

Carestream Molecular Imaging Software, Version 5.0 User's Guide

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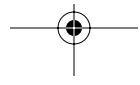
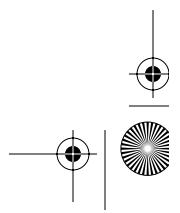
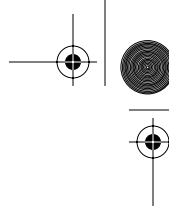


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Getting Started

Thank you for purchasing Carestream Molecular Imaging (Carestream MI) Software. We are sure you'll be pleased with our easy to use, powerful image analysis software. As a leader in scientific imaging software, Carestream Molecular Imaging, a division of Carestream Health, Inc. continues to develop innovative, quality applications for scientists in thousands of laboratories throughout the world.

Designed to support the Carestream Imaging Systems, this flexible software can also analyze images that are acquired using any TWAIN scanner or device. In addition, it can open and analyze TIFF or JPEG images from a variety of other imaging systems, such as video systems and phosphor imagers. Once acquired, you can:

- ✓ Document and analyze images with floating point accuracy.
- ✓ Prepare your results for publication and presentation.
- ✓ Print images, analysis results, and annotations.
- ✓ Database your images, results, and annotations as projects.

Carestream MI Software is supported on Windows and Macintosh operating systems and is driven through a series of navigation panels.

Carestream Molecular Imaging is dedicated to providing cutting edge imaging products for scientific research applications. For more information on Carestream products for scientific imaging applications, please visit us on the World Wide Web at mi.carestreamhealth.com

About the User's Guide

The Carestream Molecular Imaging Software User's Guide is a comprehensive reference manual. It is designed to be used in conjunction with your Carestream Imaging System's User's Guide, which contains complete instructions on setting up and capturing images.

If you are like most users, you will want to get started right away; but before you do, you need to have basic computer skills including:

- ✓ Launching applications
- ✓ Using a mouse
- ✓ Using pop-up menus
- ✓ Selecting and editing text
- ✓ Saving and printing your work
- ✓ Dragging and dropping objects

Refer to your computer manual to become familiar with the skills listed above.

Conventions

This User's Guide utilizes the following conventions:

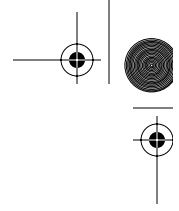
- ✓ Menus and dialog boxes are displayed using Windows XP and may appear differently on your screen. Any significant differences in commands between Macintosh and Windows platforms are noted in parenthesis in the text.
 - ✓ Menu commands, tool names, and window names are shown capitalized.
 - ✓ Warnings, tips, and notes appear in the text like this:
-  NOTE: Carestream Molecular Imaging provides maximum performance products.
- ✓ Important safety warnings appear in the text as follows:



WARNING: This symbol is used in the User's Guide to designate a warning or caution statement.



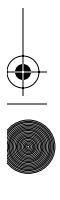
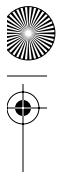
WARNING: This symbol is used in the User's Guide to designate where electrical shock is possible.



Navigating Through the User's Guide

The User's Guide is divided into the chapters listed below.

- ✓ Chapter 1: *Getting Started* describes the system requirements, describes the system components and navigates you through the installation process.
- ✓ Chapter 2: *Windows and Tools* describes the software menus, windows, tools, and preferences.
- ✓ Chapter 3: *Optimizing Images* reviews techniques to enhance your image. Once acquired, images can be cropped, rotated, contrasted, pseudo-colored and filtered for publication.
- ✓ Chapter 4: *Defining Lanes* shows you how to define experimental and standard lanes prior to band analysis.
- ✓ Chapter 5: *Generating Band Data* describes the analysis process of finding the band, generating the profile, assigning standards, reviewing the analysis data, and describes how to improve your results by modeling the data as Gaussian.
- ✓ Chapter 6: *Manual ROIs* guides you through the process of manually defining regions of interest on your image to generate positional, intensity, and volume measurement data from your selection.
- ✓ Chapter 7: *Auto ROIs* describes the process of automatically defining regions of interest on your image using various detection algorithm and generate positional, intensity, and volume measurement data.
- ✓ Chapter 8: *Grid ROIs* describes how to analyze regions of interest that appear in regularly spaced rows and columns such as slots, spots, arrays and microplates and generate positional, intensity, and volume measurement data using previously defined or custom grids.
- ✓ Chapter 9: *Annotating Images* describes how you can annotate your image and generate publication quality output.
- ✓ Chapter 10: *Managing Projects* shows you how to save, print, and export projects.
- ✓ Appendix A: *Keyboard Shortcuts* outlines Windows and Macintosh keyboard shortcuts.
- ✓ Appendix B: *Software Conventions* reviews basic conventions.
- ✓ Appendix C: *Scientific Units of Measurement* describes useful scientific units of measurement.
- ✓ Appendix D: *Standard Files* lists the standards data included with the software.
- ✓ *Glossary* defines keywords used in this guide.



What's in the Package

Before you begin the installation, check the contents of your package. Each package contains:

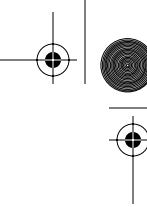
- ✓ Carestream Molecular Imaging Software Version 5.X CD (1)
 - ✓ Copy Protection Device(s)
 - ✓ User's Registration Card (1)
 - ✓ Serial Number Card (1)
 - ✓ User's Guide(s)
- (or)
- ✓ Carestream Molecular Imaging Software Network Edition (Custom Package)
 - Molecular Imaging Software Network Edition Version 5.X CD (1)
 - Molecular Imaging Software Network Edition Administrator's Manual (1)
 - Molecular Imaging Software User's Guide(s)

Registering Your Software

It is important for you to register your Carestream software. Once registered, you will receive information on maintenance releases, upgrades, and exciting new products.

Register by filling out and returning the registration card included with your software package.

You can also register any time on-line. If your computer connects to the Internet, select Register MI from the Help menu (Windows) or Register Online from the Apple menu item (Macintosh).



System Requirements

These are minimum specifications, however, we cannot ensure that all hardware and software systems are compatible. For optimal performance with a Carestream Imaging System, we strongly suggest dedicating a computer exclusively for use with any imaging system.

Minimum System Requirements—Windows

- ✓ Personal computer with a USB port
- ✓ Pentium IV (or equivalent) processor greater than 2 GHz
- ✓ Windows XP (Service Pack 3 or greater) or Windows Vista Business (Service Pack 1 or greater) operating system software

NOTE: Check your operating system version by right-clicking on the My Computer icon and then on Properties.

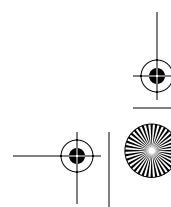
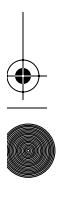
- ✓ 17 in. display—1280 x 1024 resolution
- ✓ Minimum 2 GB of available RAM
- ✓ Minimum 10 GB of available hard disk space
- ✓ CD drive, CD-RW drive recommended
- ✓ TCP/IP
- ✓ Internet Explorer 7.0 web browser

Minimum System Requirements—Macintosh

- ✓ Intel Macintosh
- ✓ Personal computer with a USB port
- ✓ Mac OS 10.5

NOTE: Check your operating system version by selecting About This Mac under the Apple menu item.

- ✓ 17 in. display—1280 x 1024 resolution
- ✓ Minimum 2 GB of available RAM,
- ✓ Minimum of 10 GB of available hard disk space
- ✓ CD drive, CD-RW drive recommended
- ✓ TCP/IP
- ✓ Safari 3.0 web browser



Obtaining Technical Support

For technical support, contact Technical Support or your Carestream Molecular Imaging dealer. For up to date dealer information, visit our web site at www.carestreamhealth.com/go/molecular. When contacting technical support, please have the following information available:

- ✓ The serial number and version number of your Carestream Software.
- ☞ NOTE: With the software running, select About Carestream MI under the Help menu (Windows) or select About Carestream MI under the Apple menu item (Macintosh).
- ✓ The type of computer you are using (make, model).
- ✓ The imaging capture system you are using with the software.
- ✓ Operating system software version.
- ☞ NOTE: Check your operating system version by right-clicking on the My Computer icon and then on Properties (Windows) or select About This Mac under the Apple menu item (Macintosh).
- ✓ The type of image you are analyzing.
- ✓ The problem you are having and what you were doing when the problem occurred. Please note the exact wording of any error messages, including any error numbers displayed.

Carestream Molecular Imaging Technical Support

Contact Carestream Molecular Imaging Technical Support by:

- ✓ Utilizing our World Wide Web support pages at:
mi.carestreamhealth.com
- ✓ Calling Carestream Molecular Imaging Technical Support at:
877-747-4357 or 203-786-5657, between the hours of 8:00 a.m. and 6:00 p.m.
(Eastern Standard Time) Monday through Friday
- ✓ E-mailing Carestream Molecular Imaging Technical Support at:
molecular-support@carestreamhealth.com
- ✓ Faxing Carestream Molecular Imaging Technical Support at:
203-786-5656

Installing Carestream Software—Windows

Any prior versions of Carestream and/or camera software that are loaded on the computer must be uninstalled prior to installation. If you are a new user, proceed to *Carestream Molecular Imaging Software Installation—Windows*. Windows 2000, Windows XP and Windows Vista installations require administrator privileges.

- ☞ NOTE: If you have purchased the Carestream Molecular Imaging Software Network Edition Package, follow the installation procedures as illustrated in the Carestream Molecular Imaging Software Network Edition Administrator's Manual.
- ☞ NOTE: If you have purchased this software for use with an Carestream Imaging System: Follow the instructions in your system's user guide for installation.

Uninstalling a Previous Version of Carestream MI or 1D Software—Windows

1 Remove your Carestream MI or 1D copy protection device from your computer.

2 Inactivate any virus protection software.

- ☞ NOTE: Norton Utilities and Norton Antivirus software must be deactivated before you uninstall the Carestream Software. The software might not uninstall properly with the virus protection left running. After installation, you can restart your virus protection software.

3 Close all software applications that may be running on your computer.

- ☞ NOTE: Carestream cameras/or imaging systems should not be connected to the computer while uninstalling the software.

4 Move any customized standards or templates and any projects from their respective subfolders in your existing MI or 1D X.X folder to a temporary folder. If you are currently using the MI database, also move the database folder.

5 Uninstall previous version(s) of Carestream MI or 1D Software.

- ✓ Uninstall MI by choosing Control Panel from the Start menu and Add/Remove Programs. Scroll to locate Carestream MI 5.X and click the Remove button. You can also uninstall the application using your Carestream MI CD. The installer shield automatically detects that you have the software loaded and offers an uninstall option.
- ✓ Uninstall 1D by choosing Programs from the Start menu and selecting Remove 1D X.X from the 1D X.X submenu.

- ✓ Uninstall Carestream MI NE or 1D NE following the instructions provided in your Network Administrator's Manual.

6 Uninstall additional Carestream Software by choosing Control Panel from the Start menu and Add/Remove Programs. Scroll to locate Sentinel System Driver. Click to select.

7 Click the Add/Remove button and follow the on-screen instructions to uninstall.

 NOTE: Automated uninstall features on some systems may not remove all previous program elements. Check drives for residual folders and files. Manual deletion of these folders and files may be necessary.

8 Restart your computer. Proceed to *Carestream Molecular Imaging Software Installation—Windows*.

Carestream Molecular Imaging Software Installation—Windows

Carestream Molecular Imaging Software is installed like most Windows application programs and requires administrator privileges.

-  NOTE: If you have purchased this software for use with an Carestream Imaging System: Follow the instructions in the your system's user guide for installation.

1 Inactivate any virus protection software.

-  NOTE: Norton Utilities and Norton Antivirus Software must be deactivated before you install the Carestream Software. The installation might not run properly with the virus protection left running. After installation, you can restart your virus protection software.

2 Close all software applications that may be running on your computer.

-  NOTE: Carestream cameras should not be connected to the computer while installing the software.

-  NOTE: Remove any copy protection devices attached to your computer during installation.

3 Insert the Carestream Molecular Imaging Software Version 5.X CD into your CD drive and double-click on the Carestream MI SE 5.X Installer.exe icon to launch the installer.

4 The installer leads you through the installation process. Make sure to select the Complete Setup to install the software in the default directory to ensure full functionality of the system.

-  NOTE: The Select Components window allows you to install MI without the database. Only install the database in the location that you want to maintain images acquired from the system.

5 While the software installation is occurring, complete your Carestream Software Registration Card and return the card to Carestream. This ensures that you receive information on new software releases, periodic maintenance releases, and technical bulletins.

6 A dialog box appears when the installation is complete. Select the option to restart the computer and click Finish.

7 Proceed to *Copy Protection Device Installation*.

Copy Protection Device Installation

Carestream MI Software is copy protected using a device that plugs into the USB port of your computer. It will not launch unless this device is attached to your computer. If you are installing the system for the first time, locate and install the copy protection device according to the instructions below. If you are upgrading your system, your package may or may not contain a new copy protection device.

To install the copy protection device:

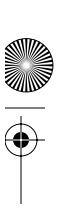
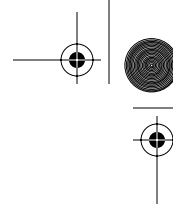
- 1** Plug the copy protection device into a USB port of your computer. Please make sure that the connection is secure.

 NOTE: Windows XP Users: A Welcome to the Found New Found Hardware Wizard dialog box appears on screen. You will be asked if you want the Windows update to search for software. Click No not at this time and advance through the installation wizard.

 NOTE: If you are upgrading from a previous version of the Carestream MI or 1D Software and received a new copy protection device, you must attach both the old and new copy protection devices to your computer. After you launch the newest version for the first time, your old copy protection device is deactivated. Remove and discard the old key.

 NOTE: If your computer has multiple USB ports, you can plug the copy protection device into any of them. The software, when launched, checks all available USB ports.

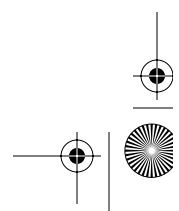
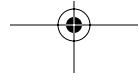
- 2** Proceed to *Setting Windows Power Settings*, later in this chapter.



Setting Windows Power Settings

Carestream Imaging Systems require that your computer Energy Saver features be disabled.

- 1** Click the Power Options icon from the Start menu and the Control Panel.
- 2** Click Power Schemes tab and select Always On from the Power Schemes pop-up menu.
- 3** The settings for Turn off monitor, Turn off hard disks, System standby and System hibernates functions become *Never*.
- 4** Click OK



Installing Carestream Software—Macintosh

Any prior versions of Carestream and/or camera software that are loaded on the computer must be uninstalled prior to installation. If you are a new user, proceed to *Carestream Molecular Imaging Software Installation—Macintosh*. Macintosh OS X installations may require authentication of permissions.

- ☞ NOTE: If you have purchased the Carestream Molecular Imaging Software Network Edition Package, follow the installation procedures as illustrated in the Carestream Molecular Imaging Software Network Edition Administrator's Manual.
- ☞ NOTE: If you have purchased this software for use with an Carestream Imaging System: Follow the instructions in your system's user guide for installation.

Uninstalling a Previous Version of Carestream MI or 1D Software—Macintosh

- 1 Remove your Carestream MI or 1D copy protection device from your computer.
- 2 Inactivate any virus protection software.
 - ☞ NOTE: Norton Utilities and Norton Antivirus software must be deactivated before you uninstall the Carestream Software. The software might not uninstall properly with the virus protection left running. After installation, you can restart your virus protection software.
- 3 Close all software applications that may be running on your computer.
 - ☞ NOTE: Carestream cameras should not be connected to the computer while uninstalling the software.
- 4 Move any customized standards or templates and any projects from their respective subfolders in your existing Carestream MI 5.X or 1D X.X folder to a temporary folder outside the Carestream folder. If you are currently using the Carestream MI database, also move the database folder. Click the Applications folder and locate the database folder labeled GMPDB located in the Carestream MI folder CarestreamAdminPortal subfolder.
- 5 Place the Carestream MI 5.X or 1D X.X folder in the Trash.
- 6 Restart your computer.
- 7 Empty the Trash.
- 8 Proceed to *Carestream Molecular Imaging Software Installation—Macintosh*.

Installing the Carestream Molecular Imaging Software—Windows

Any prior versions of MI, 1D and/or camera software that are loaded on the computer must be uninstalled prior to installation. If you are a new user, proceed to *Carestream Molecular Imaging Software Installation—Windows*. Windows Vista and Windows XP installations may require administrator privileges.

If you have purchased the Carestream Molecular Imaging Software Network Edition (Carestream MI NE) to use with your GL112:

- ✓ Follow the instructions in the Carestream Molecular Imaging Software Network Edition Administrator's Manual to install Carestream MI NE Software.
- ✓ Then proceed to *Launching Carestream Molecular Imaging Software for the First Time*, later in this chapter.

If you have purchased the Carestream Molecular Imaging Software, Regulatory Edition with Network Licensing (Carestream MI RE) to use with your Gel Logic 112 Imaging System:

- ✓ Follow the instructions in the Carestream Molecular Imaging Software, Regulatory Edition Network Administrator's Manual to install Carestream MI RE Software.
- ✓ Then proceed to proceed to *Launching Molecular Imaging Software for the First Time*, later in this chapter.

Uninstalling a Previous Version MI or 1D Software—Windows

1 Remove your MI or 1D copy protection device from your computer.

2 Inactivate any virus protection software.

 NOTE: Norton Utilities and Norton Antivirus software must be deactivated before you uninstall the software. The software might not uninstall properly with the virus protection left running. After installation, you can restart your virus protection software.

3 Close all software applications that may be running on your computer.

 NOTE: Cameras should not be connected to the computer while uninstalling the software.

- 4** Move any customized standards or templates and any projects from their respective subfolders in your existing MI or 1D X.X folder to a temporary folder outside the MI or 1D folder. If you are currently using the MI database, also move the database folder. The database folder labeled GMPDB is located in the program's folder.
 - 5** Uninstall previous version(s) of MI or 1D software.
 - ✓ Uninstall MI by choosing Control Panel from the Start menu and Add/Remove Programs (Windows XP) or Uninstall a Program from the Program menu (Windows Vista). Scroll to locate MI X.X. Click to select. Click the Add/Remove button (Windows XP) or Uninstall/Change (Windows Vista) and follow the on-screen instructions to uninstall. You can also uninstall the application using your MI CD. The installer shield automatically detects that you have the software loaded and offers an uninstall option. A window informs you as each software has successfully been removed.
 - ✓ Uninstall 1D by choosing Programs from the Start menu and selecting Remove 1D X.X from the 1D X.X submenu.
 - ✓ Uninstall MI NE or 1D NE following the instructions provided in your Network Administrator's Manual.
 - 6** Uninstall additional software (Windows XP) by choosing Control Panel from the Start menu and Add/Remove Programs or Uninstall a Program from the Program menu (Windows Vista). Scroll to locate Sentinel System Driver. Click to select. Click the Add/Remove button (Windows XP) or Uninstall/Change (Windows Vista) and follow the on-screen instructions to uninstall. A window informs you as each software has successfully been removed.
-  NOTE: Automated uninstall features on some systems may not remove all previous program elements. Check drives for residual folders and files. Manual deletion of these folders and files may be necessary.
- 7** Restart your computer.
 - 8** Proceed to *Carestream Molecular Imaging Software Installation—Windows*.

Carestream Molecular Imaging Software Installation—Windows

Carestream Molecular Imaging software is installed like most Windows application programs and requires administrator privileges.

1 Inactivate any virus protection software.

 NOTE: Norton Utilities and Norton Antivirus software must be deactivated before you install the software. The installation might not run properly with the virus protection left running. After installation, you can restart your virus protection software.

2 Close all software applications that may be running on your computer.

 NOTE: Cameras should not be connected to the computer while installing the software.

 NOTE: Remove any copy protection devices attached to your computer during installation.

3 Insert the Carestream Molecular Imaging Software Version 5.X CD into your CD drive and double-click on the Carestream MI SE Installer.exe icon to launch the installer.

 NOTE: To install Carestream MI 5.X on Windows Vista, right-click on the Installer and select Run as Administrator. In the User Account Control pop up menu, select All, to obtain administrator privileges to allow the installer to run.

4 The installer leads you through the installation process. Make sure to select the Complete Setup to install the software in the default directory to ensure full functionality of the system.

 NOTE: The Select Components window allows you to install Carestream MI without the database. Only install the database in the location that you want to maintain images acquired from the system.

