cleaned up during the scanning process. The data from the raster scan method are not vectorized and the data array is not in sequential order. This raster scan digitizing mode can fill memory very quickly as large numbers of pixels are digitized. The automatic digitizing process can be paused at any point by pressing the **Enter** key or selecting the Pause button.



Symbols/Scatter Plot

(Auto Raster Scan w/ Contiguous Data Points Converted to Single Point)

This mode is the same as Shapes/Drawings, except that this mode searches the entire rectangular region specifically for groups of contiguous pixels, and records only the position of the center of each pixel group as a data point. This mode is most useful for images that contain scattered non-connected data points or symbols (e.g. circles, squares, etc). The digitized data is non-vectorized. Although this digitizing mode can be useful for scatter plots, it may be quite slow for some images. A faster alternative to this mode may be the Symbols option in Manual mode. The automatic digitizing process can be

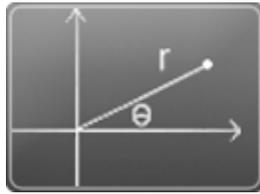
paused at any point by pressing the **Enter** key or selecting the Pause button.



(x,y) Digitizing Tablet

(Manual Mode for Standard (x,y) Coordinates)

In this mode, the user moves the cursor to each individual point on the graph that they wish to digitize, and presses the **Space Bar** (or right mouse button) to store each point. If the Automatic X Increments feature is selected, the crosshair automatically increments one resolution unit in the X direction after each point is digitized. In addition, a symbol mode option is available where simply clicking near a symbol/data point will cause the software to do a small raster search, and find the center of the data point. This mode is most useful for digitizing scatter plots, graphs, pictures, or drawings that cannot easily be digitized by any of the automatic digitizing methods.



Polar Digitizing Tablet

(Manual Mode for Polar Coordinates)

This mode is the same as (x,y) Digitizing Tablet, except that this mode is for digitizing graphs that have been recorded using a polar (r,theta) coordinate system. The user moves the cursor to each individual point on the graph that they wish to digitize, and presses the **Space Bar** (or right mouse button) to store each point. In addition, a symbol mode option is available where simply

clicking near a symbol/data point will cause the software to do a small raster search, and find the center of the data point. This mode is useful for digitizing any graph that contains polar (r,theta) coordinates. All other UN-SCAN-IT gel digitizing modes are for digitizing standard (x,y) coordinates.

6.5 Graph Digitizing Options

The Graph Digitizing Options allow you to select from the available UN-SCAN-IT gel digitizing options for the Graph Digitizing Mode you have selected. Only the options that are associated with the Graph Digitizing Mode selected will be displayed.

Main Image View – *graph digitizing options*

Maintain Aspect Ratio: This method maintains the original image aspect ratio in the main image viewing area. Although the image may not fill the entire viewing area, the image can be zoomed (1x, 2x, 4x, 8x, and 100%) to increase or adjust the size of the image within the viewing area. The zoom feature allows a closer inspection of the positioning of the setup icons, and also provides a more detailed view of the positions of the digitized data points. Note: Automatic Digitizing may be slower while image is zoomed.

Stretch to Fit: This method stretches the image to fill the entire main viewing window, making full use of the entire image viewing area. Although this method may cause some images to appear distorted, the actual digitized data values will not be affected by the stretching.

Color Type – *graph digitizing options*

Although UN-SCAN-IT gel automatically determines the default Color Type for the current image, this option can be used to modify the color type selection.

Black and White (Line Art): This type of image contains only black and white (no grayscale or color) pixels. This type of image requires the least amount of memory, and is often the easiest to digitize.

Grayscale: This type of image contains only grayscale pixels. The grayscale intensity range can be selected to limit the range of pixel intensities accepted as data, and ignore pixels outside that range.

Color: This type of image contains colored pixels. This type of image allows only pixels of a defined color to be digitized, and thus can differentiate between data lines (or points) based on color.

Digitize All Except Background: This color type digitizes all pixels, except for pixels of a defined background value. This can be useful in ignoring background and digitizing all other pixel values.

Scale – *graph digitizing options*

This option allows you to select the scale on which your original graph was drawn.

Linear: This standard type of graph contains linear scaling in both the X and Y directions. This is the most common scale for graphs.

Semi-Log (X-Linear): This type of graph contains linear scaling in the X direction, and logarithmic scaling in the Y direction.

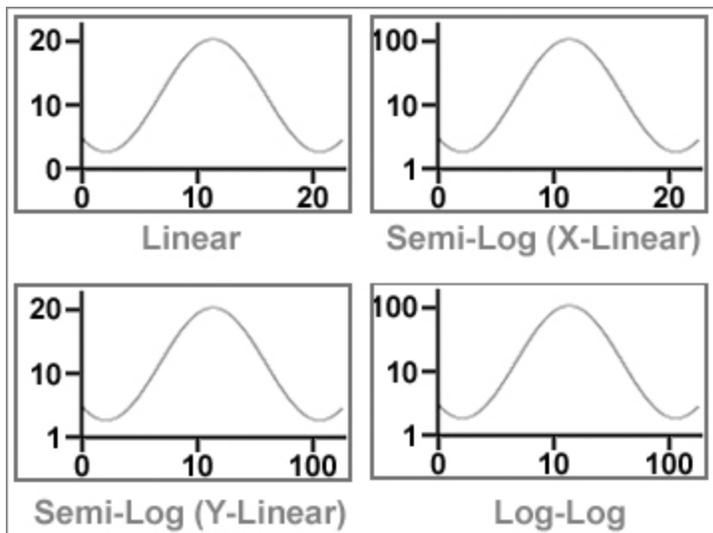
Semi-Log (Y-Linear): This type of graph contains logarithmic scaling in the X direction, and linear scaling in the Y direction.

Log-Log: This type of graph contains logarithmic scaling in both the X and Y directions.

Other Scales: Non-linear and Non-log formats must be input logically.

Example: Jan 1 to Dec 31 should be entered as Day 1 to Day 365

Example: 10:00am to 2:30pm should be entered as Hour 10.0 to Hour 14.5



Grid Line Filter – *graph digitizing options*

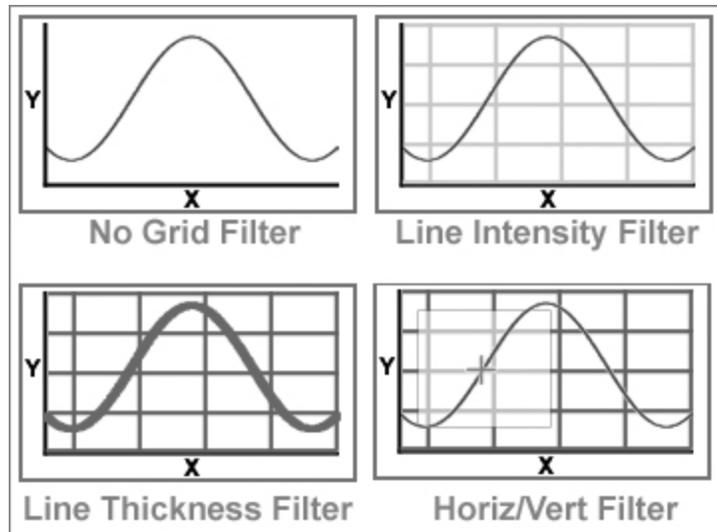
This option is used to filter out the background grid lines on the image so UN-SCAN-IT gel can follow the data line more easily.

None: This method provides no grid line filtering.

Line Intensity (Grayscale): This method provides grid line filtering based on the grayscale intensities of the data line being greater than the intensities of surrounding grid lines (requires grayscale image).

Line Thickness: This method provides grid line filtering based on the line thickness of the data line being greater than the line thickness of surrounding grid lines.

Horiz/Vert Filter: This method provides grid line filtering by ignoring horizontal and vertical lines within an adjustable search range.



Line Side | Point Assignment – *graph digitizing options*

This option allows you to select the method that UN-SCAN-IT gel will use to follow the data curve and assign the exact location in (x,y) coordinates for each point. During the digitizing process, the UN-SCAN-IT gel software will draw a colored dot at each digitized data point location, and this dot can be used to gauge the accuracy of that particular point assignment method for your particular graph type.

Upper Surface of Line: This method follows the top surface of the data line, and then subtracts half the general flat-line thickness to estimate the middle of the data line... best for upward peaks.

Advantage: Very precise and accurate for graphs with upward peaks, and graphs with relatively flat curves.

Disadvantage: Does not correct for changing line thickness on sharp peaks, resulting in a maximum possible error on sharp peaks of up to one line thickness in any direction, and may have some problem properly

following the bottom surface of sharp downward peaks.

Lower Surface of Line: This method follows the bottom surface of the data line, and then adds half the general flat-line thickness to estimate the middle of the data line... best for downward peaks.

Advantage: Very precise and accurate for graphs with downward peaks, and graphs with relatively flat curves.

Disadvantage: Does not correct for changing line thickness on sharp peaks, resulting in a maximum possible error on sharp peaks of up to one line thickness in any direction, and may have some problem properly following the top surface of sharp upward peaks.

Variable Surface of Line: This method follows the top surface when the data line is above a threshold, and the bottom surface when the data line is below a threshold... best for up and down peaks.

Advantage: Very precise and accurate for graphs with both upward and downward peaks.

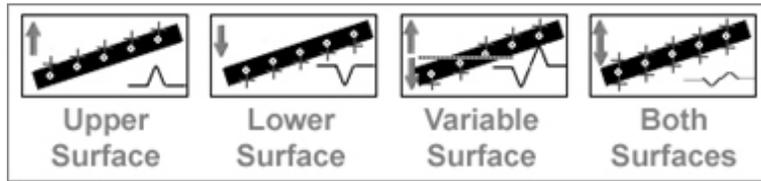
Disadvantage: Does not correct for changing line thickness on sharp peaks, resulting in a maximum possible error on sharp peaks of up to one line thickness in any direction.

Both Surfaces (Mid-Line): This method follows both the top and bottom surfaces, and digitizes the average value... due to overlapping line thickness, this may greatly reduce peak heights.

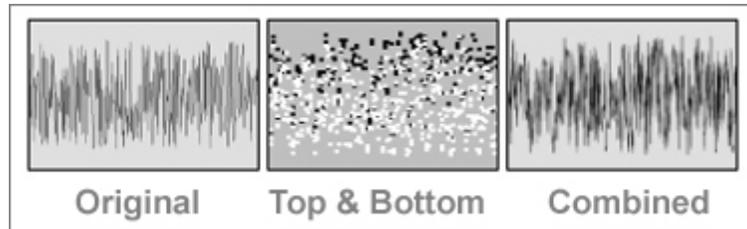
Advantage: Very precise and accurate for estimating the center of the data line in regions where sharp peaks are not present, as this method corrects for changing line thickness throughout the graph.

Disadvantage: May cause peak heights to be significantly reduced for very sharp peaks, due to the lines becoming extremely thick at the top and

bottom of sharp peaks.



If the data are extremely noisy, some of the data was lost when the noisy regions were originally printed. Although this data cannot be recovered, the noise can be approximated by digitizing both the top surface and the bottom surface of the data line. These combined data sets can then be sorted in the **Graph** portion of the UN-SCAN-IT gel software to best approximate the original data.