5 While the software installation is occurring, complete your Carestream MI Software Registration Card and return the card by mail. This ensures that you receive information on new software releases, periodic maintenance releases, and technical bulletins.

6 A dialog box appears when the installation is complete. Select the option to restart the computer and click Finish.

7 Proceed to *Copy Protection Device Installation—Windows*.

Copy Protection Device Installation—Windows

Carestream MI is copy protected using a device that plugs into the USB port of your computer. It will not launch unless this device is attached to your computer. If you are installing the system for the first time, locate and install the copy protection device according to the instructions below. If you are upgrading your system, your package may or may not contain a new copy protection device.

To install the copy protection device:

- 1** Plug the copy protection device into a USB port of your computer. Please make sure that the connection is secure.

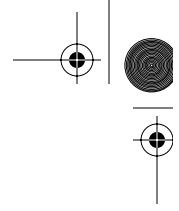
 NOTE: Windows XP Users: A Welcome to the Found New Found Hardware Wizard dialog box appears on screen. You will be asked if you want the Windows update to search for software. Click No not at this time and advance through the installation wizard.

 NOTE: Windows Vista Users: Installing Device Driver Software dialog box will appear in the lower right hand corner. Upon completing installation, a message appears informing you that Rainbow USB Superpro Device Driver Software installed successfully.

 NOTE: If you are upgrading from a previous version of the MI or 1D software and received a new copy protection device, you must attach both the old and new copy protection devices to your computer. After you launch the newest version for the first time, your old copy protection device is deactivated. Remove and discard the old key.

 NOTE: If your computer has multiple USB ports, you can plug the copy protection device into any of them. The software, when launched, checks all available USB ports.

- 2** Proceed to *Windows Power Settings*, later in this chapter.



Windows Power Settings

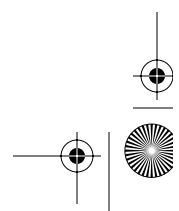
The GL112 requires that your computer Energy Saver features be disabled. This process by may differ depending upon the Windows software installed.

Windows XP

- 1** Click the Power Options icon from the Start menu and the Control Panel.
- 2** Click Power Schemes tab and select Always On from the Power Schemes pop-up menu.
- 3** The settings for Turn off monitor, Turn off hard disks, System standby and System hibernates functions become *Never*.
- 4** Click OK and proceed to *Launching Carestream Molecular Imaging Software for the First Time*.

Windows Vista

- 1** Select System and Maintenance from the Start menu and the Control Panel.
- 2** Click Power Options, select to Create a power plan and choose High Performance from the list of protocols.
- 3** Proceed to *Launching Carestream Molecular Imaging Software for the First Time*.



Installing the Carestream Molecular Imaging Software—Macintosh

Any prior versions of MI, 1D or camera software that are loaded on the computer must be uninstalled prior to installation. If you are a new user, proceed to *Carestream Molecular Imaging Software Installation—Macintosh*. Macintosh OS X installations may require authentication of permissions.

If you have purchased the Carestream Molecular Imaging Software Network Edition (Carestream MI NE) to use with your Carestream Gel Logic 112 Imaging System:

- ✓ Follow the instructions in the Carestream Molecular Imaging Software Network Edition Administrator's Manual to install Carestream MI NE Software.
- ✓ Then proceed to *Launching Carestream Molecular Imaging Software for the First Time*, later in this chapter.

If you have purchased the Carestream Molecular Imaging Software, Regulatory Edition with Network Licensing (Carestream MI RE) to use with your Carestream Gel Logic 112 Imaging System:

- ✓ Follow the instructions in the Carestream Molecular Imaging Software, Regulatory Edition Network Administrator's Manual to install Carestream MI RE Software.
- ✓ Then proceed to *Launching Carestream Molecular Imaging Software for the First Time*, later in this chapter.

Uninstalling a Previous Version of MI or 1D Software—Macintosh

- 1** Remove your MI or 1D copy protection device from your computer.
- 2** Inactivate any virus protection software.

 NOTE: Norton Utilities and Norton Antivirus software must be deactivated before you uninstall the software. The software might not uninstall properly with the virus protection left running. After installation, you can restart your virus protection software.

- 3** Close all software applications that may be running on your computer.

 NOTE: Cameras should not be connected to the computer while uninstalling the software.
- 4** Move any customized standards or templates and any projects from their respective subfolders in your existing MI X.X or 1D X.X folder to a temporary folder outside the folder. If you are currently using the MI database, also move the database folder. Click the Applications folder and locate the database folder labeled GMPDB located within the MI folder.
- 5** Place the MI X.X or 1D X.X folder in the Trash.

 NOTE: If uninstalling MI version 4.5 or greater, double-click the uninstall application in the Applications folder and the MI subfolder. Follow the on-screen instructions to uninstall MI.
- 6** Restart your computer.
- 7** Empty the Trash.
- 8** Proceed to *Carestream Molecular Imaging Software Installation—Macintosh*.

Carestream Molecular Imaging Software Installation—Macintosh

1 Inactivate any virus protection software.

 NOTE: Norton Utilities and Norton Antivirus software must be deactivated before you install the software. The installation might not run properly with the virus protection left running. After installation, you can restart your virus protection software.

2 Close all software applications that might be running on your computer.

 NOTE: Cameras should not be connected to the computer while installing the software.

 NOTE: Remove any copy protection devices attached to your computer during installation.

3 Insert the Carestream Molecular Imaging Software Version 5.X CD into your CD drive and double-click the Carestream MI SE Installer.mpkg icon to launch the installer application.

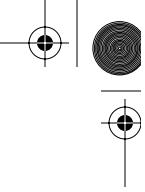
4 The installer leads you through the installation process. Make sure to select the Easy Install to install the software in the default directory and ensure full functionality of the system.

 NOTE: The Select Components window allows you to install MI without the database. Only install the database in the location that you want to maintain images acquired from the system.

5 While the software installation is occurring, complete your Carestream MI Software Registration Card and return the card by mail. This process takes only a few minutes to complete and ensures that you receive information regarding new software releases, periodic maintenance releases, and technical bulletins.

6 The installer notifies you when the installation is complete. Click Restart.

7 Proceed to *Copy Protection Device Installation—Macintosh*.



Copy Protection Device Installation—Macintosh

Carestream Molecular Imaging Software is copy protected using a device that plugs into the USB port of your computer. Carestream MI will not launch unless this device is attached to your computer. If you are installing the system for the first time, locate and install the copy protection device according to the instructions below. If you are upgrading your system, your package may or may not contain a new copy protection device.

To install the copy protection device:

- 1** Plug the copy protection device into a USB port of your computer. Please make sure that the connection is secure.

NOTE: If you are upgrading from a previous version of the MI or 1D Software and received a new copy protection device, you must attach both the old and new copy protection devices to your computer. After you launch the newest version for the first time, your old copy protection device is deactivated. Remove and discard the old key.

- 2** Proceed to *Macintosh Power Settings*.

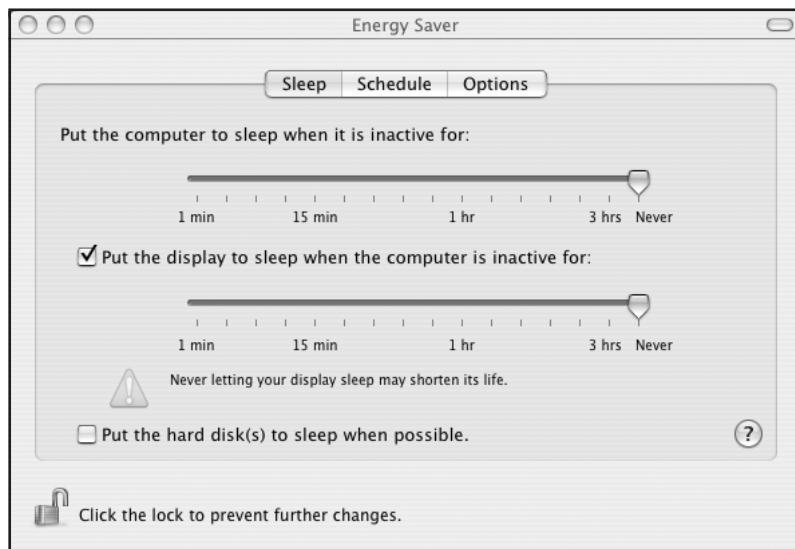


Macintosh Power Settings

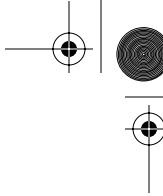
The GL112 requires that the Energy Saver settings on your computer be disabled.

To disable the system sleep features:

- 1 Choose Energy Saver from the File menu and the System Preferences submenu or from System Preferences on the Dock. The Energy Saver window opens.



- 2 Click the Sleep tab and set the Computer and Display sleep sliders to Never. Ensure the Put the hard disk(s) to sleep when possible checkbox is unchecked.
- 3 Proceed to *Launching Carestream Molecular Imaging Software for the First Time*.



Launching Carestream Molecular Imaging Software for the First Time

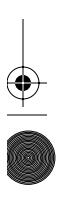
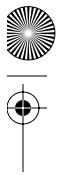
- 1 Launch Carestream MI by clicking on the MI icon found on the desktop.
- 2 The Carestream MI Security Setup dialog box appears. You must select either *No Login* or *Login Required* from the pop-up menu. Click Continue.



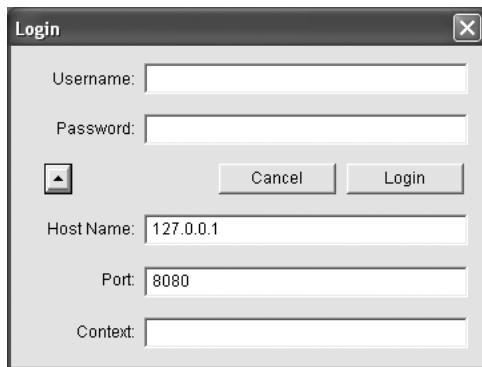
NOTE: The selected Carestream MI Security feature applies to your new Carestream MI installation for all users.

- ✓ In *No Login Mode*—all users gain access to Carestream MI bypassing the login screen.
- ✓ In *Password Mode*—requires you to enter your User Name and Password every time Carestream MI starts up.

NOTE: The initial installation of Carestream MI comes with a single user with the User Name “Admin” and the Password “password”. Use this User name to gain access to Carestream MI Image Database to set up users.



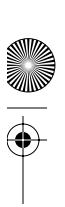
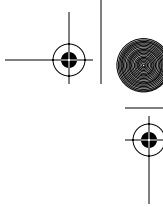
- 3** The Login window appears. Enter “Admin” as the Username and “password” as the Password. The Host Name default value will cause Carestream MI to search your local drive for the Carestream MI Image Database. If the Carestream MI Image Database is located on another machine or file server, enter the IP address of that machine in the Host Name field. Click Login.



NOTE: The initial installation of Carestream MI comes with a single user with the User Name “Admin” and the Password “password”. Use this User name to gain access to Carestream MI Image Database to set up users.

NOTE: If no Carestream MI database is detected at the Host Name location, you will be asked if you would like to continue without connection to a database.

- 4** If you selected No Login in the Carestream MI Security Setup dialog box, future launches of Carestream MI will skip the login windows and open to a Carestream MI Project window. Proceed to Step 6 to enter your User Name, Company and Carestream MI serial number.
- 5** If you selected Login Required in the Carestream MI Security Setup dialog box, you will be prompted to contact database administrator. Click OK to open Carestream MI. Future launches of Carestream MI will require both Username and Password to gain access to Carestream MI and the Carestream MI Image Database.
- 6** Type your Name and Organization in the boxes provided.



-
- 7** Enter your Serial Number exactly (including dashes) as provided on your registration card or serial number card included in your Carestream MI package.

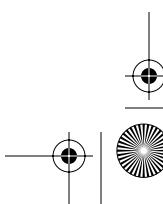
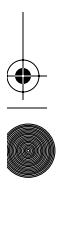
NOTE: The serial number is required when installing Carestream MI or when contacting Carestream Molecular Imaging Technical Support. Keep the serial number in a safe location.

- 8** Click OK. The Carestream MI Project window appears. Your installation is complete.

NOTE: Previous MI or 1D users may move any customized standards or templates, any projects, and database folders back into the new MI folder.

NOTE: If you selected Login Required in the Carestream MI Security Setup dialog box when Carestream MI was first launched, you can set up User Names and Passwords for each Carestream MI user. Proceed to *Carestream MI Image Database*, later in this chapter.

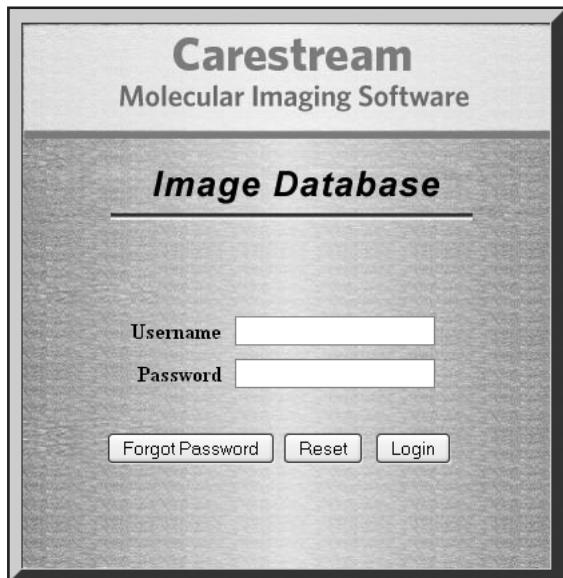
1 Getting Started



Carestream MI Image Database

The initial installation of Carestream MI comes with a single user with the User Name “Admin” and the Password “password”. Use this User name to gain access to Carestream MI Image Database to set up additional users.

- 1** Select Database from the Navigation panel.
- 2** Your web browser launches and displays the Image Database window opens.



- 3** Select the default Username, “Admin”, type the Password “password” (No quotation marks) and click Login.

1 Getting Started

- 4** Click the Users tab to access the User Administration page, where you can add users. Select Add Users.

Select	Username	First Name	Last Name	Email	Job Function	Status
<input type="checkbox"/>	Admin	Admin	Administrator	yourname@yourcompany.com	Administrator	Active

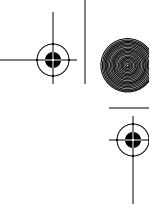
- 5** The User Information page appears. Fill out the information including First Name, Last Name, Username (enter a temporary password), Password, Job Function (Administrator, Principal Investigator, Research Scientist), an e-mail address. Click Create.

NOTE: New users are automatically added to the default Investigation and default Study.

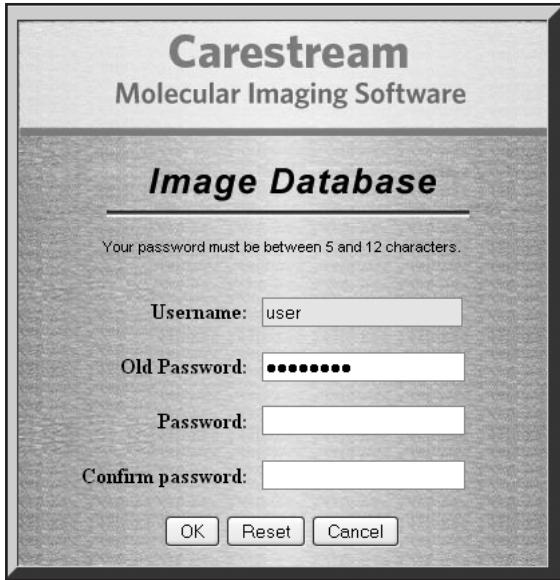
- 6** You may want to take some time to click on the Server Setup button to give your database server a name and specify a SMTP mail server.

- ✓ Server ID**—is a unique name for your machine. The Server ID can either be a name or number.

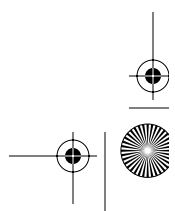
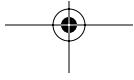
- ✓ Administrator E-mail—this indicates the e-mail address of the Administrators's.
 - ✓ *Days until password expire*—allows administrators to determine how frequently passwords expire. When a user password expires, you will be prompted to enter a new password. the minimum is 2 days.
 - ✓ *SMTP Server*—This mail server can be used for sending out e-mails and broadcasting messages. This is a mandatory field that must be entered. The SMTP mail server is required when users click the Forgot Password button.
 - ✓ If the checkbox *Enable the forget password button* for administrator is checked. A user with system administrator privileges can use the forget password function.
- 7 An additional feature of Carestream MI Image Database is the ability to broadcast e-mail messages to users. Administrators can enter a subject and message in the fields provided and click Send. All users receive the broadcast message. To send/receive a message, the user must have a valid e-mail address entered.



- 8** Click Logout to exit Carestream MI Image Database. On the next restart of Carestream MI, new users must enter their username and password. The user will then be prompted to access the web portal to change their password.



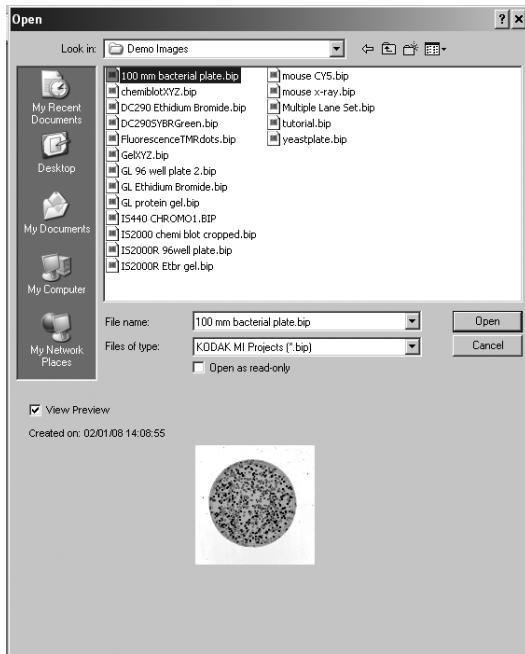
- 9** Shut down Carestream MI and your computer. Proceed to *Setting Up the Hardware*.



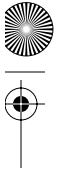
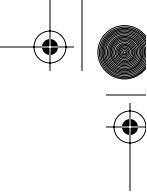
Opening a File—Projects, JPEG, TIFF Images

When you acquire an image or open a TIFF or JPEG file, the KODAK MI Software automatically creates a new project. Once saved, the project contains the original image as acquired, in addition to file information gathered during capture, analysis results, standards information, annotations, and preference settings. In addition, you can analyze images saved in TIFF or JPEG file formats. To open a file:

- 1 Choose Open from the File menu.



- 2 Choose the folder in which the file is located using the Look in pop-up menu.
- 3 Select the type of file you want to open Files of type pop-up menu.
 - ✓ MI Projects (*.bip) files are saved as projects for use in KODAK MI Software.
 - ✓ TIFF (*.tif)
 - ✓ JPEG (*.jpg)
- 4 Select the image file and click Open or double-click on the name of the file. If the image is not a KODAK MI project, the image file opens into an Untitled window. Be sure to save the image as a project before beginning your analysis.



Using Carestream Molecular Imaging Software with a TWAIN Device

You can acquire images from many types of media including autoradiograms, gel photographs, blots, negatives, and even protein gels with a TWAIN scanner or camera from within Carestream MI Software. Flatbed scanners vary widely in quality. The software is compatible with all commercial scanners that use a TWAIN acquire module.

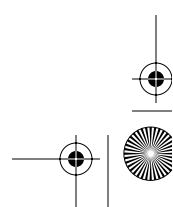
Install the imaging device and software, including the TWAIN module, according to the manufacturer's instructions. When scanning an image, here are some suggestions for improving image quality and to get the best results.

- ✓ Most scanners are designed for the graphic arts, not for scientific analysis. You will need to change the default setting to those more appropriate for scientific analysis. In general, avoid any software "enhancement" or interpolation of data.
- ✓ Following settings are recommended—scanning mode should be grayscale or 8-bit gray. Set to 300 dpi (both horizontal and vertical).
- ✓ Position your sample on the scanner straight.
- ✓ Don't overexpose your film; the analysis algorithms work best with low to medium contrasted images.
- ✓ For scanned autoradiograms, use Carestream BioMax MR or MS Film which provide high quality images with low background.