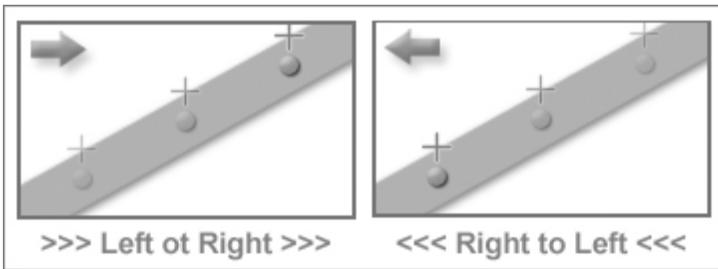


Line Follow Direction – *graph digitizing options*

The Line Follow Direction option allows you to choose the direction in which the crosshair will follow along the data line.

>>> Left to Right >>>: This method digitizes the data points from left to right across the image. The user defined ending point must be to the right of the user defined beginning point.

<<< Right to Left <<<: This method digitizes the data points from right to left across the image. The user defined ending point must be to the left of the user defined beginning point.

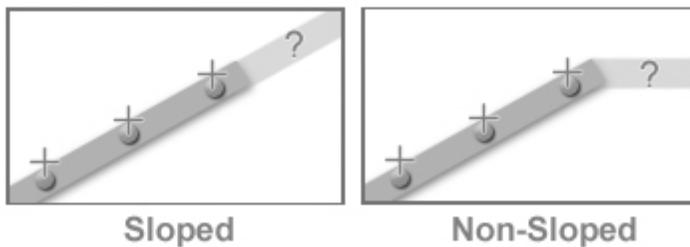


Line Follow Algorithm – *graph digitizing options*

This option is to estimate the location UN-SCAN-IT gel will use to begin searching for the next sequential point along the curve.

Sloped: This method estimates the location of the next point along the data curve based upon the slope of the previous points, resulting in faster line digitizing.

Non-Sloped: This method simply moves horizontally to estimate the location of the next point along the data curve, resulting in slower and more controlled line digitizing.



Digitizing Speed – *graph digitizing options*

This option is set the digitizing speed used during the automatic digitizing process.



Fast: The faster digitizing speeds provide identical results to the slower speeds, but may present difficulty in allowing user interaction (pausing, backing up, erasing data points, manual digitizing, etc.) due to the rapid speed.

Slow: The slower digitizing speeds provide identical results to the faster speeds, and allow easier user interaction (pausing, backing up, erasing data points, manual digitizing, etc.) due to the slower speed.

Turbo: This mode does not display the crosshair movement, digitized data points, or the data spreadsheet as the points are being digitized. This mode should only be used when maximum digitizing speed is required and no user interaction will occur.

Multi-Line Method – *graph digitizing options*

This option determines the method used by UN-SCAN-IT gel digitize graphs with intersecting lines.

Manual Pause Bars: This method allows the user to place Pause Bar icons at the line intersection points. The UN-SCAN-IT gel software will pause when it encounters these icons.

Overlap Search With Auto Pause: This method causes the UN-SCAN-IT gel software to pause automatically when an overlapping line is encountered.

Overlap Search With Auto Continue: This method causes the UN-SCAN-IT gel software to estimate a probable continuance point, and skip to that point, when an overlapping line is encountered.

Cyclic Line Follow Method | Contour Line Start – *graph digitizing options*

This option determines the starting Line Follow Direction and Line Follow Side when digitizing cyclic graphs or contour graphs.

Follow Upper Surface >>> Left to Right >>> then Reverse: This mode starts at the leftmost point's upper surface, reverses at the rightmost point, and finishes back at the leftmost point.

Follow Upper Surface <<< Right to Left <<< then Reverse: This mode starts at the rightmost point's upper surface, reverses at the leftmost point, and finishes back at the rightmost point.

Follow Lower Surface >>> Left to Right >>> then Reverse: This mode starts at the leftmost point's lower surface, reverses at the rightmost point, and finishes back at the leftmost point.

Follow Lower Surface <<< Right to Left <<< then Reverse: This mode starts at the rightmost point lower surface, reverses at the leftmost point, and finishes back at the rightmost point.

Cyclic Reversal Method – *graph digitizing options*

This option determines the method used for reversing at the turn around point when digitizing cyclic graphs.

Auto Reversal at Turn Around Point: This method automatically reverses the Line Follow Side and Direction when the turn around point is encountered, and then continues automatically.

Pause at Turn Around Point: This method automatically reverses the Line Follow Side and Direction when the turn around point is encountered, and then pauses.

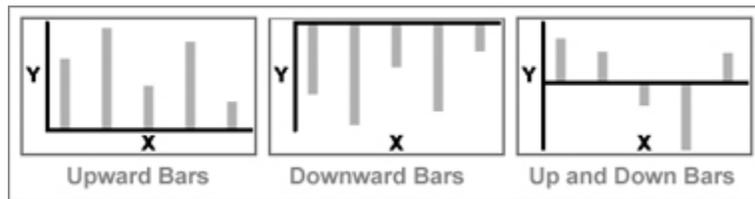
Bar Graph Type – *graph digitizing options*

This option allows you to select the type of bar graph to be digitized. UN-SCAN-IT gel only digitizes bar graphs that contain vertical bars.

Upward Bars: This method follows the top surface of the bars in a vertical bar graph.

Downward Bars: This method follows the bottom surface of the bars in a vertical bar graph.

Up and Down Bars: This method follows the top surface of the bars when the bar value is above a user defined threshold, and the bottom surface when the bar value is below the user defined threshold.



Dashed/Dotted Line Follow Method – *graph digitizing options*

This option allows you to the select method used to follow a dashed/dotted line, and to select the vertical search window size used for the digitizing process.

Semi-Automatic - Interactive Vertical Limits: This method requires the user to guide the digitizing cursor and associated vertical limits along the data line as the data points are being automatically digitized. The Vertical Search Window setting determines the vertical limits of the search window in which data can be digitized.

Automatic - Auto Estimating Vertical Limits: This method causes the software to automatically move the digitizing cursor and associated vertical limits as the data points are being automatically digitized. The Vertical Search Window setting determines the vertical limits of the search window in which data can be digitized.

Contour Line Follow Method – *graph digitizing options*

This option allows you to select method used to follow a contour line, and to select the vertical search window size used for the digitizing process.

Semi-Automatic - Interactive Vertical Limits: This method requires the user to guide the digitizing cursor and associated vertical limits along the data line as the data points are being automatically digitized. The Vertical Search Window setting determines the vertical limits of the search window in which data can be digitized.

Auto Estimating Vertical Limits with Auto Pause: This method automatically moves the cursor and vertical limits while automatically digitizing. Encountering a data gap pauses the software. The Vertical Search Window setting determines the vertical limits of the search window in which data can be digitized.

Raster Scan Direction – *graph digitizing options*

This option determines the starting point and scan direction used in raster scan digitizing.

Lower Left Corner to Upper Right Corner: This method begins digitizing at the lower left corner of the raster scan region, and digitizes

points as the crosshair moves upward and then to the right through each column of pixels in the digitizing region.

Upper Left Corner to Lower Right Corner: This method begins digitizing at the upper left corner of the scan region, and digitizes points as the crosshair moves downward and then to the right through each column of pixels in the digitizing region.

Lower Right Corner to Upper Left Corner: This method begins digitizing at the lower right corner of the scan region, and digitizes points as the crosshair moves upward and then to the left through each column of pixels in the digitizing region.

Upper Right Corner to Lower Left Corner: This method begins digitizing at the upper right corner of the scan region, and digitizes points as the crosshair moves downward and then to the left through each column of pixels in the digitizing region.

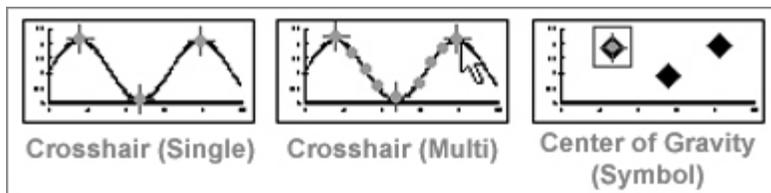
Manual Point Assignment Method – *graph digitizing options*

This option determines the method used to digitize points when using UN-SCAN-IT gel in Manual Mode (Digitizing Tablet Mode).

Crosshair Position (Single Point Click): This method digitizes one single (x,y) point at the current crosshair position each time the space bar (or right mouse button) is pressed.

Crosshair Position (Multi-Point Drag): This method digitizes multiple (x,y) points as the right mouse button is pressed and the mouse cursor is dragged.

Center of Gravity (Symbol): Pressing the space bar (or right mouse button) digitizes one single (x,y) point which represents the average data pixel location within a raster scan box. The Search Window setting determines the size of the raster search window box to be used for extracting the Center of Gravity data value.



Automatic X Increments (Manual Mode) – *graph digitizing options*

This option determines whether the manual digitizing crosshair will automatically increment in the X direction after each point is digitized in Manual Mode (Digitizing Tablet Mode).

On: This method increments the manual digitizing crosshair one resolution unit in the current direction after each point is manually digitized.

Off: This method causes the manual digitizing crosshair to remain at the current location after each point is manually digitized.

Angle Units – *graph digitizing options*

This option determines the whether a polar graph will be digitized using degree units or radians.

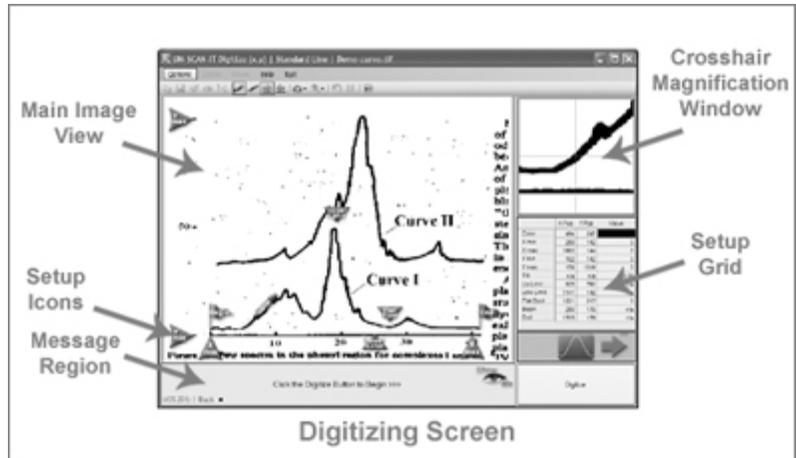
Degrees (0-360): This selection will digitize the chart in standard degrees with a required range of 0 to 360 degrees.

Radians (0-2pi): This selection will digitize the chart in radians with a required range of 0 to 2 pi radians.

6.6 Digitizing Screen

After the setup options have been selected, the main digitizing screen will appear. The main digitizing screen displays the entire image, either

maintaining the aspect ratio or stretched to fit within the resizable main window (depending on the Main Image View selected). A crosshair magnification window in the upper right corner displays a full scanner resolution view of the region surrounding the digitizing crosshair (use this window for fine adjustment of the crosshair position in order to maximize accuracy during the setup procedure). A grid on the right side of the digitizing screen displays the Setup Icon positions and values. A text box at the bottom of the screen provides instructions that guide the user through the digitizing setup process.



The main menu that appears at the top of the digitizing screen contains the following menus:

- Options** Use this menu to adjust digitizing resolutions, change line following direction, change speeds, save the digitized data, etc. These options will be discussed later in this chapter.
- Delete** Select **Delete** to sequentially delete the most recently digitized point(s).
- Pause** The **Pause** menu is only visible during the automatic

digitizing process. The automatic digitizing process can be paused at any point by selecting **Pause** menu option, or by selecting the **Pause** button. When automatic digitizing is paused, the software will temporarily switch to Manual Mode, allowing the graphics crosshair to be repositioned, points added, points deleted, digitizing options changed, etc.

Help Select **Help** to display help topics relating to the setup, options, or general digitizing process. In addition, the customer support contact information is available from the **Help** menu.

Exit Select **Exit** to exit the digitizing process and go to the Main Menu, or to exit the digitizing process and go to the Graphing Screen (only available after points have been digitized).

Options – *digitizing screen*

By selecting the **Options** menu, many of the default and previously selected digitizing options can be modified, and other additional functions performed. The options available will depend on the Graph Digitizing Mode selected earlier in the digitizing process. The **Options** menu may include many of the following options:

Point Assign Method Select this option to change the point assignment method used during automatic digitizing. Details regarding the different point assignment methods can be found in the Graph Digitizing Options portion of this manual.

Manual Point Assign Use this option to change the method used for determining the digitized point location in manual mode. Details regarding the different manual point assignment methods can be found in the Graph Digitizing Options portion of this manual.

- Bar Graph Type** Use this option to change the bar graph type setting used for digitizing. The bar graph type only applies to vertical bar graphs (horizontal bar graphs cannot be digitized with these methods). Details regarding the different bar graph type options can be found in the Graph Digitizing Options portion of this manual.
- Line Follow Side** Use this option to change the current line follow side used for automatic digitizing. Details regarding the different line follow side options can be found in the Graph Digitizing Options portion of this manual.
- Line Follow Direction** Use this option to change the current line follow direction used for digitizing. Details regarding the different line follow direction options can be found in the Graph Digitizing Options portion of this manual.
- Automatic X Increment** Use this option to change the current automatic x increments setting. Using automatic x increments can be useful when manually digitizing a continuous curve. Details regarding the different automatic x increment options can be found in the Graph Digitizing Options portion of this manual.
- Change Side and Dir** Use this option to reverse both the line follow side and line follow direction simultaneously. Details regarding the different line side and line direction options can be found in the Graph Digitizing Options portion of this manual.
- Clear Pause Bars** Use this option to clear the positions of all the current pause bars used for digitizing intersecting curves.

- Set Digitizing Speed** Use this option to change the current digitizing speed used for automatic digitizing. Slowing the digitizing speed can be useful for allowing easier user interaction with the digitizing process. Details regarding the different digitizing speed options can be found in the Graph Digitizing Options portion of this manual.
- Set Main Image View** Use this option to change the current main image view to either Maintain Aspect Ratio or Stretch to Fit. This option can also be used to set the zoom level and increase the size of the image within the viewing area. Note: Automatic Digitizing may be slower while image is zoomed.
- Set Digitizing Resolution** Use this option to change the current digitizing resolution used by the software. The default resolution is 1 scanner unit. The resolution can be set anywhere from 1 to 10 scanner units. The resolution will directly affect the number of data points digitized (e.g. a 10 inch long curve scanned at 300 dpi will produce 3000 data points when digitized at a resolution of 1 scanner unit). Generally the most useful method for adjusting resolution is to digitize the image using a resolution setting of 1. Then later use the **Adjust X Data Spacing** option in the main graphing screen to fit a cubic spline curve to the data and adjust the x spacing.
- Set Eraser/Shifter Size** Use this option to change the size of the UN-SCAN-IT gel digitized data eraser, and the vertical data shifter. The eraser can be useful for deleting stray points that were erroneously digitized during the automatic digitizing process. The vertical data shifter can be useful for adjusting the vertical position of selected digitized data points.

Set Vert Search Window Use this option to change the size of the vertical search window used by the software for following dashed/dotted graphs or contour plots. This setting determines the vertical limits beyond which the software will not search for data. Properly setting this limit will allow the software to remain on the curve of interest without jumping to another curve or skipping data.

Set Sym Search Window Use this option to change the size of the symbol search window used by the software in manual mode (symbols). This setting determines the square size of the raster region in which the software will average the data pixels to determine a single data value.

Set Data Line Color Use this option to change/set the current pixel color that the software accepts as data. This option is useful for selecting a particular colored data line to digitize among multiple colored curves.

Set Screen Colors Use this option to change the current screen colors and graphic options used by the software. The crosshair color, digitized point color, and digitized point size can be adjusted. Setting these display options can be useful in making the digitizing crosshair and data points more visible on the digitizing screen.

Relocate Setup Icons Use this option to reposition any of the setup icons to new locations. This feature can be useful for redefining the data line color, setting new axis values, etc.

Slide All Axis Setup Icons Use this option to move all the current setup icons and their corresponding location settings in the desired direction. This feature can be especially

useful when used in conjunction with the Save/Load Axis Setup Parameters option. By using these options, multiple graphs with similar axis parameters can be easily digitized by simply saving and loading the axis setup parameters, and then sliding the axis setup icons to account for shifts that may have occurred during the scanning process.

- Current Settings & Info** Use this option to display the current digitizing and setup options that have been selected, as well as some general information regarding the current image.
- Copy Screen Image** Use this option to Copy the current digitizing Screen Image to the Clipboard. The screen image can then be Pasted into another program (word processor, graphics, etc.).
- Adjust X Data Spacing** The digitizing process converts scanner pixel units into user defined scaled units. The resulting X data spacing may appear arbitrary as a result of this conversion. However, the X data spacing can be adjusted by fitting the digitized data points using a cubic spline or linear fit. To Adjust X Data Spacing, go to the Graphing Screen.
- Save Current (x,y) Data** Use this option to save the current digitized (x,y) data to a standard data file (or the Macintosh clipboard). The saved data file can then be read into other programs, such as spreadsheet, data analysis, or graphics software.
- Clear Current (x,y) Data** Use this option to clear the current digitized (x,y) data from memory. This option is useful for clearing data that has been saved, and then digitizing additional data on the same image.

Clearing data can also be useful for deleting erroneous data before returning to the digitizing process.

Save Axis Setup Param Use this option to save the current axis setup parameters (Xmin, Xmax, Begin, End, etc.). This option can be very useful for saving and retrieving the setup icon positions and values in order to digitize the current image or another similar image at a later time, without having to re-define all of the setup icon positions and values.

Load Axis Setup Param Use this option to load previously saved axis setup parameters (Xmin, Xmax, Begin, End, etc.). This option can be very useful for retrieving previously saved setup icon positions and values in order to re-digitize a previous image or digitize another similar image, without having to re-define all of the setup icon positions and values.

6.7 Positioning the Setup Icons

This section will assist you in positioning the setup icons at their appropriate locations on the graphical image. All of the required Setup Icons must be dragged to their corresponding graphical locations before digitizing can begin. The pending required Setup Icons are indicated with a gray arrow beside the icon, and can be found along the bottom edge of the main digitizing window. The accuracy of the digitized data will depend directly upon the precision with which the setup icons are located on the image.

Icon and Crosshair Movement – *positioning the setup icons*

The setup icons can be dragged to their proper locations by clicking and holding the left mouse button while dragging the setup icon to the desired

location (the hot spot on the setup icon will be shown with a digitizing crosshair). Fine icon movements can be achieved by pressing the keyboard arrow (or Ctrl+arrow) keys while the left mouse button remains depressed on the setup icon. Use the crosshair magnification in the upper right corner of the digitizing screen for precise alignment.

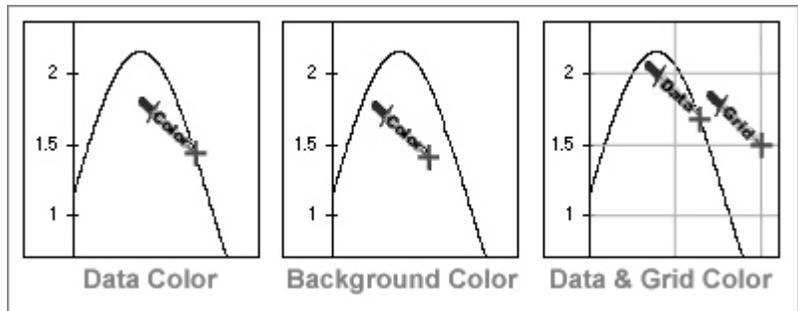
Data Color – positioning the setup icons

If **Black and White, Grayscale, or Color** was the color type selected, then move the Color Icon so that it is exactly over the data line you wish to digitize. Use the crosshair magnification window in the upper right corner of the digitizing screen for precise alignment. The color selected will be displayed in the grid at the right side of the screen. This step identifies the data line color that UN-SCAN-IT gel will accept as data.

If **Digitize All Colors Except Background** was the color type selected, then move the Color Icon so that it is exactly over the background color that UN-SCAN-IT gel will ignore and not accept as data. Use the crosshair magnification window in the upper right corner of the digitizing screen for precise alignment. The background color selected will be displayed in the grid at the right side of the screen. This step identifies the background color that UN-SCAN-IT gel will ignore and not accept as data.

Grid Line Color - If the Line Intensity (Grayscale) grid line filter has been selected, the Grid Line Color must be also identified. Move the Grid Icon so that it is exactly over a major grid line. Use the crosshair magnification in the upper right corner of the digitizing screen for precise alignment. The grid line color selected will be displayed in the grid at the right side of the screen. This identifies the grid line intensity.

Note: Incorrectly identifying the data (or background) color will result in errors during the digitizing process.



Locating Axis Scaling Points – *positioning the setup icons*

Cartesian (x,y) Coordinates

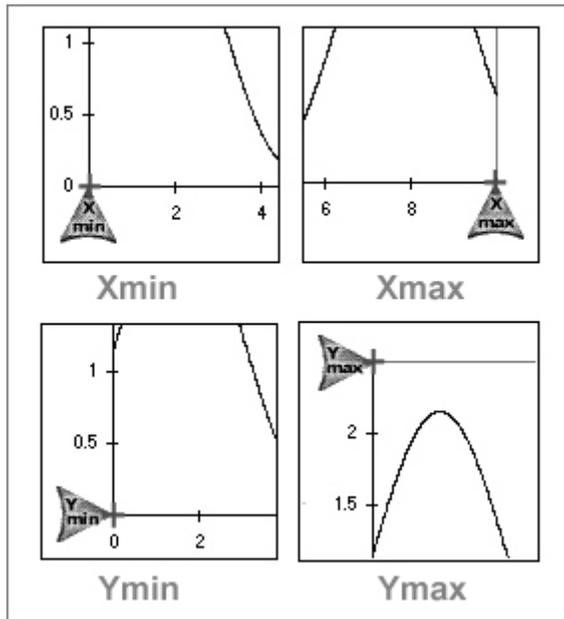
Xmin: Move the X min Icon so that it is exactly over the lowest (leftmost) X axis scaling mark. The X min Icon should be located on a scaling mark where the X value is known (not on the data line where the X value is not known). Use the crosshair magnification in the upper right corner of the digitizing screen for precise alignment. Incorrectly identifying the scaling marks will result in errors in the digitized data. The X min Icon should generally be placed over the center of the scaling mark for best precision and accuracy.