1 Getting Started

Acquiring Images with a TWAIN Scanner or Camera

- 1 After verifying that the TWAIN device software has been properly installed, launch Carestream MI Software.
- 2 Choose Select TWAIN Source from the File menu. The Select Source dialog box appears.
- 3 Click the Name of your imaging device's TWAIN module to select. Click Select.
 NOTE: If the device name does not appear in the dialog box, reinstall the TWAIN acquire module software included with the device.
- 4 Choose your device's TWAIN Acquire from the File menu. The TWAIN Scan dialog box appears. Modify the acquire module software to use the following settings:
 - ✓ Scanning Mode: Grayscale or 8-bit grays



- ✓ Brightness/Contrast setting: Use device defaults
- ✓ Scan with horizontal and vertical resolution at the same dpi
- ✓ Resolution: Between 200–300 dpi

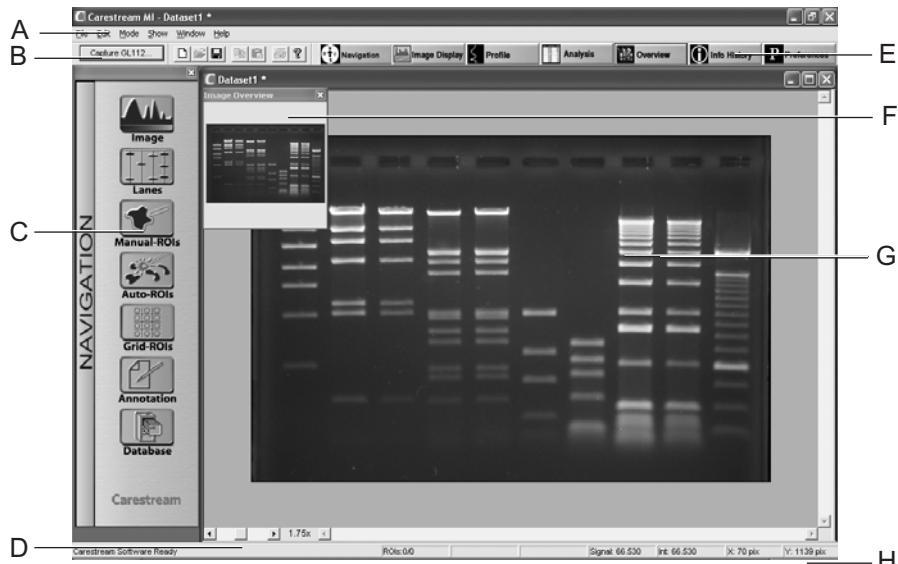
Since each TWAIN module is different, the exact terminology may differ from the above.

- 5 Capture your image following standard TWAIN acquire instructions.
- 6 After you have successfully acquired an image, the image opens as a new untitled Project window. Be sure to save the project before beginning your analysis.

Windows and Tools

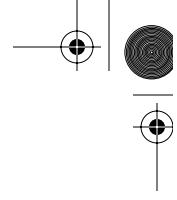
The Project Window

After acquiring a new image or selecting a saved image, Carestream MI Software opens the Project window. From this window, you can control how the image is displayed on-screen and what Quick Access features and tools appear. Lets review the Project window.



- A The *Menu bar* contains the Carestream MI Software commands. The Menu bar is organized under 6 items—File, Edit, Mode, Show, Window, and Help (Windows) or Carestream MI, File , Edit, Mode, Show, and Window (Macintosh).
- B The *Capture button* accesses your Carestream Imaging System or TWAIN Image Acquire window. If installed, the Capture button displays the currently selected device.
- C Use the *Navigation panel* to optimize the image display, quantify lanes, regions or grids, and annotate and database your image.
- D The *Magnification slider* provides digital magnification from 0.25X to 32X. The Magnification slider maintains the center of the image. This differs from the Zoom tool which shifts the center of the image to wherever the tool is clicked.

- E The *Quick Access bar* accesses frequently used windows—Navigation, Image Display, Profile, Analysis, Overview, Info History, and Preferences.
- F The *Overview Image* contains the scaled version of the entire image. A red box indicates the portion of the image appearing in the Image Section. You can navigate what is displayed in the Image Section by repositioning the rectangle. This feature is especially useful when you are zoomed in on the image.
- G The *Image Section* represents the image as acquired. This is where you work with the image.
- H The *Status bar* provide important information as you work through your project. The Status bar changes as you perform various functions.



The File Menu

The Menu bar is organized under 6 items—File, Edit, Mode, Show, Window, and Help (Windows) or Carestream MI, File , Edit, Mode, Show, and Window (Macintosh). Lets review the selection under each item.



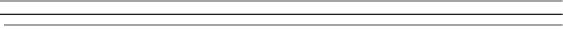
- ✓ *Digital Camera Capture*—opens a new Image Acquire window. This command is specific to the Carestream Imaging System(s) you have installed.
- ✓ *New TWAIN Acquire*—supports any TWAIN-compliant camera or scanner. This command opens the TWAIN Acquire window.
- ✓ *Open*—accesses previously saved Carestream MI projects, TIFF, or JPEG images.
- ✓ *Search*—launches the Search Database window from which you can employ an advanced search strategy to locate and open a specific stored project.
- ✓ *Select Digital Camera*—allows you to define the current digital camera system. Once selected, the New Digital Camera Capture button is updated to reflect the current camera name.
- ✓ *Select TWAIN Source*—allows you to select from the list of installed TWAIN-compliant cameras or scanners.
- ✓ *Close*—exits the active project. If the project has not been previously saved or if you have made changes to it since it was last saved, a Save dialog box appears.
- ✓ *Close All*—exits the all open projects. If any of the projects have not been previously saved or if you have made changes to it since it was last saved, a Save dialog box appears.

- ✓ *Save*—saves the active project. It is a good idea to periodically save your work during a session. If the project has been previously saved, the project is overwritten with the same file name.
- ✓ *Save All*—saves all open projects. It is a good idea to periodically save your work during a session. If the projects have been previously saved, the project is overwritten with the same file name.
- ✓ *Save As*—allows you to save the project under a new name. The new version becomes the active project and the original project is preserved as the last saved version.
- ✓ *Revert to Saved*—reverts to the last saved project. Keep in mind that you will lose any changes you have made since the last save.
- ✓ *ROI Templates*—opens the ROI Templates window where you can save a region of interest template and apply a template to images.
- ✓ *File Info*—opens the File Information window which stores archival information concerning the project. In addition, a History file is maintained tracking any changes that are destructive to the image file, e.g., cropping.
- ✓ *Export Data*—exports your image and/or all related analysis data for use in other programs. The Export Image option supports a variety of image formats including TIFF, BMP, JPEG, and PICT. You can also export the analysis data, lane profile, and histogram information. This data is saved as a tab-delimited text file that can be read by most spreadsheet programs.
- ✓ *Export Multiple File Image(s)*—exports multiple images at one time for use in other programs. The option supports TIFF, JPEG and BMP image formats.
- ✓ *Add/Import File(s) to Database*—adds selected images to the MI database.
- ✓ *Page Setup*—opens your printer's Print Setup window where you can select options such as paper size and page orientation. The window is specific to your installed printer. If no printer is install, a default page is defined.
- ✓ *Print Selection*—only prints what is contained in a selection rectangle, if one is defined.
- ✓ *Print*—prints the current image and/or analysis data. The window is specific to your printer.
- ✓ *Exit*—exits the program. You will be offered an opportunity to save any open projects that have been altered, prior to closing the program.

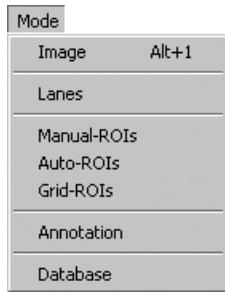
The Edit Menu

Edit	
Undo	Ctrl+Z
Cut	Ctrl+X
Copy	Ctrl+C
Paste	Ctrl+V
Clear	Delete
Select All	Ctrl+A
Duplicate ROI	Alt+D
Standards...	
Image Field Correction...	
Project Preferences...	Alt+;
New Project Prefs...	Alt+[
Application Preferences...	Alt+\

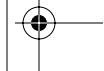
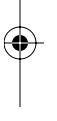
- ✓ *Undo*—undoes the last executed edit.
- ✓ *Cut*—copies the selected text or object to the clipboard and removes it from the annotations.
- ✓ *Copy*—copies the selected text or object to the clipboard, but does not delete the selection from the project.
- ✓ *Paste*—places a cut or copied text or object to the annotation page.
- ✓ *Clear*—deletes a label or annotation.
- ✓ *Select All*—selects the entire image in the Image Section or all the annotations when in the Annotation panel.
- ✓ *Duplicate ROI*—copies an ROI selection.
- ✓ *Standards*—accesses the Standards window used for mass and/or molecular weight determination in lane analysis.
- ✓ *Image Field Correction*—applies special lens and illumination corrections to images previously captured using a Carestream Image Station 1000, 2000, or 4000 systems.
- ✓ *Project Preferences*—sets the preference for the current project.
- ✓ *New Project Prefs*—defines the preferences for all new project.
- ✓ *Application Preferences*—defines application preferences, including desktop and database set-ups

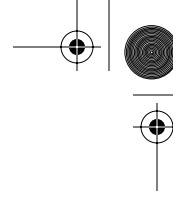


The Mode Menu

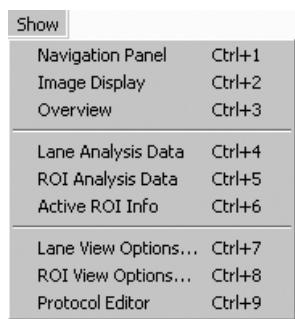


- ✓ *Image*—opens the Image panel where you can crop, rotate, and flip your image. In addition, you can access the Image Display window where you can adjust the appearance of the on-screen and printed image.
- ✓ *Lanes-Bands*—opens the Lanes panel where you analyze gels or blots.
- ✓ *Manual ROI*—opens the Manual ROI panel where you can draw regions of interest and generate intensity, geometric and location data.
- ✓ *Auto Find ROI*— opens the Auto Find ROI panel where you can automatically define ROIs using various boundary detection algorithms.
- ✓ *Grid ROI*—opens the Grid ROI panel where you can automatically set up an ROI grid.
- ✓ *Annotation*—opens the Annotation panel where you can annotate, create custom views, and label your data.
- ✓ *Database* —opens the Carestream MI Security Manager where you set up users with search and organize projects, perform advance differential display and gel comparisons.



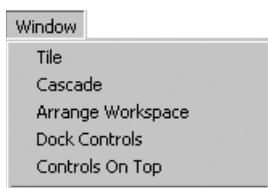


The Show Menu



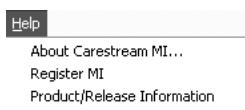
- ✓ *Navigation Panel*—click to show or hide the Navigation panel.
- ✓ *Image Display*—opens the Image Display window where you can adjust the appearance of the on-screen and printed image.
- ✓ *Overview*—click to hide or show the Overview Image.
- ✓ *Lane Analysis Data*—opens the Lane Analysis Data window which displays the data for all defined lanes.
- ✓ *ROI Analysis Data*—opens the ROI Analysis Data window which displays the data for all defined ROIs.
- ✓ *Active ROI Info*—opens the Active ROI Info window which displays analysis data for the selected ROI.
- ✓ *Lanes View Options*—opens the View Options dialog box where you can select the lanes that you want to display and what features you want displayed on your lane set, e.g., Band Labels or Lane Lines).
- ✓ *ROI View Options*—opens the View Options dialog box where you can select the ROIs that you want to display and what feature you want displayed on your ROIs, e.g., labels).
- ✓ *Protocol Editor* accesses the Protocol dialog where you can automate and execute multiple imaging steps associated with Carestream Image Station and In-Vivo Pro Imaging Systems.

The Window Menu



- ✓ *Tile*—arranges (and resizes if necessary) all open (non-minimized) windows so that they are not overlapping.
- ✓ *Cascade*—arranges and resizes the open windows so they are overlapping with their title bars visible (Windows only).
- ✓ *Arrange Workspace*—maximizes all open (non-minimized) windows and stacks.
- ✓ *Dock Controls*—rearranges the workspace by maximizing all open (non-minimized) windows and docks them to the Navigation panel. If the Overview Image is displayed, it also docks to the Navigation panel.
- ✓ *Controls on Top*—maximizes the workspace and overlays the Navigation panel onto the active window.

The Help Menu (Windows Only)

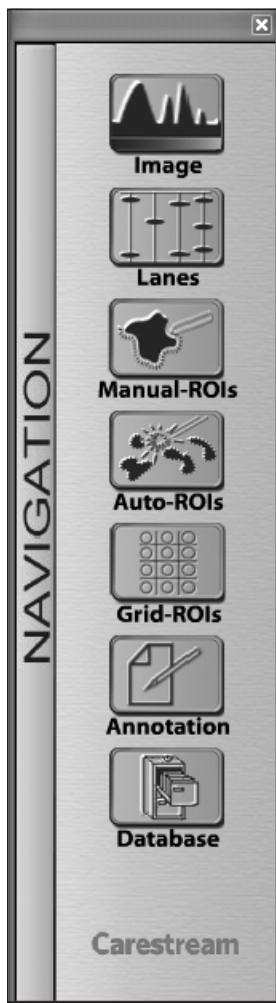


- ✓ *About Carestream MI*—provides information about the product, including the serial number and the version.
- ✓ *Register MI*—navigates you to the Carestream Molecular Imaging web site where you can complete your on-line registration of your software. A connection to the Internet is required for on-line registration.
- ✓ *Product/Release Information*—navigates you to the Carestream Molecular Imaging web site where you can view available updates to your software. A connection to the Internet is required.

The Carestream MI Menu (Macintosh Only)

- ✓ *About Carestream MI*—provides information about the product, including the serial number and the version.
- ✓ *Register OnLine*—navigates you to the Carestream Molecular Imaging web site where you can complete your on-line registration of your software. A connection to the Internet is required for on-line registration.
- ✓ *MIS Product Info*—navigates you to the Carestream Molecular Imaging web site where you can view available updates to your software. A connection to the Internet is required.

The Navigation Panel



The Navigation panel guides you through optimizing the image display, quantification of lanes, regions or grids, annotation and databasing of your image. Click the buttons on the Navigation panel to access the desired mode. To return to the Navigation panel, click your cursor along the Navigation bar on the left side of the panel.

You can choose to display or hide the Navigation panel by selecting Navigation from the Quick Access bar or choosing Navigation from the Show menu. Hide the Navigation panel to maximize your viewing area when you want to display images on-screen.

Most of the panels have three different sections. The top region, contains items for major analysis steps/features. The middle section contains items related to editing the results. The bottom section provides the tools that you will need within the mode.

- ✓ *Image*—opens the Image panel where you can crop, rotate, or flip your image. In addition, you can access the Image Display window where you can adjust the appearance of the on-screen and printed image.
- ✓ *Lanes*—opens the Lanes panel where you can analyze gels or blots as lane sets.
- ✓ *Manual ROIs*—opens the Manual ROIs panel where you can draw regions of interest and generate intensity, geometric and location data.
- ✓ *Auto ROIs*—opens the Auto ROIs panel where you are guided through the process of automatically defining ROIs using different methods.
- ✓ *Grid ROIs*—opens the Grid ROIs panel to automatically set up an ROI grid.
- ✓ *Annotation*—opens the Annotation window and Annotation panel where you can annotate, create custom views, and label your image with data.
- ✓ *Database*—opens the Database panel where you can set search criteria to find images using your project's File Information.

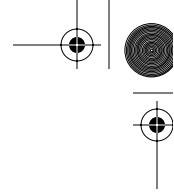
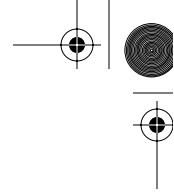
The Image Panel

The Image panel offers you the option of cropping, rotating, and flipping your image. In addition, you can access the Image Display window where you can adjust the appearance of the on-screen and printed image. You can also perform mathematical calculations on a single or pair of images using Image Math.

Image Panel Buttons



- ✓ *Image Display*—opens the Image Display window where you can adjust the appearance of the on-screen and printed image.
- ✓ *Rotate 90° CW*—rotates the image 90° clockwise.
- ✓ *Rotate 90° CCW*—rotates the image 90° counter-clockwise.
- ✓ *Flip Horizontal*—flips the image horizontally.
- ✓ *Flip Vertical*—flips the image vertically.
- ✓ *Signal Orientation*—defines the feature of interest as either white or black. This preference affects the finding algorithms.
- ✓ *Image Math*—opens the Image Math dialog box. You can perform complex calculations on a single or pair of images. The resulting image becomes a new project, with Image History documenting how you created the image. Image Math has three different types of options—Tasks, Formula, and Image Processing Filters.
- ✓ *Image Correction*—applies a lens or illumination correction to an Image Station 1000, 2000 or 4000 image.
- ✓ *Info/History*—opens the File Information/History window, which stores archival information concerning the project. In addition, a History file is maintained tracking any changes that are destructive to the image file, e.g., cropping.



The Image Panel Tools



Use the Zoom tools to adjust the size of the image through levels of magnification (0.25X, 0.33X, 0.50X, 1X, 1.5X, 2X, 4X, 6X, 8X, 16X, and 32X). To increase the magnification, click the image with the + Zoom tool. To reduce magnification, click the image with the - Zoom tool.

When you change the magnification with the Zoom tools, the software positions the point where you clicked in the center of the Image Section. This differs from the Magnification slider which maintains the center of image in the window.



Selection Rectangle Tool

Click and drag the Image Selection tool to select a rectangle to make a selection for printing, exporting a selection as a TIFF or JPEG file, or copying to the clipboard.



White Point /Black Point Tools

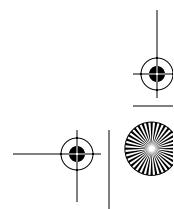
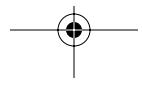
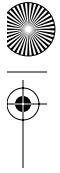
Use the White Point/Black Point tools to select the white or black point values used to adjust the contrast in the image. To use, click the Black Point tool over the pixel in which you want to represent black point. Click the White Point tool over the pixel in which you want the to represent the white point. The image is then remapped using these points.

NOTE: The white point and black point can also be adjusted using the Image Display window.



Set Resolution Tool

Resizes the image using a calibration line that you define. You may also set the horizontal and vertical resolution (pixels per inch) of the image. If you specify resolution, you can lock it so that the horizontal and vertical is the same with a checkbox. This is especially useful if you did not record your zoom settings in the Acquire window of the Carestream Imaging Systems. This tool becomes inactive if you have performed any analysis on the image.





Rotation Tool

Use the Rotation tool to straighten an image on the screen prior to analysis. Drag the Rotation tool to draw a line parallel to any feature in the image. When you release the mouse button, the image rotates to the nearest 90° axis. This tool becomes inactive if you have performed any analysis. You can also perform 90° and 180° rotations and flips using Edit menu commands. Rotation events are recorded in the history.

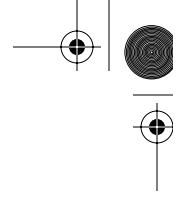
 NOTE: If you are not satisfied with the rotation, select Undo in the Edit menu and try again.



Crop Tool

You can use the Crop tool to select a part of the image and discard the rest. To use, click the tool on the image and drag across the specified area you want to retain. When you release the mouse button, the selected area appears. You can adjust the positioning of the selection and the size of the selection. To crop, move the cursor to the inside of the selection and double-click or click on the Crop tool. This tool becomes inactive if you have performed any analysis on the image. Cropping the image is destructive to the image file and is recorded in the history.

 NOTE: If you are not satisfied with the crop, select Undo in the Edit menu and try again.



The Lanes Panel

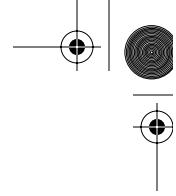
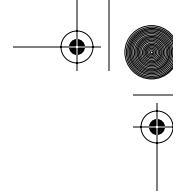
The Lanes panel walks you through the process of lane analysis of gels and blots for intensity and mobility. If you have designated a standard, molecular weight, and mass is calculated.