Xmax: Move the X max Icon so that it is exactly over the highest (rightmost) X axis scaling mark. The X max Icon should be located on a scaling mark where the X value is known (not on the data line where the X value is not known). Use the crosshair magnification in the upper right corner of the digitizing screen for precise alignment. Incorrectly identifying the scaling marks will result in errors in the digitized data. The X max Icon should generally be placed over the center of the scaling mark for best precision and accuracy.

Ymin: Move the Y min Icon so that it is exactly over the lowest (bottom) Y axis scaling mark. The Y min Icon should be located on a scaling mark where the Y value is known (not on the data line where the Y value is not known). Use the crosshair magnification in the upper right corner of the

digitizing screen for precise alignment. Incorrectly identifying the scaling marks will result in errors in the digitized data. The Y min Icon should generally be placed over the center of the scaling mark for best precision and accuracy.

Ymax: Move the Y max Icon so that it is exactly over the highest (top) Y axis scaling mark. The Y max Icon should be located on a scaling mark where the Y value is known (not on the data line where the Y value is not known). Use the crosshair magnification in the upper right corner of the digitizing screen for precise alignment. Incorrectly identifying the scaling marks will result in errors in the digitized data. The Y max Icon should generally be placed over the center of the scaling mark for best precision and accuracy."

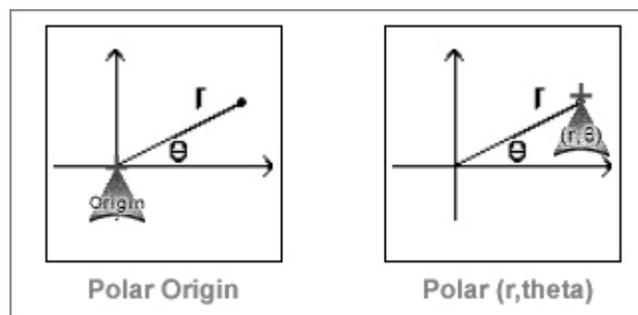


Note: It is important to be consistent in locating the positions of the tick marks (e.g. always use the center of the tick mark when defining the axes points). Careful attention to defining the axes scaling points is critical for obtaining accurate digitizing results.

Polar (r,theta) Coordinates

Origin: Move the Origin Icon so that it is exactly over the origin ($r=0$, $\theta=0$) of the polar coordinate system. Use the crosshair magnification in the upper right corner of the digitizing screen for precise alignment. Incorrectly identifying the scaling marks will result in errors in the digitized data.

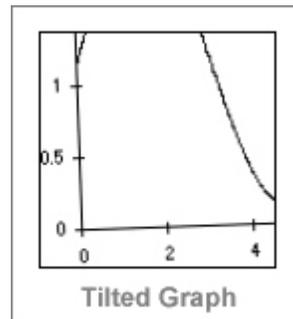
(r, theta): Move the (r, theta) Icon so that it is exactly over an axis point or a data point where the (r, theta) value is known. Use the crosshair magnification in the upper right corner of the digitizing screen for precise alignment. Incorrectly identifying the scaling marks will result in errors in the digitized data. Scaling points with horizontal and vertical components of the (r, theta) location will be required for proper scaling.



Numerical Values - After defining each axis location, UN-SCAN-IT gel will prompt you to enter the numerical value corresponding to that particular scaling mark.



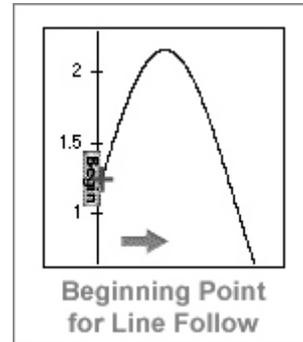
Tilt Correction: Based upon the difference in the vertical pixel locations and the difference in the horizontal pixel locations at the Xmin and Xmax setup icons, UN-SCAN-IT gel will determine if the graph is tilted. If the graph is tilted, UN-SCAN-IT gel will determine the tilt angle and allow the user to choose to correct the data values. Although the software will correct the digitized data for graphs that are slightly tilted, graphs that are tilted more than 5 degrees may cause errors in the digitized data, and should be scanned again (or rotated to the proper alignment using your scanner or image processing software). Although UN-SCAN-IT gel will correct for tilted graphs, graphs that have no tilt or a very small tilt will product the most accurate and symmetric digitized data. The axis lines must be orthogonal (i.e. at right angles to each other) to obtain accurate results.



Locating Beginning Point – *positioning the setup icons*

Automatic Line Follow Modes

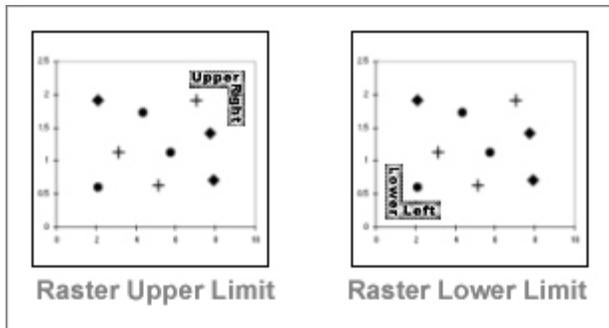
Begin: Move the Begin Icon to the location where you wish the digitizing process to begin. If the current Line Follow Direction is >>> Left to Right >>>, the Begin Icon should be located at the leftmost point to digitize. If the current Line Follow Direction is <<< Right to Left <<<, the Begin Icon should be located at rightmost point to digitize.



Raster Scan Modes

Raster Upper Limit: Move the Upper Right Icon to the upper right corner of the area you wish to raster scan. The Upper Right Icon should be vertically located slightly ABOVE the highest point to be raster scanned, and horizontally located slightly to the RIGHT of the rightmost point to be raster scanned.

Raster Lower Limit: Move the Lower Left Icon to the lower left corner of the area you wish to raster scan. The Lower Left Icon should be vertically located slightly BELOW the lowest point to be raster scanned, and horizontally located slightly to the LEFT of the leftmost point to be raster scanned.



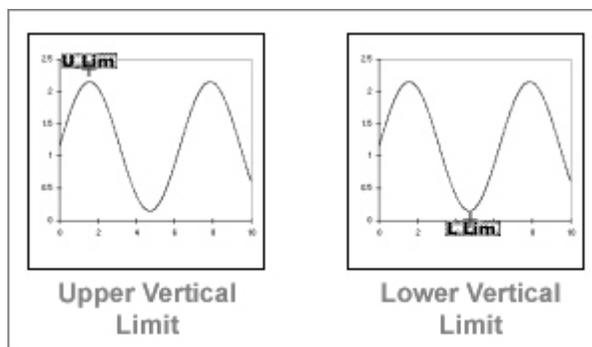
Defining Vertical Limits – positioning the setup icons

Defining the vertical limits prevents the system from accepting or searching for data outside the specified vertical digitizing region. This feature speeds the digitizing process and reduces the possibility of finding extraneous data or following axes lines.

Automatic Line Follow Modes

Upper Vertical Limit: Move the Upper Limit Icon to a vertical location slightly ABOVE the highest vertical point on the data curve. This step sets the upper vertical limit beyond which UN-SCAN-IT gel will not search. Setting the Upper Limit Icon too high may allow the software to erroneously follow another data line or the upper axis line. Setting the Upper Limit Icon too low will cause the software to erroneously ignore the data line above that limit.

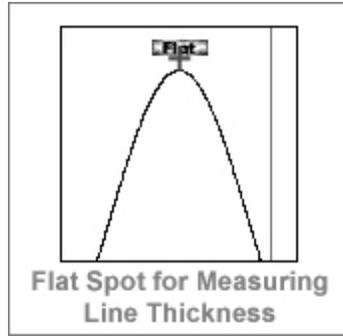
Lower Vertical Limit: Move the Lower Limit Icon to a vertical location slightly BELOW the lowest vertical point on the data curve. This step sets the lower vertical limit beyond which UN-SCAN-IT gel will not search. Setting the Lower Limit Icon too low may allow the software to erroneously follow another data line or the lower axis line. Setting the Lower Limit Icon too high will cause the software to erroneously ignore the data line below that limit.



Flat Spot / Line Thickness – *positioning the setup icons*

Automatic Line Follow Modes

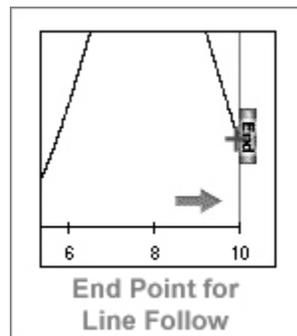
Flat Spot: Move the Flat Spot Icon to a vertical location slightly ABOVE a relatively flat portion of the data line. This step is used to measure the flat line thickness of the data curve, in order to best estimate the location of the middle of the data line during digitizing. The Flat Spot Icon should be located above the data line that matches the selected Data Line Color. Locating the Flat Spot Icon over a steep portion of the data line will cause the measured line thickness to be too large and will result in a vertical shift error in the digitized data. If there is no flat portion of the data line available, the Flat Spot icon can be placed above a flat portion of any horizontal line of similar thickness and color as the data line. To disable or re-enable the line thickness correction, press the Esc key while positioning the Flat Spot Icon.



Locating Ending Point – *positioning the setup icons*

Automatic Line Follow Modes

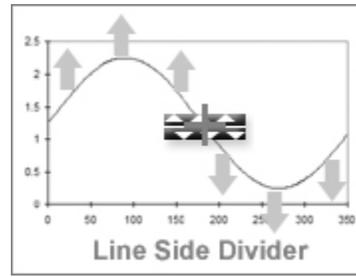
End: Move the End Icon to the location where you wish the digitizing process to end. If the current Line Follow Direction is >>> Left to Right >>>, the End Icon should be located at the rightmost point to digitize. If the current Line Follow Direction is <<< Right to Left <<<, the End Icon should be located on leftmost point to digitize.



Variable Surface – positioning the setup icons

Automatic Line Follow Modes

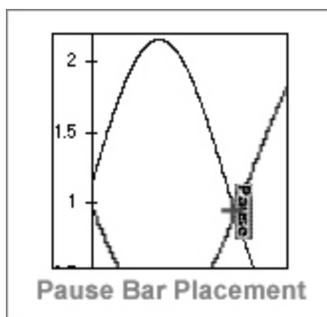
Variable Surface: If Variable Surface of Line was selected, then position the Line Side Divider Icon (red and blue icon with arrows) where the software should automatically switch Line Follow Sides. The software will follow the Upper Surface of the line when it is above this icon, and the Lower Surface of the line when it is below this icon.



Pause Bars – positioning the setup icons

Intersecting Line Follow Mode

Pause Bars: If Manual Pause Bars was selected, then move the Pause Icon so that it is exactly over the point on the data line where the software should pause during the automatic line following process. A maximum of 10 pause bars can be inserted. Automatic digitizing can also be paused (and then resumed) at any time by selecting the Pause button during automatic digitizing.



6.8 Digitizing Modes

The process in which the image is digitized depends upon which Graph Digitizing Mode was selected during the digitizing setup process.

Automatic Line Follow Modes - *digitizing modes*

After all of the setup icons have been positioned and digitizing has begun, a graphics crosshair will appear on the image. As the crosshair follows the data line, the current number of data points digitized (**N:**), the x value of the most recent digitized point (**X:**), and the y value of the most recent digitized point (**Y:**) will be displayed at the bottom of the screen. These values will also be stored and displayed in the data spreadsheet at the right side of the digitizing screen.

The crosshair will automatically follow along the data line being digitized, and will move in the current line follow direction as each digitized data point is assigned. The digitized data points will appear graphically as colored dots along the data line, and these dots can be used to gauge the accuracy of the digitized data. The color and size of the dots can be changed in the **Set Screen Colors** portion of the **Options** menu.

The digitizing process can be paused at any point by selecting the **Pause** button (or by pressing **Enter**). The software will temporarily switch to Manual Mode. In Manual Mode the graphics crosshair to be repositioned, points added, points deleted, digitizing options changed, etc. Select **Continue**

to resume automatic line follow digitizing.

If the crosshair is moving too quickly along the data line, the digitizing process can be slowed by selecting **Set Digitizing Speed** in the **Options** menu

The digitizing process can be discontinued at any time by selecting **Exit**.

Upon completion of the digitizing process, a window will appear that will prompt you regarding the digitized data.

Accept the Data Points and Return to Digitizing Screen: Choose this option if the digitized data points appeared acceptable and you wish to save, view, erase, shift, or digitize additional data points on the graph.

Accept the Data Points and Go To Graphing Screen: Choose this option if the digitized data points appeared acceptable and you wish to go directly to the Graphing Screen where you can edit, sort, smooth, integrate areas, etc. (see the Graphing / Modifying (x,y) Data chapter for details).

Do not Accept the Data Points and Return to Digitizing Screen: Choose this option if the digitized data points are not acceptable, and you wish to return to the Digitizing Screen and adjust the setup icons, change digitizing options, or exit the digitizing process.

Raster Scan Modes - *digitizing modes*

After all of the setup icons have been positioned and digitizing has begun, a graphics crosshair will appear on the image. As the crosshair moves through the region being raster scanned, the current number of data points digitized (**N:**), the x value of the most recent digitized point (**X:**), and the y value of the most recent digitized point (**Y:**) will be displayed at the bottom of the screen. These values will also be stored and displayed in the data spreadsheet at the right side of the digitizing screen.

The digitized data points will appear graphically as colored dots overlapping each data point. The color and size of the dots can be changed in the **Set Screen Colors** portion of the **Options** menu.

The digitizing process can be paused at any point by selecting **Pause** (or by pressing **Enter**). The software will temporarily switch to Manual Mode. In Manual Mode the graphics crosshair to be repositioned, points added, points deleted, digitizing options changed, etc. Select **Continue** to resume raster scan digitizing.

If the crosshair is moving too quickly throughout the data, the digitizing process can be slowed by selecting **Set Digitizing Speed** in the **Options** menu

The digitizing process can be discontinued at any time by selecting **Exit**.

Upon completion of the digitizing process, a window will appear that will prompt you regarding the digitized data.

Accept the Data Points and Return to Digitizing Screen: Choose this option if the digitized data points appeared acceptable and you wish to save, view, erase, shift, or digitize additional data points on the graph.

Accept the Data Points and Go To Graphing Screen: Choose this option if the digitized data points appeared acceptable and you wish to go to the Graphing Screen where you can edit, sort, smooth, integrate areas, etc. (see the Graphing / Modifying (x,y) Data chapter for details).

Do not Accept the Data Points and Return to Digitizing Screen: Choose this option if the digitized data points are not acceptable, and you wish to return to the Digitizing Screen and adjust the setup icons, change digitizing options, or exit the digitizing process.

Manual Modes - *digitizing modes*

After all of the setup icons have been positioned and digitizing has begun, a graphics crosshair will appear on the image. The current number of data points digitized (**N:**), the current x position of the graphics crosshair (**X:**), and the current y position of the graphics crosshair (**Y:**) will be displayed at the bottom of the screen (if digitizing in polar coordinates, the r and theta coordinates will be displayed). These values will also be stored and displayed

in the data spreadsheet at the right side of the digitizing screen.

The crosshair can be positioned using the left mouse button, the keyboard arrow keys for fine movement, or the **Ctrl** + arrow keys for coarse movements. As each point is digitized, a colored dot will appear at that position. The color and size of the dots can be changed in the **Set Screen Colors** portion of the **Options** menu.

The digitizing process can be discontinued at any time by selecting **Exit**.

Crosshair Position Mode - Press the **Space Bar** (or right mouse button) to digitize the point currently located at the graphics crosshair. Use the crosshair magnification window in upper right corner of the screen for precise crosshair alignment. If **Automatic X Increments** were selected in the **Options** menu, the graphics crosshair will increment automatically along the x-axis after each point is digitized. To delete the most recently digitized point(s) select **Delete**.

Center of Gravity (Symbol) Mode - Press the **Space Bar** (or right mouse button) to digitize the point or symbol within the raster scan region of the crosshair (this raster region is indicated by a box around the center of the crosshair). Use the crosshair magnification window in upper right corner of the screen for precise crosshair alignment. The software will raster scan the search region, find the average location of the digitized pixels in that region, and assign a single digitized value based upon that average. To delete the most recently digitized point(s) select **Delete**.

6.9 Erasing Digitized Data Points

Stray marks, other data lines, axes lines, symbols, etc. may occasionally be incorrectly identified as data points during the digitizing process. A data eraser can be activated by selecting the **Erase Data** button near the bottom of the digitizing screen. Position the data eraser box over the data points you wish to delete, and press the left mouse button to delete the underlying data points from memory and the digitizing screen. The data eraser size can be modified by selecting **Set Data Eraser Size** in the **Options** menu. Press **Esc** to exit the data erasing mode, and return to the digitizing mode.

6.10 Shifting Data Vertically

It may be useful to adjust the vertical positions of selected digitized data points after they have been digitized. The Vertical Data Shifter can be activated by selecting the data shifter button from the digitizing toolbar. Position the Shift Data box over the points to shift. Pressing the left mouse button will shift the points and their values down. Pressing the right mouse button will shift the points and their values up. The data shifter size can be modified by selecting **Set Data Shifter Size** in the **Options** menu. Press Esc to exit the shift data mode, and return to the digitizing mode.

6.11 Adjusting the X Spacing of the Data

When digitizing images the inherent image resolution is usually sufficient to produce excellent digitizing results. However, you may wish to increase, decrease or modify the X Data Spacing between the data points to a user specified value (this will allow multiple curves to have the same x-spacing and be graphed simultaneously using other graphing software). This feature can also the user to define a set number of digitized data points with user defined spacing. To modify the x spacing of the digitized data, you may wish to use the **Adjust X Data Spacing** feature available in UN-SCAN-IT gel. To access this feature, go to the Graphing Screen. For details regarding the use of the **Adjust X Data Spacing** feature, refer to the Graphing / Modifying (x,y) Data chapter.

6.12 Exporting the Digitized Data to Other Software

The primary purpose of the UN-SCAN-IT gel software is to extract the underlying (x,y) values from graphical images. Once the digitizing process is complete, the extracted data can be exported to other software by either saving the (x,y) data as an ASCII Text data file, or copying the (x,y) data directly to the Macintosh Clipboard and pasting the data into the desired software. These **File** options are discussed later in this manual.

Chapter 7

GRAPHING / MODIFYING (X,Y) DATA

7.1 Overview

This chapter will guide you through the different **Graphing** options present in the UN-SCAN-IT gel software. The graphing options allow you to graphically view, edit, and analyze the digitized data. Additionally, the (x,y) data can be exported to other graphing or spreadsheet software for analysis.

Many of the UN-SCAN-IT gel graphing menu options can also be accessed from the toolbar located below the graphing menus. The Help menus may be used to provide helpful information regarding the graphing options and buttons.

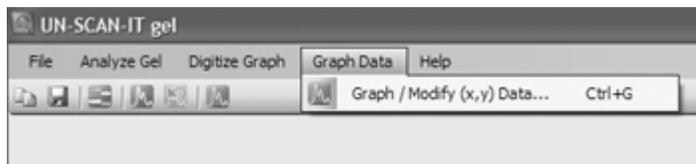
7.2 Main Menu

To enter the **Graph** portion of the UN-SCAN-IT gel program from the main menu select:

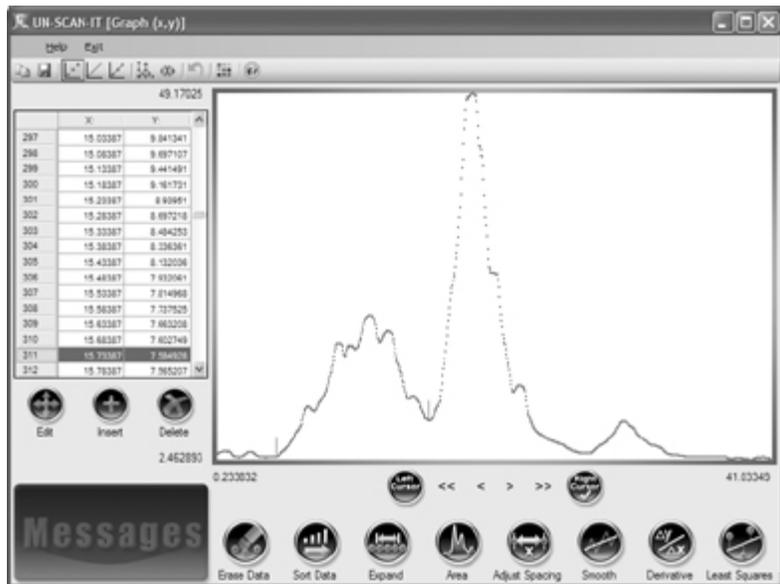
Graph Data

Graph / Modify (x,y) Data - main menu

Select **Graph / Modify (x,y) Data...** to graphically view and modify the (x,y) digitized data.



Graph Data Menu



Graphing Screen

7.3 Cursor Movement

The UN-SCAN-IT gel Graphics Screen is equipped with two interactive cursors (small vertical lines). These left and right cursors can be used for data identification, modification, and analysis.

The Left Cursor or Right Cursor can be activated by clicking the buttons at the bottom of the graph screen. The active cursor will blink, and have a checkmark displayed in the corresponding button at the bottom center of the graph.