Lanes Panel Buttons

- ✓ *Set Search Area*—defines a new lane set. When this button is selected, a selection rectangle appears on-screen that can be edited to define the area you want to analyze. The Rectangle Selection tool is automatically activated which allows you to draw a new selection rectangle or edit the default selection on-screen.
- ✓ *New Lane Set*—automatically finds lanes within an the rectangle selection defined. You can overlap lane sets and also have the ability to make an unlimited number of lane sets in an image.
- ✓ *Find Bands*—automatically creates a median profile of each lane, identifies bands, and generates band data.
- ✓ *Set Standards*—opens the Lane Information dialog box. where you can define one or more lanes as standards for analysis, set the amount of the standard loaded, and name the experimental lanes. Each Lane Set must have it's own standard lane(s).
- ✓ *Set Background*—to choose how the background is calculated. Options include using the polynomial background of the Gaussian Fit, interpolate between background points, use a line fit, or select a constant value.
- ✓ *Mass Curve*—once you have defined standard lanes, you can select the Mass Curve command to open the Mass Curve dialog box. The Mass Curve displays an interactive plot of the standard data which is used to calculate the experimental mass values (net intensity versus standard band mass). You can optimize the fit function to the data and to choose which bands to be included in the determination of mass.

- ✓ *Gaussian Fit Bands*—models the data as Gaussian and more accurately predict their mass. Data modeling may be particularly applicable in determining values for unresolved bands, over-saturated bands, and/or for images with uneven or high background.
- ✓ *Templates*—accesses the Lane Templates dialog box where you can create and apply a template from a defined lane set to a new image. The lane template recalls the number of lanes, lane designations (standard, experimental, inactive), and/or load amounts. This is especially useful if you routinely run a similar experiments.
- ✓ *Adjust Lanes*—opens the Adjust Lanes dialog box, which allows you to change the lane finding sensitivity, lane finding algorithm, and number of lanes. The algorithms correct for anomalies such as curved or slanted lanes.
- ✓ *Adjust Bands*—opens the Adjust Bands dialog box. Use the Band Sensitivity arrows to adjust the band finding sensitivity. Adjust the percentage of the Profile Width to encompass the entire band.
- ✓ *Adjust Gaussian Fit*—allows you to choose between either Gaussian or asymmetric Gaussian for a specific band or for the entire Lane Set using the Band Information dialog box.
- ✓ *Set Iso-MW Lines*—offer control points (on each lane) that can be used to accurately adjust for any localized mobility variations. When the Set Iso-MW Lines button is pressed the Add Iso-MW Lines dialog box opens where you can define how you want intermediate iso-molecular weight lines drawn. Options include automatically generated, manually placed, and position lines for each standard band.
- ✓ *Re-Map Standard*—accesses the Band Info dialog box where you can reassign standards to specific bands, view the mass curve and redefine fit function used for the mass calculations, and view the standard and combine band data for standard bands that did not separate.
- ✓ *Delete Lane Set*—deletes the current active lane set.
- ✓ *Delete Bands*—deletes all the bands in the active lane set. To delete a single band, select the band with the Pointer tool and press the Delete key. Shift-click to select multiple bands or click and drag the mouse over an area of the image containing more than one band and then press the Delete key.
- ✓ *View Options*—define what lane sets are displayed and what features are displayed (e.g., Lane Lines, Lane Markers, Band Labels).



The Lanes Panel Tools



Use the Zoom tools to adjust the size of the image through levels of magnification (0.25X, 0.33X, 0.50X, 1X, 1.5X, 2X, 4X, 6X, 8X, 16X, and 32X). To increase the magnification, click the image with the + Zoom tool. To reduce magnification, click the image with the - Zoom tool.

When you change the magnification with the Zoom tools, the software positions the point where you clicked in the center of the Image Section. This differs from the Magnification slider which maintains the center of image in the window.



Use the arrow-shaped Pointer tool to select Lane Sets, Lane Markers, Lane Lines, and Band Labels. Shift-click to select multiple objects with the Pointer tool or click and drag the mouse over an area of the image containing more than one object.

White Point /Black Point Tools



Use the White Point/Black Point tools to select the white or black point values used to adjust the contrast in the image. To use, click the Black Point tool over the pixel in which you want to represent black point. Click the White Point tool over the pixel in which you want the to represent the white point. The image is then remapped using these points.

NOTE: The white point and black point can also be adjusted using the Image Display window.



Selection Rectangle Tool

Click and drag the Image Selection tool to select the area of the image to be analyzed. The Selection Rectangle tool is also used to select a rectangle to make a selection for printing, exporting a selection as a TIFF or JPEG file, or copying to the clipboard.



Lane Marker Tool

Use the Lane Marker tool to mark the lane area. Drag the Lane Marker tool to manually draw a Lane Marker across the top of the image. When you release the mouse button, a dialog box prompts you to enter the number of lanes on the image. After entering the number of lanes, Lane Marker are drawn with that number of equally spaced lanes. After creating the first Lane Markers, you can use the Lane Marker tool to add additional markers. For more information on using the Lane Marker tool, see Chapter 4: *Defining Lanes*.

Add Band Label Tool

After you've used the Find Bands command to find bands in your image, you can use the Band tool to add new Band Labels. Click on the position on the lane where you want to place a band label. The band position, mobility and area of bands need to be adjusted. It is best to use the Profile window to make these adjustments.

Reference Band Tool

Use the Reference Band tool to draw an ROI that bounds a typical band within the gel. The location, width, height, and intensity of the band is automatically be used in the Find Lanes algorithms. Once a reference box is drawn, the box can be edited using any of the four corners to resize prior to choosing Find Bands. This is especially useful when the automated finding tools are not producing good results.

The Manual ROIs Panel

You can perform measurements by creating regions of interest (ROIs) on the image. To manually draw ROIs, Carestream MI Software provides tools to both area and volume measurements. Once drawn, these ROIs are editable, movable, and can be duplicated.



The Manual ROIs Buttons

- ✓ *New ROI Set*—defines a series of region of interests as a set. You have the ability to make an unlimited number of ROI sets within an image.
- ✓ *Set Standards*—opens the ROI Analysis Data window where you assign multiple ROIs as standards for the Mass Curve calculations. Select an ROI and click in the Std column in the spreadsheet. The ROI Mass window opens so that you define the name of the standard and the Total Mass in the ROI. Each ROI set must have its own set of standards.
- ✓ *Set Background*—lets you define how background for an ROI is calculated. Options include using the perimeter intensity values (median, mean, minimum, maximum) around each ROI, using any constant value, or using the median, mean, minimum or maximum intensity of a selection.
- ✓ *Mass Curve*—once you have defined your standards, select Mass Curve to open the ROI Mass Curve dialog box. Mass Curve displays an interactive plot of the standard data which is used to calculate the experimental mass values (net intensity versus mass). You can optimize the fit function to the data and can choose which ROIs to be included in the determination of mass.
- ✓ *Templates*—accesses the ROI Templates dialog box where you can create and apply a template from a defined ROI set to a new image. The ROI template recalls the number of ROIs, and Standard load amounts. This is especially useful if you routinely run a similar experiments.



- ✓ *Center ROIs*—automatically centers ROIs based on the centroid (center of mass or the 2nd moment of the intensity distribution).
- ✓ *Delete ROI Set*—deletes the active ROI set.
- ✓ *View Options*—define what ROI sets and what analysis data are displayed (e.g. ROI Boundaries, ROI IDs). In addition you can choose to show the ROI Analysis window and/or the Active ROI Info window.

The Manual ROIs Panel Tools



Zoom Tools

Use the Zoom tools to adjust the size of the image through levels of magnification (0.25X, 0.33X, 0.50X, 1X, 1.5X, 2X, 4X, 6X, 8X, 16X, and 32X). To increase the magnification, click the image with the + Zoom tool. To reduce magnification, click the image with the - Zoom tool.

When you change the magnification with the Zoom tools, the software positions the point where you clicked in the center of the Image Section. This differs from the Magnification slider which maintains the center of image in the window.



Pointer Tool

Use the arrow-shaped Pointer tool to select ROIs and to resize selected ROIs. Shift-click on multiple objects with the Pointer tool or click and drag the mouse over an area of the image containing more than one object.



White Point /Black Point Tools

Use the White Point/Black Point tools to select the white or black point values used to adjust the contrast in the image. To use, click the Black Point tool over the pixel in which you want to represent black point. Click the White Point tool over the pixel in which you want the to represent the white point. The image is then remapped using these points.

NOTE: The white point and black point can also be adjusted using the Image Display window.



Selection Rectangle Tool

Click and drag the Image Selection tool to select a rectangular selection area for printing, exporting as a TIFF or JPEG file, or copying to the clipboard.



ROI Ellipse Tool

The ROI Ellipse tool is used to draw a round or elliptically shaped ROI and is used to analyze objects like dot blots, arrays, or spots. To access ROI Ellipse tool options, double-click on the tool.



NOTE: To draw a perfect circle press, the Shift key while defining the ROI using the ROI Ellipse tool.



ROI Rectangle Tool

The ROI Rectangle tool is used to draw a box or rectangular shaped ROI and is useful when analyzing bands in a gel or blot. To access ROI Rectangle tool options, double-click on the tool.



NOTE: To draw a perfect square press the Shift key while defining the ROI using the ROI Rectangle tool.



ROI Polygon Tool

The ROI Polygon tool is used to draw a polyshape ROI and is useful for analyzing irregularly shaped objects like tissue sections or tumors. The ROI Polygon tool draws a series of line segments that are connected by points that can be edited.



ROI Free Form Tool

The ROI Free Form tool draws a continuous free form object and is useful for analyzing irregularly shaped objects like tissue sections or tumors. The free form ROI is always a closed shaped.



ROI Line Tool

The Line tool is used to draw line as ROIs. This tool can be useful when measuring distances between objects.



NOTE: To draw a straight line, press the Shift key while defining the ROI using the ROI Line tool.



Reactivate ROI Tool

The Reactivate ROI tool reactivates the selected ROI so that it can be replicated and repositioned as a new ROI.

Rotate Selected ROI Tool

The Rotate Selected ROI tool is used to rotate a selected ROI. Click on the tool and then select a grab handle on an ROI and rotate in any direction.

Magic Wand Tool

The Magic Wand tool automatically defines an ROI for you. To use, click and drag along a small portion of the edge of the ROI that you want to define. The gray level information from these pixels is used to define the boundary.

Alternately, you can click in the center of an ROI and the finder determines the edge using the threshold level value selected in the Magic Wand options. You can adjust how close the Magic Wand tool sets the threshold level with respect to its estimate of the background. To access the Magic Wand tool options, double-click on the tool.

Separate ROIs Tool

The Separate ROIs tool selects an ROI cutting tool. Click and drag across the boundary of two connected ROIs to cut. The Separate ROIs tool only works with free form ROIs drawn by the ROI Free Form tool, the Magic Wand tool or the Auto ROI methods.

The Auto ROIs Panel

You can perform measurements by creating regions of interest (ROIs) on the image.

The Auto ROIs Buttons

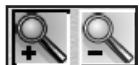


- ✓ The *Set Search Area*—defines the search area for a new ROI set. When this button is selected, a selection rectangle appears on-screen that can be edited to define the area to be analyzed. Use the Rectangle Selection tool or Ellipse Selection tool to draw a new search area.
- ✓ *New ROI Set*—launches the Auto Find panel where you can use various automatic boundary detection methods.
- ✓ *Set Standards*—opens the ROI Analysis Data window where you assign multiple ROIs as standards for the mass curve calculations. Select an ROI and click in the Standards (Std) column in the spreadsheet. The ROI Mass window opens so that you define the name of the standard and the total mass in the ROI. Each ROI set must have its own mass curve and set of standards.
- ✓ *Set Background*—lets you define how background for an ROI is calculated. Options include using the perimeter intensity values (median, mean, minimum, maximum) around each ROI, using any constant value, or using the median, mean, minimum or maximum intensity of a selection.
- ✓ *Mass Curve*—once you have defined your standards you can select Mass Curve to open the ROI Mass Curve dialog box. An interactive plot of the standard data is generated to calculate the experimental mass values (net intensity versus standard band mass). You can optimize the fit function to the data and to choose which ROIs are included in the determination of mass.
- ✓ *Templates*—accesses the ROI Templates dialog box where you can create and apply a template from a defined ROI set to a new image. The ROI template recalls the number of ROIs and standard load amounts. This is especially useful if you routinely run similar experiments.



- ✓ *Delete ROI Set*—deletes the active ROI set.
- ✓ *View Options*—defines what ROI sets and what analysis date are displayed (e.g., ROI Boundaries, ROI IDs). In addition you can choose to show the ROI Analysis window and/or the Active ROI Info window.

Auto ROIs Tools



Zoom Tools

Use the Zoom tools to adjust the size of the image through levels of magnification (0.25X, 0.33X, 0.50X, 1X, 1.5X, 2X, 4X, 6X, 8X, 16X, and 32X). To increase the magnification, click the image with the + Zoom tool. To reduce magnification, click the image with the - Zoom tool.

When you change the magnification with the Zoom tools, the software positions the point where you clicked in the center of the Image Section. This differs from the Magnification slider which maintains the center of image in the window.



Pointer Tool

Use the arrow-shaped Pointer tool to select ROI Sets and ROIs. Shift-click to select multiple objects with the Pointer tool or click and drag the mouse over an area of the image containing more than one object.



White Point /Black Point Tools

Use the White Point/Black Point tools to select the white or black point values used to adjust the contrast in the image. To use, click the Black Point tool over the pixel in which you want to represent black point. Click the White Point tool over the pixel in which you want to represent the white point. The image is then remapped using these points.



NOTE: The white point and black point can also be adjusted using the Image Display window.



Selection Rectangle Tool

Click and drag the Image Selection tool to select a rectangle to make a selection for printing, exporting a selection as a TIFF or JPEG file, or copying to the clipboard.



Reference ROI Tool

Use the Reference ROI tool to draw an ROI that bounds a typical ROI within the image. The location, width, height, and intensity of the ROI is automatically be used in the Find ROIs algorithms. Select the shape of the reference ROI (Ellipse or Rectangle) using the Reference ROI tool options. To access the options, double-click on the Reference ROI tool. This tool is especially useful when the automated finding tools are not producing good results.



Separate ROIs Tool

The Separate ROIs tool selects an ROI cutting tool. Click and drag across the boundary of two connected ROIs to cut.



Selection Ellipse Tool

Click and drag the Selection Ellipse tool to select the area of the image to be analyzed.



Rotate Selected ROI Tool

The Rotate Selected ROI tool is used to rotate a selected ROI. Click on the tool and then select a grab handle on an ROI and rotate in any direction.



Magic Wand Tool

The Magic Wand tool automatically defines an ROI for you. To use, click and drag along a small portion of the edge of the ROI that you want to define. The gray level information from these pixels is used to define the boundary.

Alternately you can click in the center of an ROI and the finder determines the edge using the threshold level value selected in the Magic Wand options. You can adjust how close the Magic Wand tool sets the threshold level with respect to its estimate of the background. To access the Magic Wand tool options, double-click on the tool.



The Grid ROIs Panel

The Grid ROI panel walks you through setting up a grid for analyzing slots, spots, arrays, or microplates that have ROIs in regularly spaced rows and columns. You can choose to apply a pre-set grid or create your own grid.

The Grid ROIs Buttons



- ✓ *Set Grid Area*—defines the size of the grid. When this button is selected, a selection rectangle appears on-screen that can be edited to define the area you want to analyze. Use the Rectangle Selection tool to draw a new area. You can create an unlimited number of grids in an image.
- ✓ *Set Reference ROI*—defines the ROI shape (ROI Ellipse or ROI Rectangle). Use the ROI Ellipse or Rectangle to draw a boundary around the ROI within the grid.
- ✓ *Make New Grid*—uses the Reference ROI and the Set Grid Area to create a grid.
- ✓ *Set Standards*—opens the ROI Analysis Data window where you assign multiple ROIs as standards for the mass curve calculations. Select an ROI and click in the Standards (Std) column in the spreadsheet. The ROI Mass window opens so that you can assign a name and total mass to a standard in the ROI. Each ROI set must have its own set of standards.
- ✓ *Set Background*—lets you define how background for an ROI is calculated. Options include using the perimeter intensity values (median, mean, minimum, maximum) around each ROI, using any constant value, or using the median, mean, minimum or maximum intensity of a selection.
- ✓ *Mass Curve*—once you have defined your standards you can select Mass Curve to open the ROI Mass Curve dialog box. Mass Curve displays an interactive plot of the standard data, which is used to calculate the experimental mass values (net intensity versus standard band mass). You can optimize the fit function to the data and to choose which ROIs to be included in the determination of mass.



- ✓ *Templates*—accesses the ROI Templates dialog box where you can create and apply a template from a defined ROI set to a new image. The ROI template recalls the number of ROIs and standard load amounts. This is especially useful if you routinely run similar experiments.
- ✓ *Center ROIs*—automatically centers ROIs based on the centroid (center of mass or the 2nd moment of the intensity distribution).
- ✓ *Delete ROI Set*—deletes the active ROI set.
- ✓ *View Options*—defines what ROI sets and what analysis data are displayed (e.g., ROI Boundaries, ROI IDs). In addition you can choose to show the ROI Analysis window and/or the Active ROI Info window.

The Grid ROIs Tools



Zoom Tools

Use the Zoom tools to adjust the size of the image through levels of magnification (0.25X, 0.33X, 0.50X, 1X, 1.5X, 2X, 4X, 6X, 8X, 16X, and 32X). To increase the magnification, click the image with the + Zoom tool. To reduce magnification, click the image with the - Zoom tool.

When you change the magnification with the Zoom tools, the software positions the point where you clicked in the center of the Image Section. This differs from the Magnification slider which maintains the center of image in the window.



Pointer Tool

Use the arrow-shaped Pointer tool to select ROI Sets and ROIs. Shift-click to select multiple objects with the Pointer tool or click and drag the mouse over an area of the image containing more than one object.



White Point /Black Point Tools

Use the White Point/Black Point tools to select the white or black point values used to adjust the contrast in the image. To use, click the Black Point tool over the pixel in which you want to represent black point. Click the White Point tool over the pixel in which you want the to represent the white point. The image is then remapped using these points.

NOTE: The white point and black point can also be adjusted using the Image Display window.



Selection Rectangle Tool

Click and drag the Image Selection tool to define the grid size. The Selection Rectangle tool is also used to select a rectangle to make a selection for printing, exporting a selection as a TIFF or JPEG file, or copying to the clipboard.



ROI Ellipse Tool

The ROI Ellipse tool is used to draw a round or elliptically shaped ROI to analyze objects like dot blots, arrays, or spots. To access ROI Ellipse tool options, double-click on the tool.



NOTE: To draw a perfect circle press the Shift key while defining the ROI using the ROI Ellipse tool.



ROI Rectangle Tool

The ROI Rectangle tool is used to draw a box or rectangular shaped ROI and is useful when analyzing bands in a gel or blot. To access ROI Rectangle tool options, double-click on the tool.



NOTE: To draw a perfect square press the Shift key while defining the ROI using the ROI Rectangle tool.



Move ROI Tool

The Move ROI tool is used to move or adjust the position of an ROI within the image. You can select multiple objects clicking and dragging the mouse over an area of the image containing more than one object.



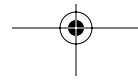
Resize All ROIs Tool

The Resize All ROIs tool is used to edit the shape of all ROIs within a grid. Click on the tool and then select an ROI and resize. All ROIs within the grid are resized.



Rotate All ROIs Tool

The Rotate All ROIs tool is used to rotate all ROIs within a grid. Click on the tool and then select a grab handle on an ROI and rotate in any direction. All ROIs within the grid are rotated.





Resize Grid Tool

The Resize Grid tool resizes the spacing of the grid. The Grid ROI is not altered.



Rotate Selected ROI Tool

The Rotate Selected ROI tool is used to rotate a selected ROI within a grid. Click on the tool and then select a grab handle on an ROI and rotate in any direction.



Resize Selected ROI Tool

The Resize Selected ROI tool is used to edit the shape of a selected ROI within a grid. Click on the tool and then select an ROI to resize. Position the tool over a control point. Click and drag the control point in the direction that you want to resize the object.



NOTE: Magnify the image if you have trouble selecting the control point.

You can select multiple ROIs by Shift-clicking objects or by dragging the Resize Selected ROI tool over the ROIs. When you resize on ROI, all selected ROIs will resize.



Magic Wand Tool

The Magic Wand tool automatically defines an ROI for you. To use, click and drag along a small portion of the edge of the ROI that you want to define. The gray level information from these pixels is used to define the boundary.

Alternately you can click in the center of an ROI and the finder determines the edge using the threshold level value selected in the Magic Wand options. You can adjust how close the Magic Wand tool sets the threshold level with respect to its estimate of the background. To access the Magic Wand tool options, double-click on the tool.



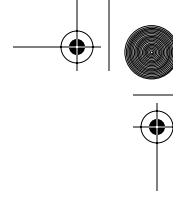
The Annotation Panel

The Annotation window provides you with a copy of the image for adding comments, labeling, or preparing the image for publication.



The Annotation Panel Buttons

- ✓ **Add Analysis Image**—adds an image to the Annotation window. When selecting Add Analysis Image, the Annotation Image Layout dialog box opens. You can customize the size, zoom in on features of interest, and choose the portion of the image you want to display.
- ☞ NOTE: You can cut and paste any image from any project or source, i.e., Powerpoint, Photoshop, JPG or TIFF)
- ✓ **Add Analysis Data**—opens the Analysis window and allows you to copy the data from the Analysis worksheet as individual values or as columns of data.
- ✓ **Add Profile**—appends a lane profile to the image. The lane profile is only available if the image has been analyzed for lanes and bands. The profile that was appended to the image is the lane(s) that is displayed in the Profile window prior to entering the Annotation mode.
- ✓ **Add Mass Diagram**—displays the mass standard curve in your annotations, if you analyzed your image and assigned mass standards.
- ✓ **Add Annotation Bar**—When acquiring images using Carestream Imaging Systems, you have the option to append an Annotation bar to your image. Once you have made the selection to append an Annotation bar, the information can be edited in the Annotation window.
- ✓ **Add Intensity Scale**—provides a visual index of intensities. The color index is defined in the Advanced Image Display window.



- ✓ *Align Objects*—lines up selected objects precisely along a horizontal or vertical axis, as well as distributes them evenly across a horizontal or vertical axis. If you are aligning to the right, all selected objects align to the right edge of the selected object furthest to the right.
- ✓ *Group*—takes individual objects and fuses them into a single unit that can be moved as one object.
- ✓ *Ungroup*—separates the “fused” objects that have been grouped. Each individual object can be moved independently.
- ✓ *Bring to Front*—you can change the stacking order of objects that are overlapped. The Bring to Front button rearranges the stacking order, placing the selected object on top of the stack.
- ✓  *Send to Back*—you can change the stacking order of objects that are overlapped. The Send to Back button rearranges the stacking order, placing the selected object behind all other objects in the stack.

The Annotation Panel Tools

Zoom Tools

Use the Zoom tools to adjust the size of the image through levels of magnification (0.25X, 0.33X, 0.5X, 0.75X, 1X, 1.25X, 1.5X, 1.75X, 2X, 2.5X, 3X, 4X, 6X, and 8X). To increase the magnification, click the image with the + Zoom tool. To reduce magnification, click the image with the - Zoom tool.



When you change the magnification with the Zoom tools, the software positions the point where you clicked in the center of the Image Section. This differs from the Magnification slider which maintains the center of image in the window.

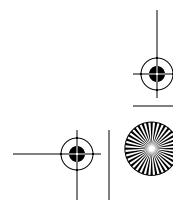
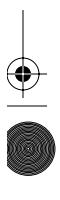
Pointer Tool



Use the arrow-shaped Pointer tool to select objects to move or resize. Shift-click on multiple objects with the Pointer tool or drag the mouse over an area of the image containing more than one object.

Text Tool

Use the Text tool to annotate with image using text. You can select custom fonts, sizes, styles, and rotation of the text. To use, select the Text tool, define the area in which you want the label to appear by clicking and dragging on the image.



 **Line Tool**

The Line tool is designed to draw lines, arrows, or brackets on the image.

 **Ellipse Tool**

The Ellipse tool is designed to draw circles or ovals which can either be filled, framed, or filled and framed. Double click on the Ellipse tool to access drawing options.

 **Rectangle Tool**

The Rectangle tool is designed to draw boxes which can either be filled, framed, or filled and framed. Double click on the Rectangle tool to access drawing options.

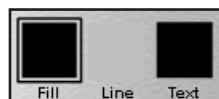
 **Crop Tool**

The Crop tool masks part of the object using a rectangle to trim the edges of the image so they are not displayed.

 **Dropper Tool**

Use the Dropper tool to select a new foreground or background color by clicking the Dropper tool on the object.

 **NOTE:** The currently selected color (as shown by the frame) corresponds to whether or not the Dropper tool selects a foreground or background color.


Fill, Line, and Text Color Options

Use the Fill, Line or Text Color option buttons to define color.

The Database Panel

When clicking Database on the Navigation panel the Security Manager is launched. The Security Manager provides easy to use interface for setting up accounts, permission levels, project and studies. In addition, you can use the Search menu to:

- ✓ Search for files—provides search criteria for specific images or data from within files using the Search criteria in the Search window.
- ✓ Gel Comparison—compares the presence or absence of bands across multiple gels, and displays the results of the analysis in a sorted order (closest match at top of list).

Differential Display—analysis uses one lane as a reference and compares the reference lane to all the other lanes in the file. Lanes are compared on a band by band basis to determine if the band masses (or if no mass calibration is available, the band net intensities) are increasing or decreasing.

Adjusting Magnification

Drag the slider to the left to decrease the magnification and to the right to increase the magnification of the image.



Magnification levels are 0.25X, 0.33X, 0.5X, 1X, 1.5X, 2X, 4X, 6X, 8X, 16X, and 32X in all panels except the Annotation panel. The Annotation panel levels are 0.25X, 0.33X, 0.5X, 0.75X, 1X, 1.25X, 1.5X, 1.75X, 2X, 2.5X, 3X, 4X, 6X, and 8X.

 NOTE: The Magnification slider maintains the center of the image. This differs from the Zoom tools, which maintains the center position based on where the tool is clicked.

The Quick Access Bar

The Quick Access bar is designed to provide easy navigation to commonly used windows and is located above the Image Section.



- ✓ *Navigation*—displays the Navigation panel that guides you through the analysis of images.
- ✓ *Image Display*—opens the Image Display window where you can adjust the appearance of the on-screen and printed image.
- ✓ *Profile*—displays the Profile window (median profile of the lanes) if lane analysis has been completed. Use the profile to compare experimental and standard lanes, or edit the bands defined by the software.
- ✓ *Analysis*—opens the Lane/ROI Analysis windows displaying analysis results for the current image.
- ✓ *Overview*—displays the scaled version of the entire acquired image. A red box identifies the area shown in the Image Section.
- ✓ *Info History*—opens the File Info dialog box and displays the history tab.
- ✓ *Preferences*—opens the Carestream MI Preferences window.



The Status Bar

The Status bar provides easy access to useful functionality as you work through your project. The Status bar changes as you move through the various steps of the analysis.

Signal: 165.000	Int: 32.000	X: 11 pix	Y: 153 pix
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- ✓ *Signal*—indicates the amount of light captured by each pixel. The signal values correspond to information in the Image Display window. These values are dependent on the bit depth of the image, i.e., 8-bit has values from 0–255.
- ✓ *Int*—indicates the intensity of the pixel selected using any tool. For calculation purposes, the software assumes that the presence of a band or ROIs has a positive value relative to the local background. For black bands/ROIs, the intensity value differs from the signal value.
- ✓ *X* and *Y*—coordinates indicate the horizontal and vertical position of the cursor on the image. Units are defined in the Preferences dialog box.

Depending on the task you are performing, you may encounter additional status fields, including:

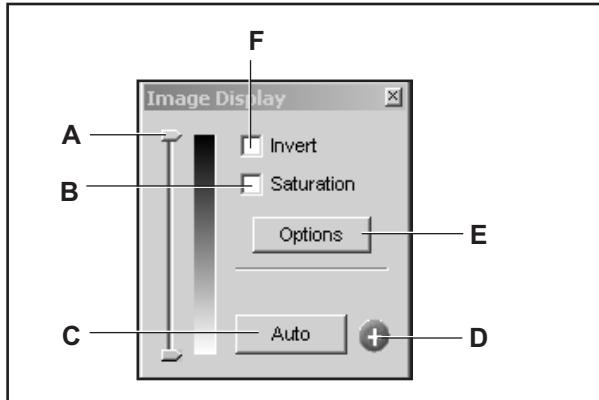
- ✓ *W* = provides the width of a selected area. Units are defined in the Preferences dialog box.
- ✓ *H* = provides the height of a selected area. Units are defined in the Preferences dialog box.
- ✓ *Band*—identifies the specific band that has been selected and is only available after you have found bands.
- ✓ *MW* and *Mass*—reflect molecular weight and mass for the selected band and are only activated after you have found bands.
- ✓ *ROI*—identifies the specific ROI that has been selected. In addition, it reports the total number of ROIs found.

The Image Display Windows

You can adjust the appearance of the on-screen and printed image to make faint features easier to see. The adjustments you make affect the on-screen and printed image only. These adjustments do not affect the image data. To access, choose Image Display from the Quick Access bar, select Image Display from the Image panel, or choose Image Display from the Show menu.

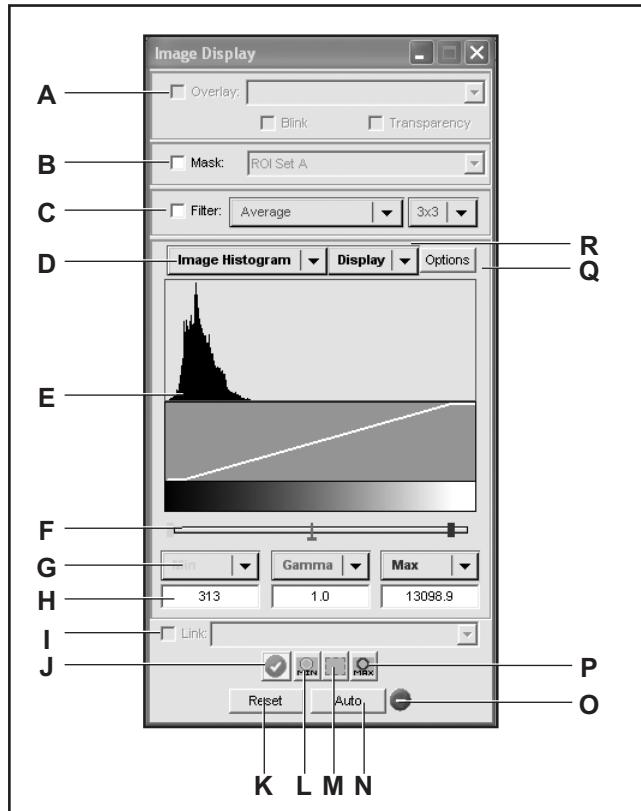
The Image Display is available as either a Basic Image Display window or an Advanced Image Display window. The Basic Image Display window, the default option, is designed to provide the most commonly used functions including brightness/contrast, inverting of the image, and saturation display. The Advanced Image Display window provides more comprehensive set of tools. The +/- icon toggles to the Advanced Image Display window. The Options button allows you to change the default option.

The Basic Image Display

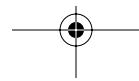
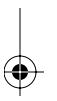


- A *Contrast sliders* adjust contrast on the on-screen and printed image. The top slider adjusts the minimum display value and the bottom slider adjusts the maximum display value.
- B *Saturation checkbox* shows any saturated pixels in the image in red.
- C *Auto (Contrast) button* chooses optimal white and black points that maximize the appearance of the image.
- D *+/- icon* toggles between the Basic Image Display window and the Advanced Image Display window.
- E *Options* offers preferences for using the Image Display window.
- F *Invert checkbox* reverses the intensity values; for example whites become black.

The Advanced Image Display Window



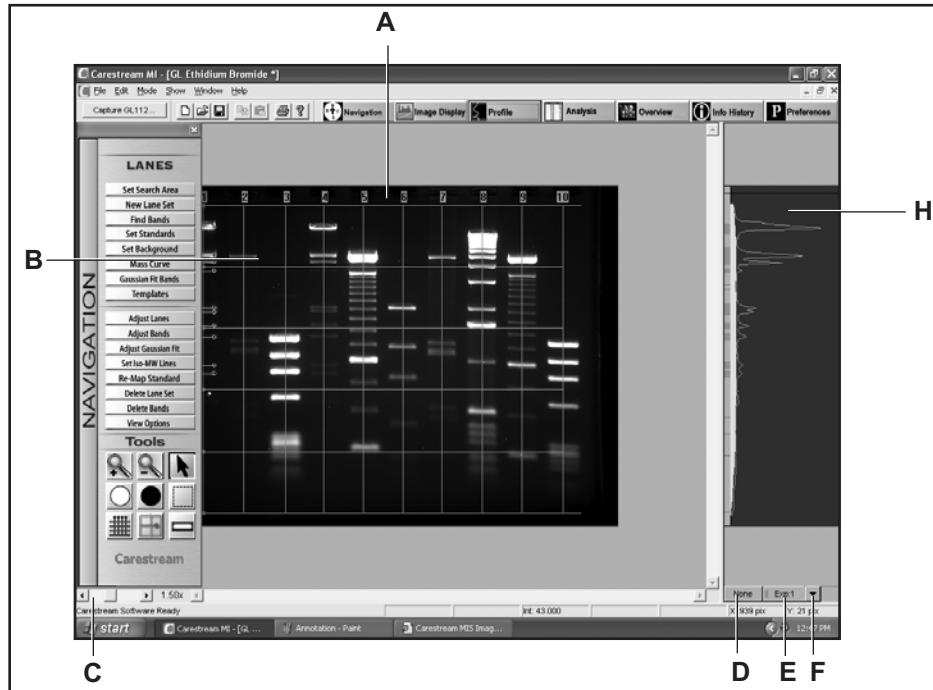
- A *Overlay checkbox* allows you to display one image on top of another image.
- B *Mask checkbox* allows you to overlay an ROI set over an image.
- C *Filter checkbox* allows you to turn on the filtering options, select a filter using the Filter pop-up menu, and the Filter Kernel pop-up menu.
- D *Image Histogram pop-up menu* allows you to select how to view the histogram, on either a linear or logarithmic scale. You can also superimpose a color palette on the histogram and control settings on how the Image Histogram is displayed.
- E *Image Histogram* displays the range of signals of the image according to the frequency distribution of pixels.



- F *Contrast slider* sets the black and white points and gamma for the displayed image.
- G *Contrast pop-up menus* allow you to adjust the black and white points and gamma settings using preset values or percentiles.
- H *Contrast text edit boxes* display and allow you to type in values for the black and white points and the gamma of an image.
- I *Link checkbox* enables you to link images together. The pop-up menu lists all the open images that you can link together. Once linked, you can adjust the image display of all the images simultaneously.
- J *Default Image Display check mark* accesses the Image Display defaults. You can set defaults by illumination type. For example, you can choose to invert all luminescent images (white on black) as new images are acquired.
- K *Reset button* returns the Image Histogram to its original state.
- L *White Point tool* allows you to set a pixel value in the image as the white point.
- M *White Point/Black Point Selection tool* takes the min/max information from an image selection and sets the Image Display min/max using these values.
- N *Auto (Contrast) button* automatically chooses the white and black points to optimize the appearance of the image.
- O *+/- icon* toggles between the Basic Image Display window and the Advanced Image Display window.
- P *Black Point tool* allows you to set a pixel value in the image as the black point.
- Q *The Option button* offers preferences for using the Image Display window.
- R *Display pop-up menu* lets you choose different pseudocolor palettes for the Image Display window.

The Profile Window

Once you define lanes and find bands, view the Profile window (median profile of a lane) by choosing Profile from the Quick Access bar. Use the profile to compare experimental and standard lanes, or edit the bands defined.



- A The *Lane Set* (labeled A) identifies current lane set displayed. You can overlap lane sets and also have the ability to make an unlimited number of lane sets in an image.
 - B The *Image Section* is where you work with the image.
 - C The *Magnification slider* provides levels of magnification. Magnification levels are 0.25X, 0.33X, 0.5X, 1X, 1.5X, 2X, 4X, 6X, 8X, 16X, and 32X.
- NOTE:** The Magnification slider maintains the center of the image. This differs from the Zoom tools which maintains the center position based on where the tool is clicked.
- D The *Std pop-up menu* is used to select a standard lane in the Profile window and highlighted in the Image section.

- E *Exp pop-up menu* is used to select an experimental lane in the Profile window and highlighted in the Image Section.
- F The *Profile pop-up menu* allows you define the appearance of the Profile.
- G The *Band Rectangle* (not shown) details the boundaries of bands in the Profile window. When a band is selected three red lines appear in the Profile window to indicate the position of the band's vertical center and upper and lower boundaries. The outer two boundaries represent the area of the band which is used for the intensity and mass measurements. The center line defines the center of signal (that is, the center of gravity) in the band rectangle and is used for the mobility and molecular weight measurements. You can adjust the both the area and the center of signal using the grab handles.
- H The *Profile window* is a median intensity profile of the selected experimental and standard lanes. Colors distinguish between standard and experimental lanes.

The Lane Analysis Data Window

The Lane Analysis Data window shows the results in a spreadsheet format. Use the Lane Set and Lanes pop-up menus and the Display dialog box in the Lane Analysis Data window to select which attributes you want to display. You can select cells in the Lane Analysis Data window, copy them, and then paste them into other programs or export them as a tab-delimited text file.

B **A**

Lane Set A Lanes Display

1: Lambda-Hi... 2: Untitled 3: Untitled 4: Untitled

	MW (bp)	Mass (ng)	MW (bp)	Mass (ng)	MW (bp)	Mass (ng)	MW (bp)
1	23130	476.9	21883.3	293	21259.9	170.4	21259.9
2	9416	194.1	9265.5	112.5	9115.1	85.52	9115.1
3	6557	135.2	6465.5	99.6	6374	55.59	6374
4	4361	88.9	4311.3	62.6	4261.5	26.49	4311.3
5	2322	47.9	2285.1	25.2	2248.2	12.56	2285.1
6	2027	41.8	1988.5	21.33	1988.5	10.04	1988.5
7	564	11.6	525.5	6.443			

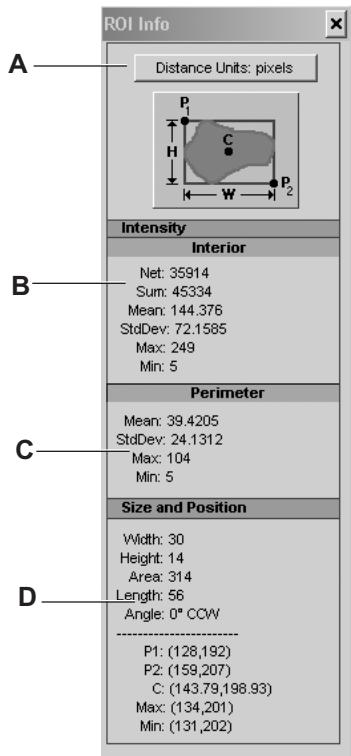
- A The *Display dialog box* allows you to choose which analysis data fields are displayed in the Lane Analysis Data window.
- B The *Lanes pop-up menu* allows you to choose the lanes you want to display.
- C The *Lane Set pop-up menu* allows you to choose the lane set you want to display. The active lane set is the default.
- D The *Lane Name text box* identifies the lane name as set in the Lanes Information dialog box.
- E *MW (bp), Mass (ng)* represent the data fields (and units) chosen in the Display dialog box.
- F *Band Labels* identify the displayed bands sequentially.
- G *Data Fields* contain the calculated data in cells. Black values are calculated using the standards curve, and are well determined measurements. Red values are calculations for bands that lie outside the molecular weight and/or mass range of the standards, and are only estimates.

The ROI Info Window

Once you have defined a region of interest, view the analysis data for the current selection using the ROI Info window.

To display the window:

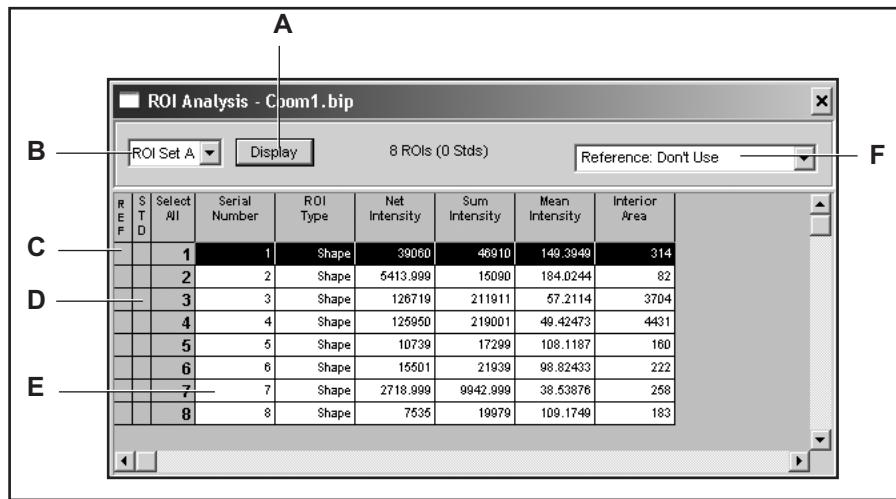
- ✓ Choose Show Active ROI from the View Options pop-up menu on the ROIs Navigation panel or the select Active ROI Info from the Show menu.