The active cursor can be moved by clicking the mouse at the desired horizontal position on the graph, clicking the arrow buttons at the bottom of the graph, using the keyboard arrow keys (**Ctrl** + arrow keys for larger movements), or by selecting the desired data point in the spreadsheet on the

left side of the screen.

The x and y values of the data point at the active cursor are highlighted in the spreadsheet on the left side of the screen.

If you have any difficulty using or moving the cursors, you may wish to click the **Sort** button to sort the data into ascending or descending array order. The Left Cursor and Right Cursors cannot cross each other (in array order), so data arrays that are not sorted may prevent proper movement of the graphic cursors.

7.4 Graph Options

After the graph is displayed on screen, an **Options** menu will be available that contains useful graphical options and features:

- Undo Delete** Select **Undo Most Recent Point Delete** to restore the most recent data point that has been deleted.
- Draw Points** Select **Draw Points** to display the (x,y) data points as dots on the screen. The data point size can be set using the Set Graph Colors option.
- Draw Lines** Select **Draw Lines** to connect the (x,y) data points with straight lines. If the data are not sorted, the lines may appear to be connected in a haphazard manner, and you may wish to sort the data. The data line size can be set using the Set Graph Colors option.
- Points & Lines** Select **Draw Points and Lines** to display the (xy) data points as dots connected with straight lines. If the data are not sorted, the lines may appear to be connected in a haphazard manner, and you may wish to sort the data. The data point and line size can be set using the Set Graph Colors option.

- X Direction** Select **X Direction... Low to High** to scale the graph with the lowest x values on the left and highest x values on the right. Select **X Direction... High to Low** to scale the graph with the highest x values on the left and lowest x values on the right.
- Y Direction** Select **Y Direction... Low to High** to scale the graph with the lowest y values on the bottom and highest y values on the top. Select **Y Direction... High to Low** to scale the graph with the highest y values on the bottom and lowest y values on the top.
- Set Scale** Select **Set Scale...** to manually choose the x and y scales used to display the graph. Full Scale is defined such that all of the data points will appear on the graph. Linear, log, and semi-log scales can be selected.
- X Data Spacing** Select **Adjust X Data Spacing...** to adjust the x data spacing by fitting the data with either a cubic spline or linear fit. A window will appear with several options. Enter the data range to be adjusted and the desired x increments. Xmin cannot be less than the lowest x data point, and Xmax cannot be greater than the highest x data point. The X Increment is the desired spacing (in user units) between adjusted x points. The New # of Points represents the number of data points after the adjustment is performed. You may select a Cubic Spline fit or Linear fit for the digitized data points. The original and adjusted data will be displayed on screen, and you will be prompted to replace the original data with the adjusted data or retain the original data. This feature is useful for aligning x spacing in order to graph multiple curves on the same scale using other graphing software or to set x data points to exact user specified values. This feature can also be used allow to user to define a set number of digitized data points.
- Graph Colors** Select **Set Graph Colors...** to change the background color, data point color, data line color, cursor color,

highlight point color, highlight line color, data point size, and data line size.

- Set Eraser Size** Select **Set Data Eraser and Data Shifter Size...** to change the size of the graphical data eraser . This eraser can be useful for deleting stray points that were obtained during the automatic digitizing process.
- Edit/Ins Mode** Select **Edit/Insert Mode... Keyboard** to use the keyboard to edit or insert data points on the graph when the **Edit** or **Insert** button has been selected. Select **Edit/Insert Mode... Mouse** to use the mouse for point and click editing and insertion of data points when the **Edit** or **Insert** button has been selected.
- Temporary File** Select **Temporary File... Add Current Data Point** to add the data point at the active cursor (or Area value if area is highlighted) to the temporary file. Select **Temporary File... Clear Temporary File** to erase all the data values in the temporary file. Select **Temporary File... Copy Temporary Data to Clipboard** to copy all the data in the temporary file to the clipboard and allow the data to be pasted into another program (spreadsheet, word processor, etc.). These options are useful for extracting just a few selected data points or area values from the graph.
- Screen Image** Select **Copy Screen Image to Clipboard...** to Copy the current digitizing Screen Image to the Clipboard. The screen image can then be Pasted into another program (word processor, graphics, etc.).
- Save (x,y) Data** Select **Save Data...** to save the (x,y) data to a file. The current (x,y) data can be saved to a standard data file, or copied to the Macintosh clipboard. The data can then be read into other programs, such as spreadsheet, data analysis, or graphics software.
- Load (x,y) Data** Select **Load (x,y) Data...** to load a previously digitized

(x,y) data file, or to load a standard (x,y) ASCII Text data file. The standard Macintosh dialog box will appear. Loading a file clears all digitized data presently in memory. The software can load data saved by UN-SCAN-IT gel; or standard comma or tab delimited (x,y) ASCII Text data saved by other programs.

7.5 Graph Buttons

After the graph is displayed on screen, a series of graph buttons will be displayed below the data spreadsheet and at the bottom of the graphing screen

Edit Point - graph buttons

The data point at the active cursor can be edited by selecting the **Edit** button. In Keyboard Edit Mode, the current x and y values at the active cursor will be displayed in the edit box. Edit these values to change the value of the selected point. In Mouse Edit Mode, simply click on the graph where you wish to locate the edited point.

Insert Point - graph buttons

A data point can be added to the data file and displayed on the screen by selecting the **Insert** button. In Keyboard Insert Mode, the current x and y values at the active cursor will be displayed in the insert box. Enter the x and y values of the new data point. In Mouse Insert Mode, simply click on the graph where you wish to locate the new point.

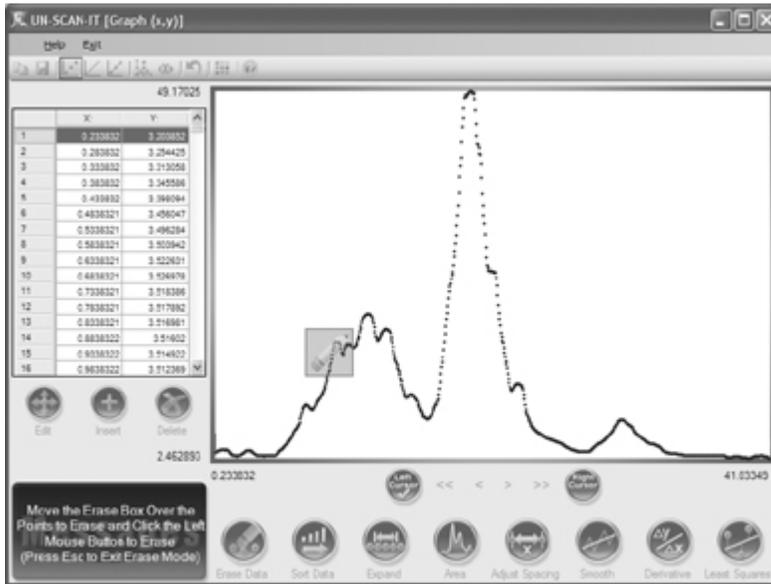
Delete Point - graph buttons

The data point at the active cursor can be deleted from the data file and erased from the graphing screen by selecting the **Delete** button. Select **Undo Most Recent Point Delete** from the **Options** menu to restore the most recently deleted data point.

Erase Data - graph buttons

The data eraser can be activated by selecting the **Erase Data** button. Position the data eraser box over the data points you wish to delete, and press the left mouse button to delete the underlying data points from memory and the graphing screen. The data eraser size can be modified by selecting **Set Data**

Eraser Size in the **Options** menu. Press **Esc** to exit the data erasing mode, and return to the regular graphing mode.



Data Eraser Box

Sort Data - graph buttons

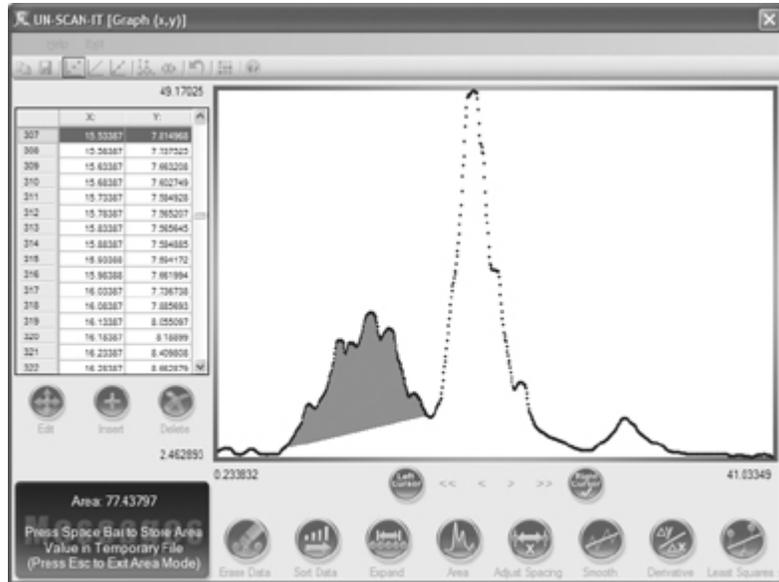
The entire (x,y) data array can be sorted into ascending or descending x order by selecting the **Sort** button. This option should be used when data have been added to the digitized file or some of the data points are not in increasing (or decreasing) x array order. Some of the cursor and graphing options may not work properly if the data are not properly sorted.

Expand - graph buttons

The region between the cursors can be expanded to fill the graphing screen by selecting the **Expand** button. To return to full scale, right click the **Expand** button (or use the **Set Scale** feature in the **Options** menu).

Area - graph buttons

The area under the curve between the graphics cursors can be integrated by selecting the **Area** button. The UN-SCAN-IT gel software uses the trapezoidal rule to determine the area. The area (x units multiplied by y units) will be displayed in the upper right message box. The background selection for the area calculation is a straight line between the cursors, and can be modified by Inserting or Deleting data points, and locating the cursor on the new data point to calculate a new background. Press **Esc** to exit the area mode, and return to the regular graphing mode.



Area Integrated Between Cursors

Adjust X Data Spacing - graph buttons

The x data spacing of the data points can be adjusted by selecting the **Adjust Spacing** button. A window will appear with several options. Enter the data range to be adjusted and the desired x increments. Xmin cannot be less than the lowest x data point, and Xmax cannot be greater than the highest x data point. The X Increment is the desired spacing (in user units) between adjusted

x points. The New # of Points represents the number of data points after the adjustment is performed. You may select a Cubic Spline fit or Linear fit for the digitized data points. The original and adjusted data will be displayed on screen, and you will be prompted to replace the original data with the adjusted data or retain the original data.

This feature is useful for aligning x spacing in order to graph multiple curves on the same scale using other graphing software or to set x data points to exact user specified values. This feature can also allow the user to define a set number of digitized data points

Smooth - graph buttons

The entire (x,y) data set can be smoothed by selecting the **Smooth** button. The program will sort the data and then prompt you to select the number of points in the smoothing function, and the type of smooth to be performed. The n-point smooth is a standard boxcar averaging smooth, while the weighted smooth statistically prioritizes each data point in order to best retain the original peak height and shape. The original and smoothed data will be displayed on screen, and you will be prompted to replace the original data with the smoothed data., or retain the original data.