- A** *Distance Units* pop-up menu allows you to define the units for position and area measurements. Use the pop-up to choose pixels, centimeters, or inches.
- B** *Interior* provides net, sum, mean, standard deviation, maximum, and minimum intensity values of the interior of the object.
- C** *Perimeter* provides mean, standard deviation, maximum, and minimum intensity values of the perimeter of the object.
- D** *Size and Position* provides width, height, area, length, and rotation angle of the object. The data provides information on positioning of the centroid and minimum and maximum intensities.

The ROI Analysis Data Window

The ROI Analysis Data window shows all the ROI data in a spreadsheet format. You can choose what ROI set and analysis data you want to display. You can select cells in the ROI Analysis Data window, copy them, and then paste them into other programs or export a tab-delimited text file.

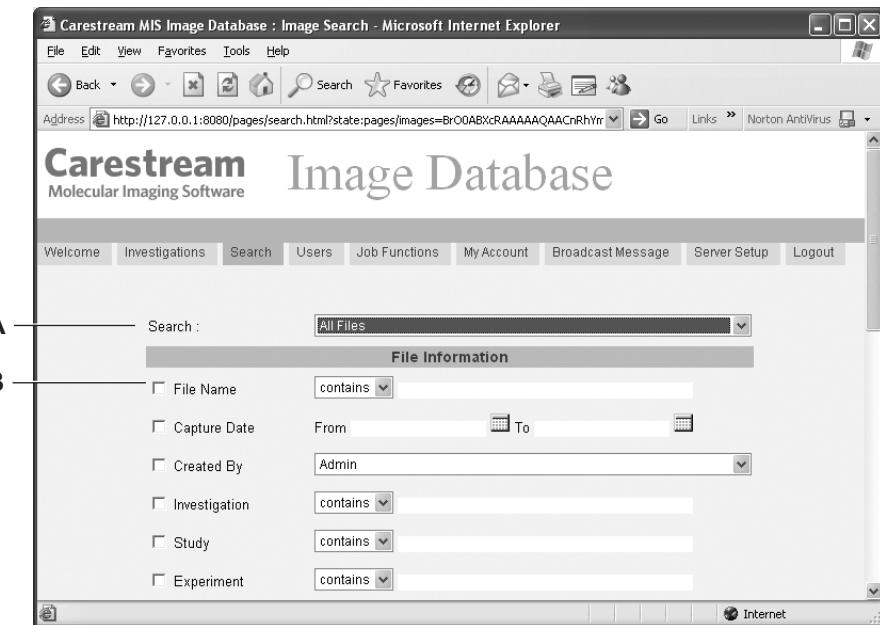


- A *Display button* accesses the Analysis Display dialog box where you can choose the type of data you want displayed in the ROI Analysis Data window. You can also choose how the background is calculated.
- B The *ROI Set pop-up menu* allows you to choose the ROI set you want to display. The active ROI set is the default.
- C *Reference Selector* designates a single ROI as a reference to measure against all other ROI's. Select a row as a reference by clicking in the adjacent REF column.
- D The *Standard Selector* designates an ROI as a standard point. Select by clicking in the adjacent STD column. A ROI Mass window opens allowing you to assign an ROI name and assign a mass load. At least two standard points are required to generate a mass curve.
- E *Data Fields* contain the measurement values and calculated data for each ROI.
- F *Reference pop-up menu* allows you to display the referenced values (if a reference has been designated) as either a ratio or a% difference. The option default is *Don't Use*.

The Image Database Windows

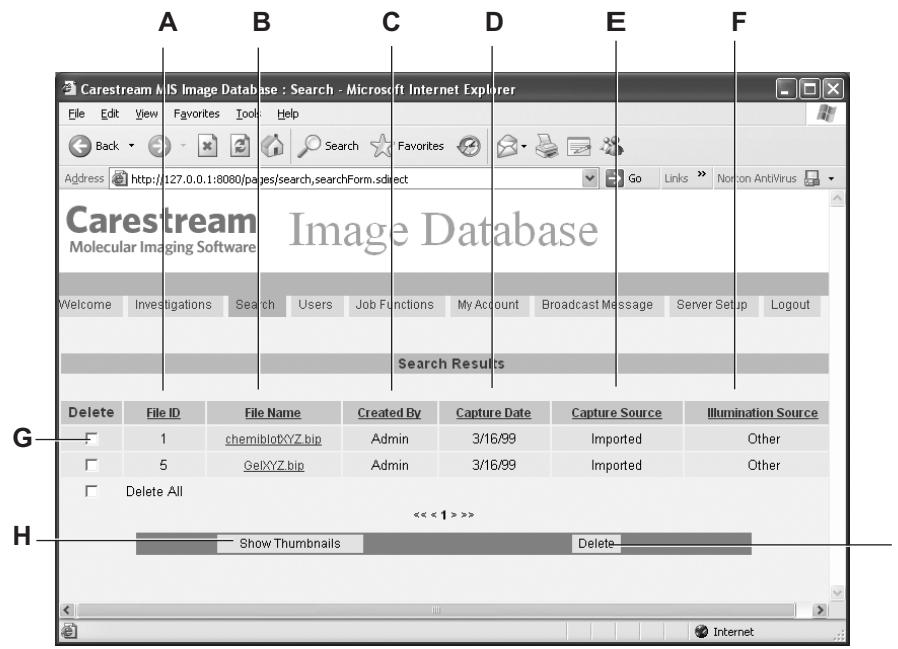
Carestream MI provides a databasing feature where you can archive important retrieval information to manage your projects. The database is made up of individual records and pointers to a source file. Information that is populated into the database record is parsed from the File Information. Once populated not only can you sort and manage projects but you can do advanced data comparisons with images containing Lane Analysis Data.

The Database Search Window



- A *Search pop-up menu* defines whether you want to search the All Files in the database or your files (My Files Only) if users have been set up. You can further refine your search criteria using the search field in the dialog box.
- B *Search Criteria* defines parameters you want to use to find specific projects.
- C *Search button (not shown)* initiates the search using criteria you have defined.
- D *Reset button (not shown)* restores the window to default selections.

The Database Search Results Window



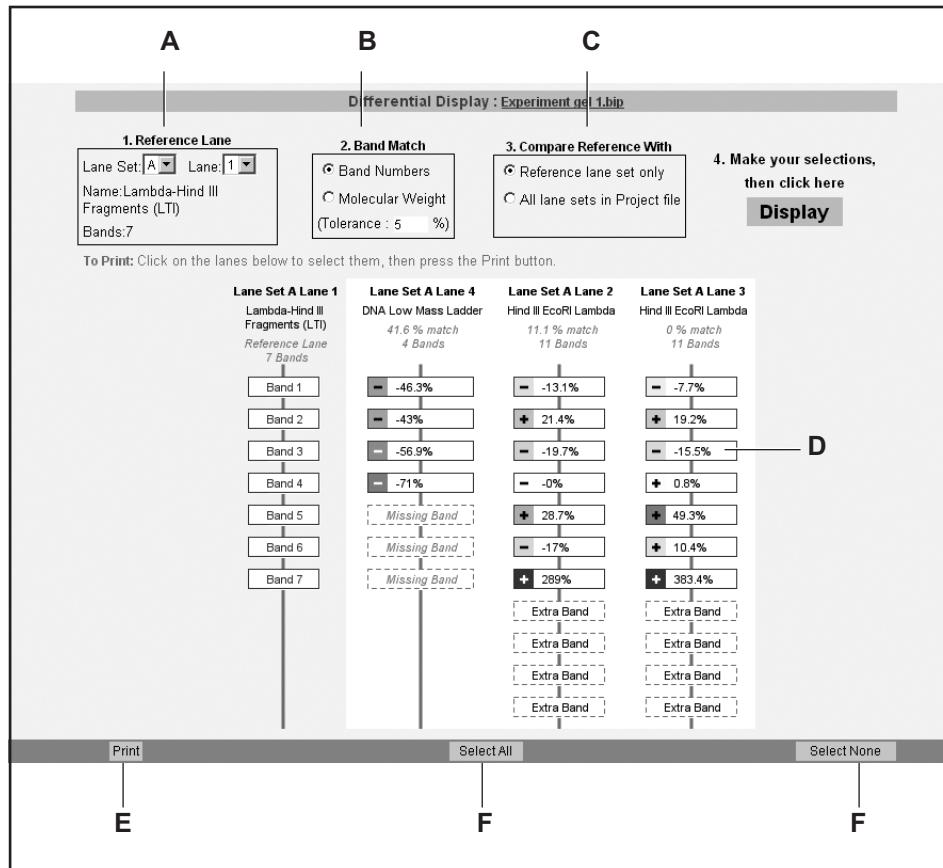
- A *File ID*—is a unique identifier that every image is automatically assigned.
- B *File Name*—identifies the image that you have selected in the Search Results window. Clicking on the File Name opens the File Information window.
- C *Created By*—identifies the user that originally saved the file.
- D *Capture Date*—identifies the date that the file was originally saved.
- E *Capture Source*—lists the Carestream camera type that was used to capture the image.
- F *Illumination Source*—lists the illumination method used when capturing the image.
- G *Delete*—marks the project for deletion from the database. Use the Delete All checkbox to mark all the files found for deletion. This action does not eliminate the file from the server but only eliminates the data from the database and relinquishes the control from the database.
- H *Thumbnails (Hide or Show)* is a visual display of your file.
- I *Delete*—eliminates the project from the database.

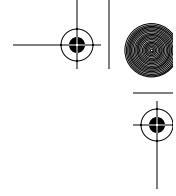
The Differential Display Window

Differential Display uses one lane as a reference and compares the reference lane to all the other lanes in the image. Lanes are compared on a band by band basis to determine if the band masses (or if no mass calibration is available, the band net intensities) are increasing or decreasing.

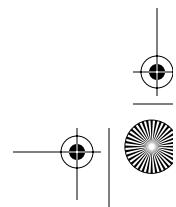
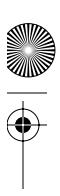
You must have an image open with lane analysis complete. In addition, if there is a selected lane, then the selected lane is designated as the reference lane, otherwise Lane 1 of the active lane set is the default reference lane. To launch, select Differential Display from the Database panel or from the Mode menu.

 NOTE: Lanes must contain band data to get valid results. If your lane set contains empty lanes make these lanes inactive.





- A *The Reference Lane box* displays the selected Reference Lane (if any, or else lane 1) and allows you to select the Reference Lane and the Reference Lane Set.
- B *The Band Match box* controls how band matching is accomplished. If “Band Numbers” is selected, then bands with the same number in the reference set are compared with bands of the same number in the comparison set. If molecular weight is selected, then the molecular weights of each band are compared to within the given tolerance level.
- C *The Compare Reference With box* selects whether or not you want to generate a plot using all the lanes in the lane set or just the lanes of the reference lane set.
- D The *Band boxes* along the lane line show each match and the percent difference in net intensity (or band mass) between the reference bands and the comparison bands. The boxes are scored and colored according to the following rules:
- ✓ If a band in the comparison lane matches a band in the reference lane—a box is drawn at position of reference lane band. When a standard is used the band masses are used to find the% difference, otherwise the net intensity is used. The box is shaded red if percent is negative, with pure red for 100%, 50% red for a50% difference, etc. The box is shaded blue if percent is positive, with pure blue for 100% or greater, 50% blue for a 0% difference, etc.
 - ✓ If a band is in the comparison lane but not in the band reference lane—a box is drawn at position of comparison lane band. The box is green and labeled as extra band.
 - ✓ If a band is in the reference lane but no band is in comparison lane—a box is drawn at the position of reference lane band. The box is black and is the band is labeled missing.
 - ✓ The overall score for each lane is the sum of the absolute values of the percent differences for case 1 bands, plus 100 for every missing or extra band. Therefore, if the max score is zero, the match is 100%—all the lanes are identical.
- E The *Print* button prints the selected lanes.
- F The *Select All* and *Select None* selects all or non of the lanes.

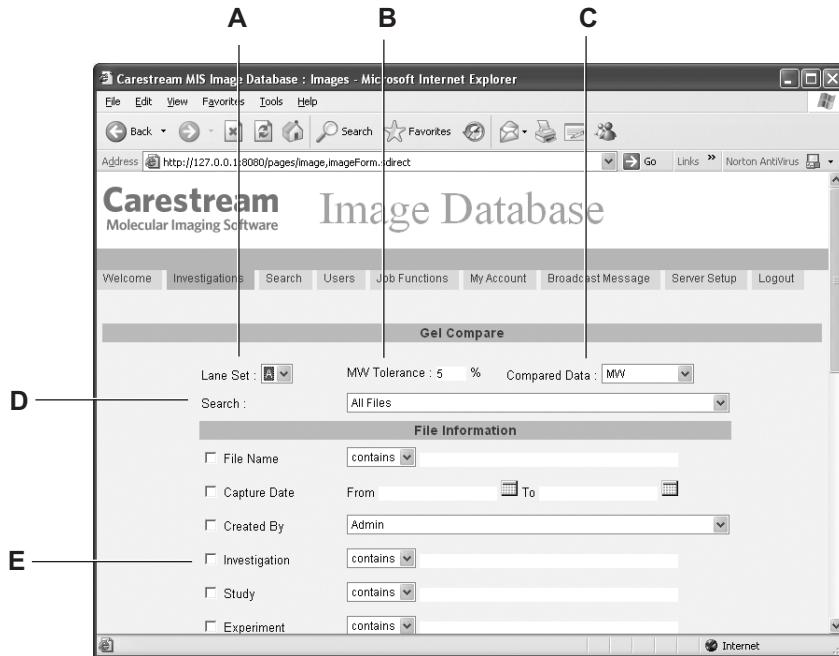


The Gel Comparison Windows

Gel Comparisons compare the presence or absence of bands across multiple gels, and displays the results of the analysis in a sorted order (closest match at top of list).

The Gel Comparison Search Window

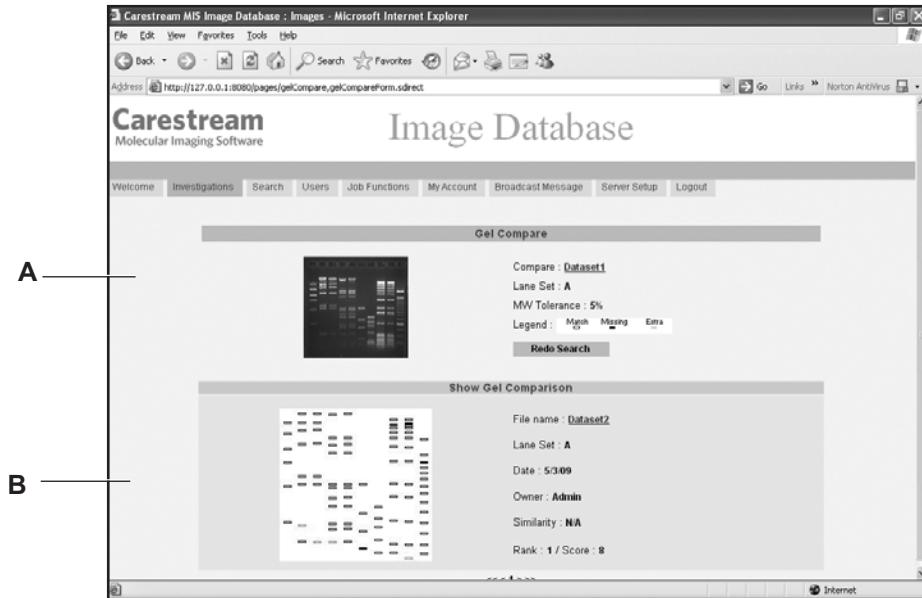
To launch, select Gel Comparison from the Database panel or from the Mode menu. The active project is assigned as the reference gel.



- A The *Lane Set* pop-up menu allows you to select the Lane Set(s) want to compare as the reference.
- B *MW Tolerance* sets the molecular weight tolerance that the gel comparison algorithms used to determine if individual bands are matches. By default this tolerance is set to 5%.
- C *Compared Data* lets you select synthetic molecular weight measurement.
- D *Search* pop-up menu defines whether you want to search the All Files in the database or your files (My Files Only) if users have been set up. You can further refine your search criteria using the search field in the dialog box.
- E *Search Criteria* defines parameters you want to use to find specific projects.
- F *Display button* (not shown) initiates the search using criteria you have defined.
- G *Reset button* (not shown) clears any selections you have made.

The Gel Comparison Results Window

Following initiation of a Gel Comparison search, the results appear in the Gel Comparison window.



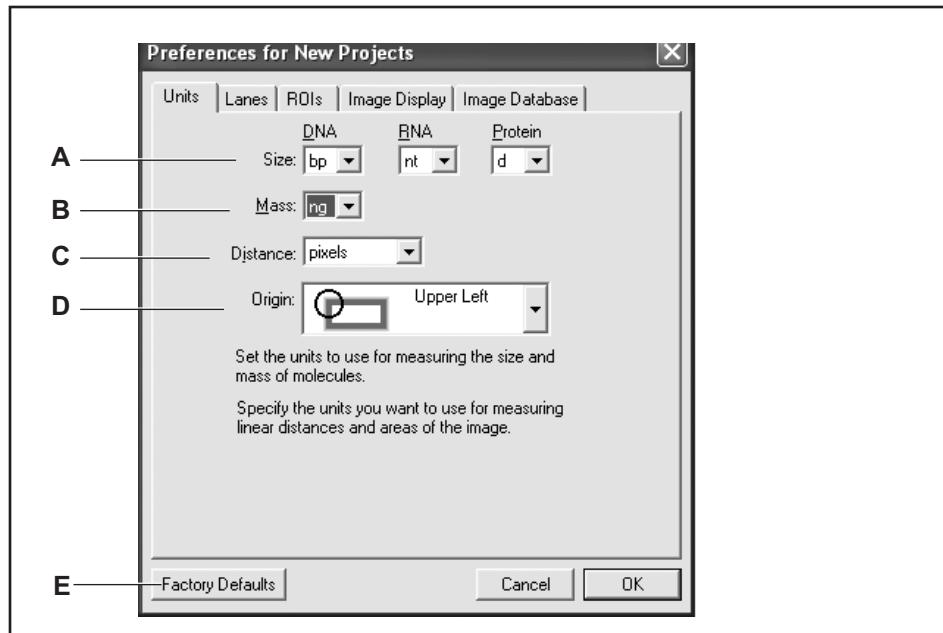
- A The *Reference Image* to which you want to compare all the other images, is placed at the top of the windows.
- B The *Results* —provides tools to scroll through the set of comparison gels and visually be able to see the differences. Each gel is identified with key information including the name of the image, lane set, date the image was created and the% similarity. The color of the bands correspond to whether or not they appear in both gels or are unique to one of the comparison gels.
 - open (filled with white) corresponding to bands in both the reference gel and comparison gel.
 - black bands corresponding to bands only found in the reference gel.
 - green bands corresponding to bands that are only found in the comparison gel.

The New Project Preferences Window

For all projects, you can set standard setting using Preferences. The Preferences are divided into five tabs. You can set a preference for an individual project or can apply a set of preferences to any new projects.

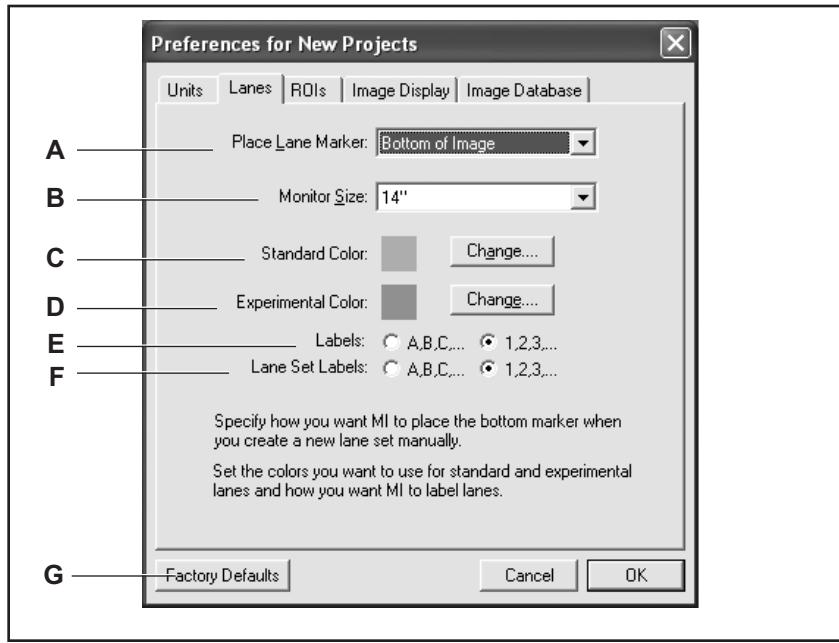
- ✓ To set the Preferences for all new projects—choose New Project Preferences from the Edit menu or click on the Preferences button on the Quick Access bar when no projects are open.
- ✓ To change the Preferences for the open project—choose Project Preferences from the Edit menu or click on the Preferences button on the Quick Access bar with the project open and in the active window.

The Units Preferences Tab



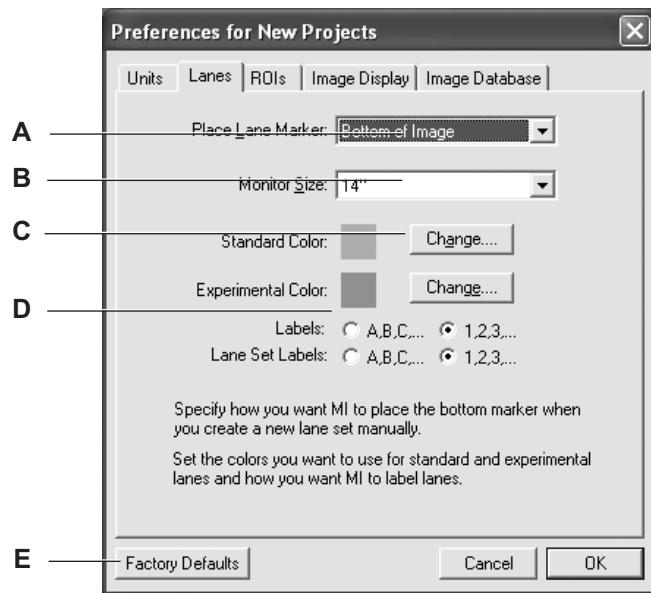
- A *Size pop-up menu* specifies the units to use for DNA, RNA, and protein molecular weights.
- B *Mass pop-up menu* specifies the units for DNA, RNA, and protein masses.
- C *Distance pop-up menu* defines units for distance measurements like mobility or the size of a selection in the image.
- D *Origin pop-up menu* defines the 0,0 pixel location.
- E *Factory Defaults button* resets the preferences to the shipped default settings.

The Lanes Preferences Tab



- A *Place Lane Marker* pop-up menu specifies whether you want the bottom Lane Marker to be placed at the bottom of the image or at the bottom of the screen when you use the Lane Marker tool on the image.
- B *Monitor Size* pop-up menu allows you to define your monitor size.
- C *Standard Color* pop-up menu specifies which of 16 colors designating standard lanes.
- D *Experimental Color* pop-up menu specifies which of 16 colors designating experimental lanes.
- E *Labels* buttons specifies the whether lanes are labeled alphabetically or numerically.
- F *Lane Set Labels* buttons specifies the whether lane sets are labeled alphabetically or numerically.
- G *Factory Defaults* button resets the preferences to the shipped default settings.

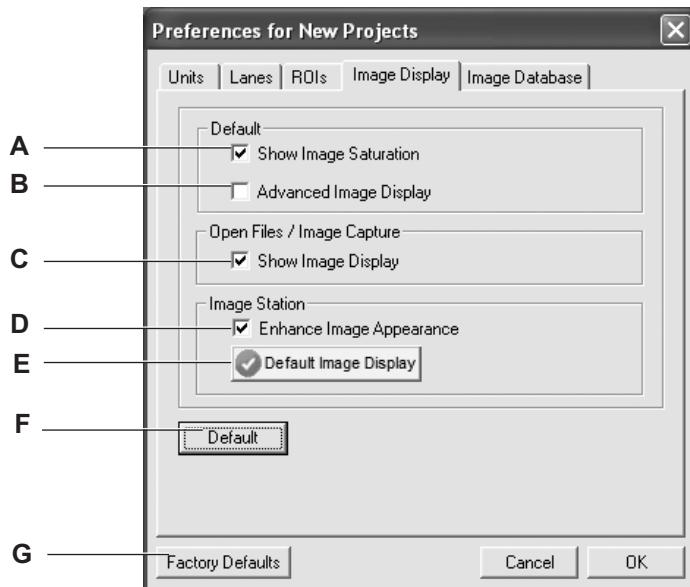
The ROI Preferences Tab



- A *Standard Color pop-up menu* specifies which of 16 colors designating standard ROIs.
- B *Experimental Color pop-up menu* specifies which of 16 colors designating experimental ROIs.
- C *Reference Color pop-up menu* specifies which of 16 colors designating single references ROIs.
- D *ROI Set Labels buttons* specifies whether ROI sets are labeled alphabetically or numerically.
- E *Factory Defaults button* resets the preferences to the shipped default settings.

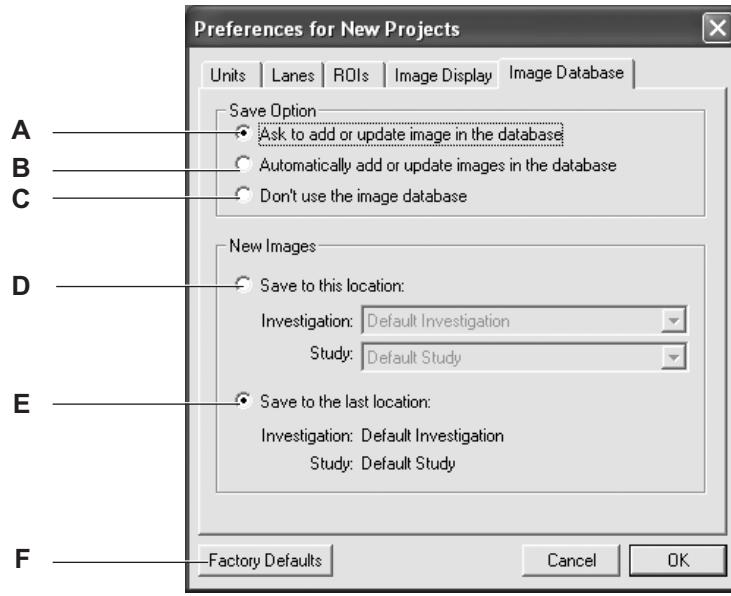
The Image Display Preferences Tab

The Image Display Preferences provide you a way to choose between Basic or Advanced features of the Image Display, the preferences can automatically apply image display functions to images as they are submitted from Image Station or In-Vivo Imaging Systems.



- A Select *Show Image Saturation* to automatically show saturated pixels on-screen when opening any new image.
- B Select *Advanced Image Display* to make the Advanced Image Display window the default.
- C Select whether or not you want to automatically launch the Image Display window when a new file is opened or new image is captured using the *Open Files/Image Capture* checkbox.
- D Select the *Enhance Image Appearance* option to improve on-screen or printed images captured with Image Station and In-Vivo Systems.
- E *Default Image Display* accesses the Image Display defaults. You can set defaults by illumination type. For example, you can choose to invert all luminescent images (white on black) as new images are acquired.
- F *Default button* sets your selections as the default.
- G *Factory Defaults button* resets the preferences to the shipped default settings.

The Image Database Preferences Tab

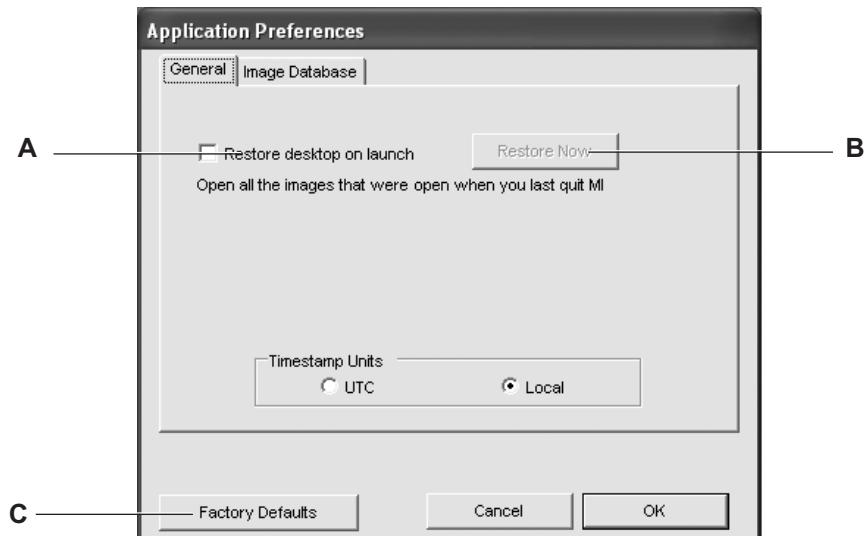


- A *Ask to add or update image in the database* selection prompts you every time you save an image if you want to save the image to the database.
- B *Automatically add or update images in the database* does not prompt you but saves and updates the database whenever an image is saved.
- C *Don't use the database* does not save any images or info in the database and cannot be searched.
- D *Save to this location* enables you to select the destination folder to save all new images. The images are saved to the selected investigation and study.
- E *Save to the last location* saves the files to the last saved destination folder. The images are saved to the selected investigation and study.
- F *Factory Defaults button* resets the preferences to the shipped default settings.

Application Preferences Window

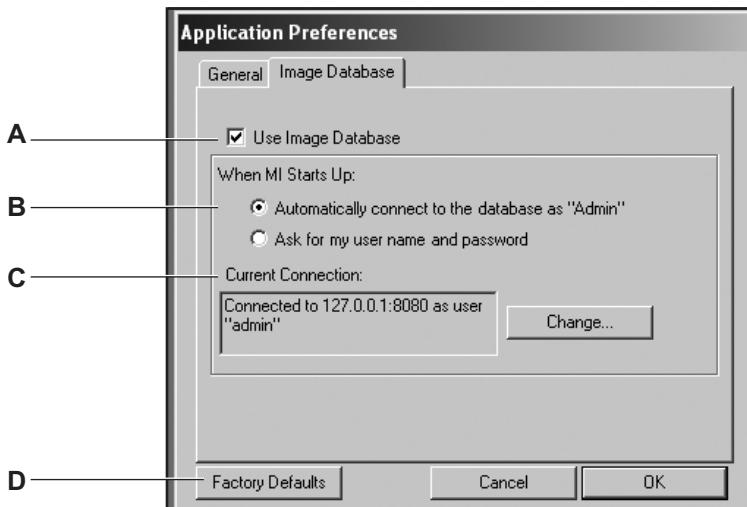
You can set application setting using the Applications Preferences window. The Application Preferences are divided into two tabs—General and Image Database. To access these Preferences, choose Application Preferences from the Edit menu when no projects are open in the MI Software window.

The General Tab



- A Select *Restore desktop on launch* to automatically restore your MI desktop and open any images that you had open to the state in which you last exited the software.
- B Click *Restore Now* restores your MI desktop and open any images that you had open to the state in which you last exited the software.
- C *Factory Defaults* button resets the preferences to the shipped default settings.

The Image Database Tab



- A *Use Image Database* checkbox to include the database in your workflow.
- B *When MI Starts Up* options provides you the option to open the database automatically or requiring a sign in.
 - ✓ *Automatically connect to the database as “Admin”* bypasses user log-in and database is open automatically.
 - ✓ *Ask for my user name and password* prompts for users to enter their name and password when launching the database. The users are established by the Administrator using Security Manager.
- C *Current Connection* indicates the location of the MI database. Use the *Change* button to redirect the software to a new location of the MI database you would like to connect when launching MI.
- D *Factory Defaults* button resets the preferences to the shipped default settings.

3*Optimizing Images*

Optimizing Images

Once an image is acquired, a new project opens and the image is displayed in the Project window. From within the Project window you can crop, rotate, or adjust how the image is displayed using the Image panel. Let's Review the Image panel.



- ✓ *Image Display*—opens the Image Display window where you can adjust the appearance of the on-screen and printed image.
- ✓ *Rotate 90° CW*—rotates the image 90° clockwise.
- ✓ *Rotate 90° CCW*—rotates the image 90° counter-clockwise.
- ✓ *Flip Horizontal*—flips the image horizontally.
- ✓ *Flip Vertical*—flips the image vertically.
- ✓ *Signal Orientation*—defines the feature of interest as either white or black. This preference affects the finding algorithms.
- ✓ *Image Math*—opens the Image Math dialog box. You can perform complex calculations on a single or pair of images. The resulting image becomes a new project, with Image History documenting how you created the image. Image Math has three different types of options—Tasks, Formula, and Image Processing Filters.
- ✓ *Image Corrections*—applies a lens or illumination correction to an Image Station 1000, 2000 or 4000 image.
- ✓ *Info/History*—opens the File Information/History window, which stores archival information concerning the project. In addition, a History file is maintained tracking any changes that are destructive to the image file, e.g., cropping.
- ✓ *Tools*—provides you the tools you need to perform functions within the Image panel.



Image Display Concepts

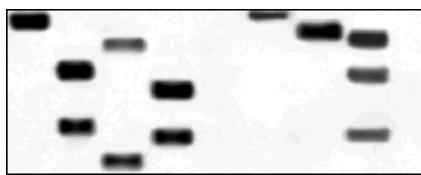
Photographs display an infinite number of shades, ranging from black to white. These shades are continuously blended together without interruption. Unlike the human eye, a computer cannot interpret continuous tone images. Digital systems translate grayscale information of an image into numbers. The digitization process is best described as placing an invisible grid over the image and interpreting the brightness, contrast, and color of the image at each grid location. The resulting numbers from each grid location are assigned to a pixel. Information from all the pixels in an image are grouped together to create a pixel matrix. This matrix contains information on:

- ✓ Coordinate location—defining the row and column (X,Y) of each pixel in the matrix.
- ✓ Brightness information—intensity information at each pixel location.

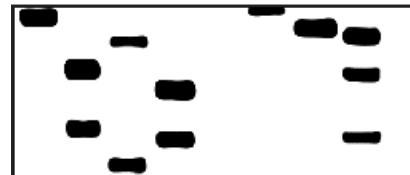
Carestream MI Software, similar to other programs, uses filtering techniques to enhance and manipulate these pixel matrices. The Image Display window allows you to alter the appearance of your image by adjusting the contrast and gamma of your image by applying a pseudocolor palette or applying other assorted predefined filters.

 NOTE: The adjustments made in the Image Display window affect the on-screen, exported, and printed image only. These adjustments do not affect the image data

The Image Display window presents the intensity data as a histogram, measured and illustrated in a graphical form. Adjusting the brightness and contrast of an image changes the shape of the histogram.

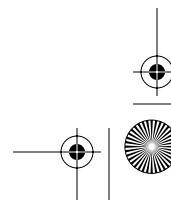
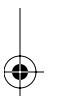


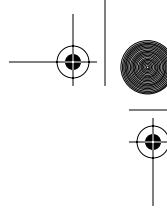
Low Contrast Image



High Contrast Image

Adjusting the brightness and contrast, as with all options within the Image Display window affects the visual display while maintaining the image data integrity. You can also adjust the gamma of the image. Gamma is a mathematical transformation function that can be used to improve image appearance by decreasing or increasing the contrast of an element of interest in an image.

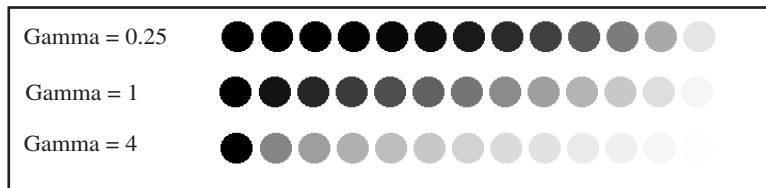




3

Optimizing Images

Adjusting the gamma of an image disproportionately skews the gray level distribution; higher gamma values lighten the image and lower gamma values darken the image.

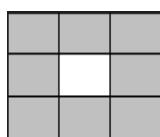


 NOTE: Adjusting the black point, white point, or gamma in the Image Display window affects the on-screen, exported, and printed image only. These adjustments do not affect the image data. When saving as a TIFF, only the image is saved; other project elements including annotations, file information, and analysis data are not saved.

The software also offers two basic types of image filtering—Single Pixel Filtering and Neighboring Pixel Filtering. These filters are designed to improve the appearance, enhance a particular feature, or add special effects to the visual appearance of your image.

Single Pixel Filtering—performs a mathematical or logical calculation on individual pixels, with the resulting value substituted back into the image for display. For example, the Invert operation reverses the intensity values of each pixel, i.e., whites become black.

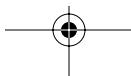
Neighboring Pixel Filtering—performs mathematical or logical calculations on groups of pixels. Neighborhood filtering differs from single point pixel processing since the filtering uses information from adjoining pixels to perform mathematical calculations on the group of pixels surrounding each pixel. The number of pixels involved in a calculation are defined by the filter kernel. The software performs these pixel calculations on 3 x 3, 5 x 5, or 7 x 7 groups of neighboring pixels.



For example, a 3×3 kernel evaluates the pixel with its surrounding eight neighbors (shaded in gray) to calculate a new value for the center pixel. This operation is repeated throughout image—pixel by pixel with its eight adjoining pixels.

Offers over 30 additional filters can be applied. Let's review.

- ✓ *Average*—filter determines the average pixel intensity within a neighborhood and assigns the averaged value to the pixel, resulting in a smoother image.
 - ✓ *Row and Column Average*—filters average a row or column of pixels within the neighborhood and assigns the averaged value to the pixel smoothing the image.





- ✓ *Low Pass*—filter smooths the appearance of the image by removing the high contrast edges (blurring the edges). This filter is useful for filtering out or reducing noise in the image.
- ✓ *Gaussian*—filter applies a weighted average to the pixels; adding low frequency detail.
- ✓ *High Pass*—filter enhances the high contrast edges in an image; high frequency values in the image brighten, while lower frequency portions darken resulting in a sharper image. This filter may also introduce more noise in your image.
- ✓ *Mean*—filter calculates the mean pixel value within a neighborhood and assigns the mean value to the pixel.
- ✓ *Unsharp Mask*—filter gives added emphasis to the edge detail. This filter can help improve an image that appears out of focus.
- ✓ *Laplacian*—filter enhances all edges within the image and is well suited for looking at the noise of an image.
- ✓ *Horizontal, Vertical, or Diagonal Edge*—filters look for horizontal or vertical edges. The filter takes an image and shifts it by one pixel over and subtracts it from the original.
- ✓ *Gradient North, NE, East, SE, South, SW, W, or NW*—filters are designed to highlight one of eight compass directions—creating edge line drawing.
- ✓ *Emboss North, NE, East, SE, South, SW, W, or NW*—filters enhance the edge, making the image features appear as raised 3D objects. The eight different directions indicate of angle for embossing.
- ✓ *Median*—filter determines the median value within a neighborhood and assigns the median value to the pixel. The median filter despeckles an image and is good for reducing noise but also causes some blurring of the image.
- ✓ *Minimum*—filter determines the darkest pixel value within a neighborhood and assigns the darkest value to the pixel.
- ✓ *Maximum*—filter determines the brightness pixel value within a neighborhood and assigns the brightest value to the pixel.
- ✓ *Normalize Histogram*—(Histogram Equalization) uses a histogram of pixel values to produce a filtered image that has more gray levels for regions of the histogram (peaks) which have the greatest number of pixels.