Derivative - graph buttons

A derivative of the entire (x,y) data set can be obtained by selecting the **Derivative** button. The program will sort the data and then prompt you to select the number of points in the smoothed derivative operation, and to select the derivative type. The standard 1st derivative (dy/dx) is a standard derivative (with no smoothing), while the weighted derivative options statistically weights and smoothes the derivative data as it is processed. The original and derivative data will be displayed on screen, and you will be prompted to replace the original data with the derivative data, or retain the original data.

Least Squares - graph buttons

A least squares polynomial can be fit to the data by selecting the **Least Squares** button. You will be prompted to enter the order of fit for the least

squares equation.

Select **Average Value** to obtain an average Y value for the data.

($y = a$)

Select **1st Order Polynomial** to obtain a linear line fit of the data.

($y = a + bx$)

Select **2nd Order Polynomial** to obtain a second order fit of the data.

($y = a + bx + cx^2$)

The original data and least squares fit will be displayed on screen, and the least squares constants and standard deviation will be displayed in the message box.

7.6 Help

Select **Help** to display help topics relating to the graphing options and buttons. In addition, the customer support contact information is available from the **Help** menu

7.7 Exit

Select **Exit Graph** to exit the graphing screen and return to the Main Menu, or to exit the graphing screen and return directly to the Digitizing Screen (only available if the graphing screen was accessed from the digitizing screen).

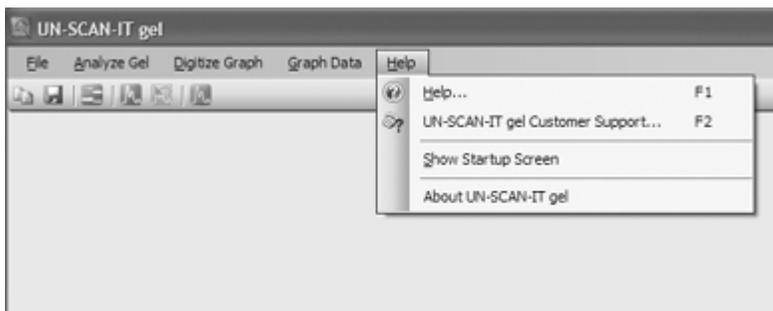
Chapter 8

UN-SCAN-IT gel HELP

8.1 Help Menu

To enter the **Help** portion of the UN-SCAN-IT gel program from the main menu select:

Help



Help Menu

8.2 Help

Context sensitive help is available throughout the digitizing and graphing screens. If you have further questions or need assistance, please contact customer support.

8.3 Customer Support

Silk Scientific, Inc.
1060 Oakmont Lane Provo, Utah 84604 USA
Tel: 1-801-377-6978 Fax: 1-801-228-2448
E-Mail: info@silkscientific.com

8.4 Show Startup Screen

Selecting this menu option will display the Startup Screen, and allow you to directly choose to Analyze a Gel, Digitize a Graph, or go to Main Menu whenever the program starts.

8.5 About UN-SCAN-IT gel

Selecting this menu option will display the current UN-SCAN-IT gel version number, Serial Number, and license holder.

Macintosh Note: The About UN-SCAN-IT gel menu is located in the UN-SCAN-IT gel main application menu.

Appendix A

SCANNING AND MODIFYING THE IMAGE

Overview

The UN-SCAN-IT gel software imports standard image files from scanners, digital cameras, the internet, etc. These images should be obtained and modified using the hardware and software provided with these devices, or by using third party imaging software. The final image should be 'right side up' with the lanes in the vertical direction (gels), or the x-axis in the horizontal direction (graphs). The image should be saved in a standard image format (tif, jpg, png, bmp, gif, etc.).

Appendix B

TROUBLESHOOTING FAQs (GEL ANALYSIS)

Overview

This appendix provides some possible solutions to questions that may be encountered while using the UN-SCAN-IT gel software to analyze gels. Please do not struggle with any technical issues that you may have regarding the use of this software, as we can often answer your question and solve your problem. Please note that the customer support information listed below can also be displayed from within the software by accessing the main **Help** menu.

Customer Support:

Silk Scientific
1060 Oakmont Lane Provo, Utah 84604 USA
Tel: 1-801-377-6978 Fax: 1-801-228-2448
E-Mail: info@silkscientific.com

Question: Is there a way to get the image to load and digitize faster?

Answer(s):

- Try scanning and saving the image at a lower dpi (dots per inch) resolution from within your scanning software.
- If you are loading the image file from a disk, copy the file to the hard drive, and load the image file from the hard drive.
- Move the software onto a faster computer with more memory.

Question: What could prevent UN-SCAN-IT gel from reading the image?

Answer(s):

- Make sure you are loading the image file from the **Analyze Gel** menu and not the **File/Load** menu.
- Make sure the file was saved in a standard scanner format accepted by UN-SCAN-IT gel (e.g. .jpg, .tif, .bmp, etc.).
- Try reading the image file on a different computer to make sure that it is not a hardware problem unique to your computer.

Question: Why does the image appear stretched or distorted in the Main Window?

Answer:

- If the Stretch to Fit option was selected, the entire image is scaled to fit within the main analysis window. Any stretching of the image is to allow the entire image to fit in the within the main window (this apparent stretching will not affect the analyzed data). If you would like to reduce the stretching in the main window, you can either resize the UN-SCAN-IT gel main window to dimensions more similar to the original image, or select the Maintain Aspect Ratio option to preserve the original image width to height ratio when displaying the image in the main digitizing window.

Question: Why does the gel image appear very small in the Crosshair Magnification Window?

Answer:

- The crosshair Magnification Window displays any portion (or all) of the image at full scanner resolution. If this image is small, it was probably scanned at a low resolution, and should be scanned at a higher resolution.

Question: What if UN-SCAN-IT gel gives inaccurate results?

Answer(s):

- Make sure the gel was scanned straight.
- Minimize the number of stray marks on the gel.
- Adjust the contrast setting on the scanner to give a more representative image.

Note: There are inherent uncertainties regarding the conversion of any image into data. UN-SCAN-IT gel and all gel analysis software simply make the best approximation for each image by converting the grayscale pixel values into meaningful quantitative data. The experience and expertise of the scientist is a crucial factor in obtaining accurate results from any gel analysis software package.

Question: What if the gel to be analyzed is on film or transparency?

Answer(s):

- Use a scanner that is designed for scanning transparencies or obtain a transparency adapter for your scanner. Scanning in transparency mode will allow light to transmit through the film, rather than simply being reflected.
- If a transparency scanner is not available, place a clean white piece of paper behind the film so the reflected light from the scanner can most closely approximate the original.

Question: What if the original gel is only visible under fluorescent light?

Answer:

- Take a photo of the fluorescent gel, and scan the film or photo (or use a digital camera to obtain the image).

Appendix C

TROUBLESHOOTING FAQs (GRAPH DIGITIZING)

Overview

This appendix provides some possible solutions to questions that may be encountered while using the UN-SCAN-IT gel software to digitize (x,y) graphs. Please do not struggle with any technical issues that you may have regarding the use of this software, as we can often answer your question and solve your problems based upon our experience. Please note that the customer support information listed below can also be displayed from within the software by accessing the main **Help** menu.

Customer Support:

Silk Scientific
1060 Oakmont Lane Provo, Utah 84604 USA
Tel: 1-801-377-6978 Fax: 1-801-228-2448
E-Mail: info@silkscientific.com

Question: Is there a way to get the image to load and digitize faster?

Answer(s):

- Try scanning and saving the image at a lower dpi (dots per inch) resolution from within your scanning software.
- If you are loading the image file from a disk, copy the file to the hard drive, and load the image file from the hard drive.
- Move the software onto a faster computer with more memory.

Question: What could prevent UN-SCAN-IT gel from reading the image?

Answer(s):

- Make sure you are loading the image file from the **Digitize** menu and not the **File/Load** menu.
- Make sure the file was saved in a standard scanner format accepted by UN-SCAN-IT gel (e.g. .jpg, .tif, .bmp, etc.).
- Try reading the image file on a different computer to make sure that it is not a hardware problem unique to your computer.

Question: Why does the image appear stretched or distorted in the Main Window?

Answer:

- If the Stretch to Fit option was selected, the entire image is scaled to fit within the main digitizing window. Any stretching of the image is to allow the entire image to fit in the within the main window (this apparent stretching will not affect the digitized data). If you would like to reduce the stretching in the main window, you can either resize the UN-SCAN-IT gel main window to dimensions more similar to the original image, or select the Maintain Aspect Ratio option to preserve the original image width to height ratio when displaying the image in the main digitizing window.

Question: What if the grid line is digitized rather than the data line?

Answer(s):

- During the scanning process try adjusting the contrast control on your scanner to ignore the grid lines but pick up the data line.
- Use one of the grid line filters available within the digitizing setup menu.
- Make a photocopy of the original graph, using the sensitivity adjustment of the photocopy machine to reduce the intensity of the grid lines.
- Erase the grid lines on one side of the data line with your scanning

software or other computer paintbrush program, and re-save the image. Then use the Surface of Line point assignment to follow only the surface of the data line.

- Digitize the graph in Manual Mode so that you can guide the crosshair along the data line, and ignore the grid lines.

Question: Why would UN-SCAN-IT gel not digitize data points near the axes lines?

Answer(s):

- Ensure that the vertical limits were properly and accurately defined at the beginning of the digitizing process. The defined values should extend beyond the lowest and highest data points being digitized.
- Make sure the line trace does not approach too closely to the axes, and if it does, define the axes slightly further away from the line trace.
- If there is a significant tilt to the graph that would result in the axes lines not being parallel to the sides of the image, re-scan the image and adjust the axes so that they are parallel to the sides of the paper.

Question: What if UN-SCAN-IT gel follows the horizontal axis line?

Answer(s):

- During the setup process, define the vertical limits slightly above the lower horizontal axis line and slightly below the upper horizontal axis line.
- If there is a significant tilt to the graph that would result in the axes lines not being parallel to the sides of the image, re-scan the image and adjust the axes so that they are parallel to the sides of the paper.

Question: What if UN-SCAN-IT gel gives inaccurate results?

Answer(s):

- Carefully and accurately define the axes locations and values during the setup process.
- Confirm that the original graph is on a continuous linear or logarithmic scale and accurately plotted.
- Ensure that there are no stray marks near or crossing the data line being digitized.

Note: There are inherent uncertainties regarding the conversion of any graphic image into data. UN-SCAN-IT gel (and all (x,y) digitizing software) simply makes the best approximation for each image by converting the pixel locations into meaningful quantitative data. The experience and expertise of the scientist is a crucial factor in obtaining accurate results from any digitizing software package.

Question: What if UN-SCAN-IT gel reduces peak heights when digitizing?

Answer(s)

- If the graph has sharp peaks, do not use the Both Surfaces (Mid-Line) point assignment. Surface of Line point assignment is far superior for graphs that contain sharp peaks.
- If the graph has upward peaks, then make sure you selected Upper Surface of Line (and conversely for downward peaks).

Question: Can the graph be digitized if the original is on paper too large to fit the scanner?

Answer(s):

- Photo-reduce the original to a size that can be easily scanned.
- Scan only the portion of the paper that contains the graphical image.
- Scan the image in pieces, and later append the digitized data points. It is crucial that each piece scanned contain proper scaling marks if the data is to be properly digitized and appended.

Question: How can additional data points be digitized when the data memory is full?

Answer(s):

- Save the data that have already been digitized using the **Save Data** feature from the **File** menu. Then clear the data from memory using the **Clear Data** feature from the **File** menu. Resume digitizing by selecting **Return to Current Image** option from the **Digitize** menu.
- Collect the digitized data at a lower resolution.
- Scan the original graph at a lower dpi resolution.

Question: What can be done if UN-SCAN-IT gel gets lost?

Answer(s):

- Re-define the single pixel Data Line Color that you wish to digitize.
- Erase grid lines or other stray marks that may cross the data line.
- If the software is skipping to other data lines or marks, ensure that the data line of interest is a continuous curve.

Appendix D

NEW VERSION 6.3 FEATURES (GEL ANALYSIS)

Overview

This appendix provides a brief overview of some of the new gel analysis features of UN-SCAN-IT gel Version 6.3. Most of these features are described in further detail in the body of this manual.

Scalable Main Image View

The main image view can be set to maintain the aspect ratio of the original image. This eliminates the undesirable stretching that sometimes occurred when the original image is stretched to fit the digitizing screen.

Zoomable Main Image View

When the main image view is set to maintain aspect ratio, the image view zoom level can be selected as 1x, 2x, 4x, 8x and 100% values. This allows a closer inspection of the positioning of the lane or segment boxes. Note: Analysis may be slower while the image is zoomed.

Distance Measuring Tool

This new tool may be used to measure distance on the image in the horizontal (x), vertical (y), and combined (z) directions. Simply click the mouse at the point where you wish to begin the measurement, and drag the mouse cursor to any point on the image. The data can be displayed in pixels, inches, or centimeters.

Gaussian Peak Resolving

The software may miss peaks, find extra peaks, or not resolve overlapping peaks. The Resolve Peaks feature can be used to overlay Gaussian peaks on the density profile to estimate where the baseline should be set if the peaks were represented by a Normal distribution. The three Gaussian peaks in the upper left corner of the density profile can be used to overlay the peaks you wish to fit. You can use one, two, or all three of the Gaussian peaks to fit single, double, or triple peaks.

New Context Sensitive Help Buttons and Tooltips

Context sensitive help buttons are now located adjacent to many of the setup and analysis options. In addition, descriptive tooltips are displayed when the mouse moves over the controls and icons.

Turbo Mode

A new digitizing speed setting (Turbo Mode) is available that allows the image to be digitized extremely quickly. Turbo mode does not display the gel digitizing process as the image is being digitized. This option can be used when maximum digitizing speed is required.

Additional Image Formats and Larger Image Support

The software supports additional gel image formats, and can load images larger than the previous 32000 x 32000 limit.

New Peak Finding Options

Additional options for user defined automatic peak finding settings are available in the density profile screen.

Band Normalization Tool

The Pixel Total values for all segments can be Normalized to a user defined segment by clicking the Normalize button above the results grid.

Enhanced (x,y) Graph Digitizing Features

UN-SCAN-IT gel also contains enhanced features for digitizing (x,y) graphical images. The features are described in the appendix that follows.

Appendix E

NEW VERSION 6.3 FEATURES (GRAPH DIGITIZING)

Overview

This appendix provides a brief overview of some of the new features of UN-SCAN-IT gel Version 6.3. Most of these features are described in further detail in the body of this manual.

Scalable Main Image View

The main image view can be set to maintain the aspect ratio of the original image. This eliminates the undesirable stretching that sometimes occurred when the original image is stretched to fit the digitizing screen.

Zoomable Main Image View

When the main image view is set to maintain aspect ratio, the image view zoom level can be selected as 1x, 2x, 4x, 8x and 100% values. This allows a closer inspection of the positioning of the setup icons, and also provides a more detailed view of the positions of the digitized data points. Note: Automatic Digitizing may be slower while the image is zoomed.

Integrated Digitizing and Graphing Screens

It is now possible to go directly from the digitizing screen to the graphing screen, and also to return directly from the graphing screen to the digitizing screen. This feature allows the addition, deletion, modification, and display of the digitized data values between the digitizing and graphing screens to be virtually seamless.

Toolbar Shortcuts

Many of the most common menu selections from the UN-SCAN-IT gel menus can now be accessed more easily using the toolbars located below the menus. Some of the toolbar options include shortcuts to the digitizing screen, graphing screen, pause button, speed settings, zoom settings, help menus, line types, and data copying and saving features.

Integrated Digitizing and Graphing Spreadsheets

Both the digitizing screen and the graphing screen contain spreadsheets that display the (x,y) data values in real time. These spreadsheets can be useful in tracking and modifying the (x,y) data values during the digitizing and graphing process.

Turbo Mode

A new digitizing speed setting (Turbo Mode) is available that allows data points to be digitized extremely quickly (30,000+ points per second). Turbo mode does not display the crosshair movement, digitized data points, or the data spreadsheet as the points are being digitized. This mode is useful when maximum digitizing speed is required and no user interaction will occur.

Increased Number of Data Points

The maximum number of digitized (x,y) data points has been increased to 100,000 data points per file.

Vertical Data Shifter

A graphical data shifter tool has been integrated in the digitizing screen to allow selected digitized data points to be shifted up or down to better fit the graphical image.

Additional Image Formats and Larger Image Support

The software supports additional graphic image formats, and can load images larger than the previous 32000 x 32000 limit.

New Context Sensitive Help Buttons and Tooltips

Context sensitive help buttons are now located adjacent to many of the setup and digitizing options. In addition, descriptive tooltips are displayed when the mouse moves over the controls and icons.

Larger Setup Icons

The digitizing setup icons are larger and clearer to improve user interaction during the setup process.

Customizable Data Eraser

The size of both the data eraser and data shifter can now be customized to improve precision.

Improved Graphing Features

The graphing screen now includes new line types, improved X data spacing adjustment, additional screen colors, and smooth point sizes.

Glossary

1-bit Image - This type of image contains only a single pixel color, and is used to represent line drawings. This format is also referred to as **Line Art** or **Black & White**.

8-bit Grayscale Image - This type of image contains 8 bits per pixel, and represents each pixel with one of 256 shades of gray. This format is also referred to as **Black & White Photo**.

24-bit Color Image - This type of image contains 24 bits per pixel, and represents each pixel with one of 16,777,216 colors.

ASCII (text) - ASCII format is a universal data format used by most computers for data and text exchange. UN-SCAN-IT gel can export data in both tab and comma delimited ASCII (text) format.

Background - The background for most gels is the average pixel intensity for a region where no bands are present. The non-zero background can present a problem when quantifying segments, as pixel sums become dependent upon the size and shape of the segment box. This background interference can be reduced or eliminated by using one of the UN-SCAN-IT gel background subtraction methods or the the *Calibrate Image Intensity* feature during the gel setup procedure.

Black & White Image - This type of image contains only a single pixel color, and is used to represent line drawings. This format is also referred to as **1-bit** or **Line Art**.

Clipboard - Both Windows and Macintosh computers have a temporary location in memory where data or graphics can be placed. This Clipboard region can be used to transfer data between programs. UN-SCAN-IT gel can export data to the computer's **Clipboard**, and the data can be pasted into other applications.

Colored Line - Most scanners and image input devices represent colored lines by a mixture of pixels of varying colors. UN-SCAN-IT gel uses a color approximation algorithm to digitize colored data lines and points.

Contiguous Data Point – A data point that is comprised of a set of contiguous (i.e. touching each other) pixels of a defined data color.

Data Eraser - A graphical eraser box used in the digitizing or graphing screen to delete underlying data points from memory.

Digitizing Process - The process of converting raw pixel locations and intensities into useful scientific data.

dpi - Dots per inch (dpi) resolution of an image. Digitizing a 10 inch long graph that was scanned at 300 dots per inch will produce approximately 3,000 data points in line follow mode.

Image Input Device - Any device (such as a scanner or digital camera) that will capture a hard copy image and convert that image into a useable computer format (e.g. jpg, tif, .bmp, etc.).

Image Stretching – When Stretch to Fit option is selected for the main image view, the entire image will be stretched to fit within the main digitizing window. Although the digitizing results will not be affected, it is often preferable to select the Maintain Aspect Ratio option to preserve the original image width to height ratio when displaying the image in the main digitizing window.

Full Scanner Resolution - The image will be digitized at full scanner resolution, and is not limited to screen resolution. Therefore, scanning the image at a higher **dpi** setting can improve the resolution and accuracy of the digitized data (i.e. digitizing a 10 inch long graph that was scanned at 300 dots per inch will produce approximately 3,000 data points).

File Types (Data) - UN-SCAN-IT gel can export data in both tab delimited and comma delimited **ASCII (text)** format. This format can be imported into nearly all spreadsheet and data analysis programs. In addition, the data can be saved to the computer's **Clipboard** and pasted into these applications.

File Types (Image) - There are a number of commonly used image file types

supported by nearly all scanners and digital cameras. UN-SCAN-IT gel can import and digitize most of these common file types such as JPG, TIFF, PNG, GIF, and BMP.

Grayscale Intensity Width – A user defined range for the grayscale values that UN-SCAN-IT gel will accept as data.

Horizontal/Vertical Range – A user defined range for the grid line filter window used to ignore grid lines during grid line filtering digitizing.

Line Art Image - This type of image contains only a single pixel color, and is used to represent line drawings. This format is also referred to as **1-bit** or **Black & White**.

Line Following Algorithm - The method that UN-SCAN-IT gel uses to follow and assign an (x,y) data value to each point along the curve. This method involves many considerations, such as line thickness, tilted graphs, intersecting curves, grid lines, etc.

Resolution - Although the resolution (number of pixels in each step) can be adjusted in the *Options* portion of the software, UN-SCAN-IT gel generally operates at a resolution of one scanner unit. Therefore, digitizing a 10 inch long graph that was scanned at 300 dots per inch will produce approximately 3,000 data points. The digitized data resolution can be further adjusted using the **Adjust X Data Spacing** option in the graphing screen.

Single Valued – A curve or function that has only a single y value for each x value along the curve. Circular graphs, drawings, etc. are not single valued.

TLC Plate - The UN-SCAN-IT gel software can also be used to digitize TLC (thin layer chromatography) plates, and is not limited to just electrophoresis gels. If the TLC plate is fluorescent, you will need to scan a photo of the plate, or use a digital camera.

Vertical Data Shifter - A graphical data shifter box used in the digitizing screen used to shift the selected digitized points vertically.

X Data Spacing - The (x,y) values of the digitized data are extracted directly from the pixel locations on the original image. Although the x values may have what appear to be arbitrarily assigned x spacing, due to the conversion of a pixel location to an (x,y) value, the spacing can be Adjusted to any user defined increment. The **Adjust X Data Spacing** feature (located in the **Graphing** portion of the UN-SCAN-IT gel software) is useful for aligning x spacing in order to graph multiple curves on the same scale using other graphing software or to set x data points to exact user specified values.