3*Optimizing Images*

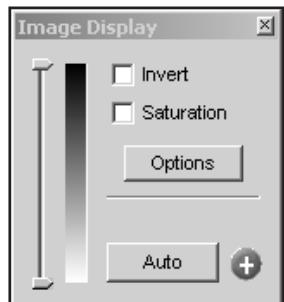
Adjusting Image Display

The Image Display window is available as either a Basic Image Display window or an Advanced Image Display window. The Basic Image Display window, the default option, is designed to provide the most commonly used functions including brightness/contrast, inverting of the image, and saturation display. The Advanced Image Display window provides more comprehensive set of tools. The +/- icon toggles to the Advanced Image Display window. The Options button allows you to change the default option.

 NOTE: The adjustments made in the Image Display window affect the on-screen, exported, and printed image only. These adjustments do not affect the image data.

The Basic Image Display Window

Adjust the image using the Basic Image Display window by:



- 1 Select the +/- icon to toggle to the Basic Image Display window. If the Image Display window is not displayed on-screen, choose Image Display from Image panel, click on the Image Display button on the Quick Access bar, or click Image Display from the Show menu.

 NOTE: Options offers preferences for using the Image Display window.

- 2 To adjust the brightness and contrast of the image, use either using the Contrast sliders or the Auto (Contrast) button.
 - ✓ *Contrast sliders*—the top slider adjusts the minimum display value and the bottom slider adjusts the maximum display value.
 - ✓ *Auto (Contrast) button*—chooses optimal white and black points that maximize the appearance of the image using the Image Histogram.
- 3 Select Invert to reverse the intensity values; for example whites become black.
- 4 Select Saturation to show any saturated pixels in the image in red.

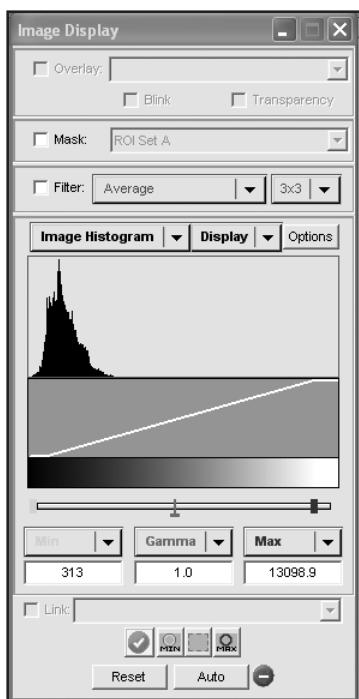
The Advanced Image Display Window

The Advanced Image Display window provides a comprehensive set of options, including brightness/contrast, gamma adjustment, filtering, and pseudocolor. The +/- icon toggles to the Basic Image Display window (default option). The Options button allows you to change the default option.

Contrasting the Image

To adjust contrast of the image:

- 1 Select the +/- icon to toggle from the Basic Image Display window to the Advanced Image Display window. If the Image Display window is not displayed on-screen, choose Image Display from Image panel, click on the Image Display button on the Quick Access bar, or click Image Display from the Show menu.
- 2 You can adjust the white, black, and gamma points in the image in several ways:



- ✓ Use the *Contrast sliders*—the left slider adjusts the minimum display value, the right slider adjusts the maximum display value, and the center slider adjusts the gamma.
- ✓ Use the *Black Point*, *Gamma*, and *White Point* text edit boxes—to assign numerical values.
- ✓ Use the *Min*, *Max*, and *Gamma*—pop-up menus to select preset choices.
- ✓ Use the *Auto (Contrast)* button—to choose optimal white and black points that maximize the appearance of the image.
- ✓ Use the *White Point* and *Black Point Dropper tools*—to assign the values by clicking on a pixel in the image—these new values are used to contrast the image.
- ✓ Use the *Black Point/White Point Selection tool*—to select the areas to use as the min/max of the selection to contrast the image.

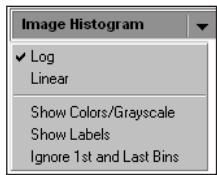
 NOTE: You can link multiple images together so that the image display setting are applied simultaneously by clicking Linked and selecting the images that apply using the pop-up menu.

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Adjusting the Image Histogram

To adjust the Image Histogram:

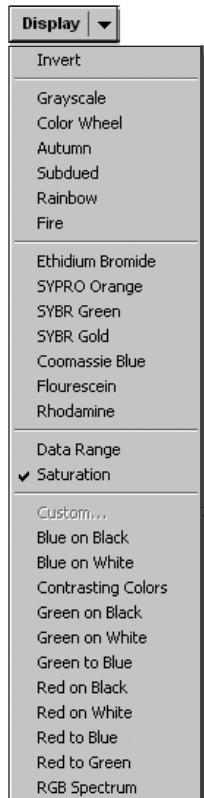


- 1 Choose the Image Histogram using the pop-up menu on the Advanced Image Display window to change the viewed histogram.
- ✓ *Log*—displays the histogram as a log function.
- ✓ *Linear*—displays the histogram as a linear function.
- ✓ *Show Colors/Grayscale*—displays the histogram in the color or grayscale color space.
- ✓ *Show Labels*—displays the units on the histogram.
- ✓ *Ignore 1st and Last Bins*—ignores the white point and black point when scaling and displaying the histogram.

NOTE: The adjustments made in the Image Histogram affect the on-screen, exported, and printed image only. These adjustments do not affect image data.

Adjusting the Display—Pseudocolor

To pseudocolor or change the color of the image as display, exported or printed:



- 1 Choose the Display pop-up menu to alter the appearance of on-screen, exported, and printed image.
- ✓ *Invert*—reverses the intensity values (white become black).
- ✓ *Grayscale*—displays the image in grayscale.
- ✓ *Color Wheel, Autumn, Subdued, Rainbow, and Fire*—assign a pseudocolor palette to the intensities in the image.
- ✓ *Ethidium Bromide, SYPRO Orange, SYBR Green, SYBR Gold, Coomassie Blue, Flourescein, and Rhodamine*—assign a pseudocolor palette based on their respective color.
- ✓ *Data Range*—highlights regions of the image that may be over-exposed (saturated) or under-exposed (data lost in background). Information in either of these regions may not be accurate.
- ✓ *Saturation*—displays saturated pixels in the image in red.

NOTE: The pseudocolor adjustment affects the on-screen, exported, and printed image only. These adjustments do not affect the image data.

- ✓ *Blue on Black, Blue on White, Contrasting Colors, Green on Black, Green on White, Green to Blue, Red on Black, Red on White, Red to Blue, Red to Green, and RGB Spectrum*—assign a pseudocolor palette based on their respective color.

Filtering Images

To apply a filter to an image using the Image Processing Options:

- 1 Click the *Filtering Checkbox* to apply filters.



- 2 Choose the filter type using the *Filter Pop-up Menu*.
- 3 Choose how many pixels are used in each filtering operation using the Filter Kernel pop-up menu. The options are 3 x 3, 5 x 5, and 7 x 7 pixels. Increasing the Filter Kernel size potentiates the effect of the filter.

NOTE: These adjustments affect the on-screen, exported, and printed image only. These adjustments do not affect the acquired image data.

Image Overlay

Using the Advanced Image Display window, you can display one image on top of another image.

- 1 Open the project in which you want to place the overlay image (background image).
- 2 Open the project you want to overlay (overlay image).
- 3 Use the contrast features in the Image Display window to best display the features of interest.

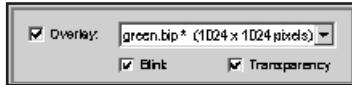
NOTE: Use the contrast features in the Image Display window to best display the features of interest. You can use pseudocolor to better display the features that you are overlaying. See *Adjusting the Display—Pseudocolor*, earlier in this chapter.

- 4 Click on the background image to make the project the active window.
- 5 Open the Advanced Image Display window by choosing Image Display from Imaging panel, clicking on the Image Display button on the Quick Access bar or clicking Image Display from the Show menu. Use the + icon to show the Advanced Image Display window.

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- 6** Click Overlay and use the pop-up menu to select the overlay image.



 NOTE: The Overlay pop-up menu is populated with the list of all currently open documents.

- 7** Click Transparency or Blink.

✓ *Blink checkbox*—when selected, the overlay image is shown and hidden at approximately 1 second intervals. This is functionally the same as selecting and deselecting the *Overlay checkbox* once every 1 second. This function is particularly useful if you are looking for change between two images (e.g., an object that changed position or brightness).

✓ *Transparency checkbox*—when selected, the overlay image is shown on top of the active document image, but wherever the overlay image has a value at far below the minimum display value for the overlay image, the overlay image is transparent to allow the active image to be seen. The transparent color could be black or white, depending on the invert setting.

- 8** You can print and export the overlay image.

✓ Overlays in “Blink” mode do not print.

✓ For “Transparency” mode, the transparent image and the active document image are printed as they are displayed. On a color printer the images should print with their respective color tables.

✓ Export Images—Windows Users can select metafile format (.emf) to export the image using the current overlay display settings and with separate color tables. Macintosh Users can export PICT files, 32-bit color image with the colors of the two images properly rendered. If greater resolution is required color output files at full image resolution can be generated.

Image Mask Overlay

You can create an ROI set and overlay just the ROI set over another image. This is similar to Overlay except that you are only overlaying the feature(s) of interest defined by an ROI.

- 1 Open the project in which you want to place the overlay image (background image).
- 2 Open the project you want to overlay (overlay image).
- 3 Define one or more ROIs on the overlay image using Manual ROIs, Auto ROIs or Grid ROIs. The ROI boundaries defines what are displayed. Do not close the project.

 NOTE. Use the Image Display window to best display the features of interest. You can use pseudocolor to better display the features that you are overlaying. Refer to earlier section titled *Adjusting the Display—Pseudocolor*.

- 4 Click on the background image to make the project the active window.
- 5 Select Mask.
- 6 Open the Advanced Image Display window by choosing Image Display from Imaging panel, clicking on the Image Display button on the Quick Access bar or clicking Image Display from the Show menu. Use the + icon to show the Advanced Image Display window.
- 7 Click Overlay and use the pop-up menu to select the overlay image.

 NOTE: The Overlay pop-up menu—is populated with the list of all currently open documents.

- 8 Click Transparency or Blink.
- ✓ *Blink checkbox*—when selected, the overlay image is shown and hidden at approximately 1 second intervals. This function is particularly useful if you are looking for change between two images (e.g., an object that changed position or brightness).
 - ✓ *Transparency checkbox*—when selected, the overlay image is shown on top of the active document image, but wherever the overlay image has a value at far below the minimum display value for the overlay image, the overlay image is transparent to allow the active image to be seen. The transparent color could be black or white, depending on the invert setting.

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9 The ROI's from the overlay appear on the background image.

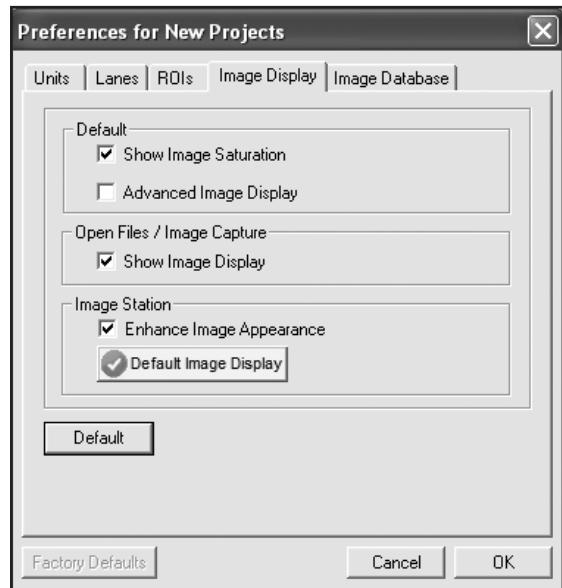
10 You can print and export the overlay image.

- ✓ Overlays for “On” and “Blink” modes do not print.
- ✓ For “Transparency” mode, the transparent image and the active document image are printed as they are displayed. On a color printer the images should print with their respective color tables.
- ✓ Export Images—Windows Users can select metafile format (.emf) to export the image using the current overlay display settings and with separate color tables. Macintosh Users can export PICT files, 32-bit color image with the colors of the two images properly rendered. If greater resolution is required color output files at full image resolution can be generated.

Setting the Image Display Options Preferences

The Image Display Preferences provide you a way to choose between Basic or Advanced features of the Image Display and to automatically apply the preferred image display functions to images as they are submitted from Image Station.

- 1 Select the Option button from Image Display or choose either the Project Preferences or New Project Prefs from the Edit menu.



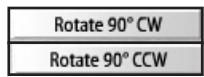
- 2 Choose your default preferences.
 - ✓ Select *Show Image Saturation* to automatically show saturated pixels on screen when opening any new image.
 - ✓ Select *Advanced Image Display* to make the Advanced Image Display window Option the default.
- 3 Select whether or not you want to automatically launch the Image Display when a new file is opened or new image is captured using the *Open Files/Image Capture Checkbox*.
 - ✓ Image Station and In-Vivo System Users—select the *Enhance Image Appearance* option to improve the on-screen, exported, and printed image.
 - ✓ Select Default Image Display to set defaults by illumination type. For example, you can choose to invert all luminescent images (white on black) as new images are acquired.
- 4 Click OK.

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Rotating Images

You can rotate the image with the Rotation tool or by using the Rotate buttons that are located on the Image panel. Use the Rotation tool when you need to straighten less than 90°. If you are analyzing lanes on a gel or blot, rotate your image so that the lanes are vertical. Any curvature to the lanes can be later adjusted using the curved lane option. Once analysis has begun, the rotation functions become inactive.

Using the Rotate Button



- ✓ Choose Rotate 90° CW or Rotate 90° CCW using the buttons on the Image panel.

NOTE: If you are not satisfied with the rotation, select Undo Rotate in the Edit menu and try again.

Using the Rotation Tool



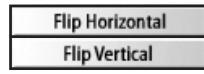
- 1 Select the Rotation tool from the Image panel.

- 2 Click and drag the Rotation tool to draw a line parallel to any feature in the image. When you release the mouse button, the image rotates to the nearest 90° axis. The degree of rotation is displayed in the Status bar.

NOTE: If you are not satisfied with the rotation, select Undo Rotate in the Edit menu and try again.

Flipping Images

You can flip images using the buttons on the Image panel tools.



- ✓ Choose Flip Horizontal or Flip Vertical using the buttons on the Image panel.

NOTE: If you are not satisfied with Flip, select Undo Flip in the Edit menu and try again.

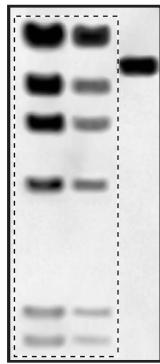
Cropping Images

You can crop your image to a selection. Cropping permanently removes a portion of your image. The History records any cropping information within the File Information window.

To crop an image:



- 1 Choose the Crop tool from the Image panel tools. The cursor changes to a crosshair.



- 2 Click and drag to select the region of interest of the image. When you release the mouse button, the selected area appears. You can adjust the positioning of the selection or the size of the selection.

- 3 To crop, move the cursor to the inside of the selection and double-click or click on the Crop tool.

NOTE: If you are not satisfied with the crop, select Undo Crop in the Edit menu and try again.

NOTE: Once you have cropped your image and saved the project, you will not be able to undo the crop. You may want to save the cropped image as a new file, using the Save As command.

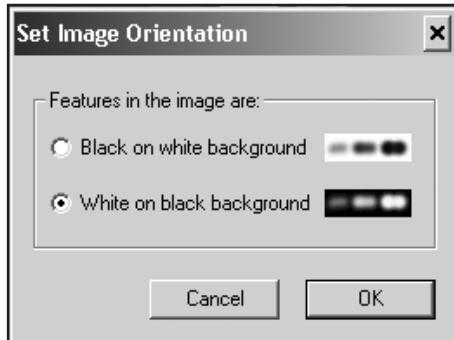
NOTE: This tool becomes inactive if you have performed any analysis on the image. Cropping the image is destructive to the image file and is recorded in the history.

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Signal Orientation

When an image is captured with a Carestream Imaging System, your image capture selections are used by the software to determine whether the features on the image have a white or black signal. If an image is captured with a TWAIN device or is a TIFF or JPEG image file, the program reviews the image histogram to determine signal type. If the image is not well contrasted, the software may have difficulty identifying the signal type. Use the Set Image Orientation on the Image panel to manually define the signal type.

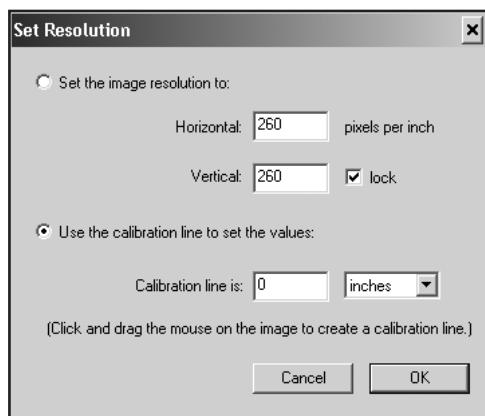


- 1 Click Signal Orientation from the Image panel. The Set Image Orientation window opens.
- 2 Select *Black on white background* or *White on black background* depending upon on the signal features.
- 3 Click OK

Set Resolution

Set Resolution resizes your image using a calibration line that you define. This is especially useful if you did not record your zoom settings in the Acquire window during capture with a Carestream Imaging System. This tool becomes inactive if you have performed any analysis on the image.

- 1 Click Set Resolution from the Image panel. The Set Resolution window opens.



- ✓ *Set the image resolution to* sets the horizontal and vertical resolution (pixels per inch) of the image.
- ☞ NOTE: If you specify resolution, you can lock it so that the horizontal and vertical is the same by clicking the checkbox.
- ✓ *Use the calibration line to set the values* resets the resolution based on a calibration line.

- 2 Click OK.

Image Math

Using Image Math, you can perform complex calculations on a single or pair of images. The resulting image becomes a new project, with Image History documenting how you created the image. Image Math has three different types of options—Tasks, Formula, and Image Processing Filters.

The Task Options

- ✓ *Invert image*—mathematically inverts all the pixel intensity values in the image. The calculation maintains the max and min value, i.e., old image max becomes image min.
- ✓ *Illumination correction*—corrects the illumination field of view of your image with an illumination reference image.
- ✓ *Convert to optical or X-ray density units* converts the intensity values in the image to optical or X-ray density values (floating point numbers). Optical or X-ray density determines the amount of matter in a substance by measuring the amount of light or X-ray, respectively, that it transmits and are expressed as follows:

$$\text{OpticalDensity} = C \left(\log_{10} \frac{A - B}{I} \right)$$

$$X - \text{RayDensity} = C \left(\log N \frac{A - B}{I} \right)$$

where:

C = user-defined constant for a particular experiment to compensate for concentration variations (default state of constant C = 1).

A = maximum observable intensity (white point)—use the max pop-up menu to adjust the value.

B = minimum observable intensity (black point)—use the Min pop-up menu to adjust the value.

I = intensity value for each pixel in the source image.

- ✓ *Convert to pico Joules/sq mm*—use this option to convert the intensity values to pico Joules per millimeter. The Joule is the SI unit of energy, so this conversion produces an image which has units of energy per square millimeter. This option improves comparison of images that were taken with different settings for FOV and f-Stop by converting intensity values to physical units.

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- ✓ *Convert to photons/sq mm*—use this option to convert the intensity values to photons (550 nm) per millimeter. The Joule is the SI unit of energy, so this conversion produces an image which has units of energy per square millimeter. This option improves the comparison of images that were taken with different settings for FOV and f-Stop by converting intensity values to physical units.
- ✓ *Convert to pico Watts/sq mm*—use this option to convert the intensity values to pico Watts per millimeter. The Watt is the SI unit of power, so this conversion produces an image which has units of energy per second per square millimeter. This option improves comparison of images that were taken with different settings for FOV and f-Stop by converting intensity values to physical units.
- ✓ *Convert to photons/sec per sq mm*—use this option to convert the intensity values to photons per second (550 nm) per millimeter. The Watt is the SI unit of power, so this conversion produces an image which has units of energy per second per square millimeter. This option improves comparison of images that were taken with different settings for FOV and f-Stop by converting intensity values to physical units.
- ✓ *Merge two images*—adds two images together ($Z = aX + bY$) where a and b are constants that can be used to scale the images prior to addition. By default these values are 1, however, you can override these values.
- ✓ *Merge two normalized images*—scales the image with the smaller dynamic range to that of the other image. These two images are then added together ($Z = X_n + Y_n$) where X_n and Y_n denote normalized images.

 NOTE: The current Image Display settings are used during the normalization.

- ✓ *Merge images with opposite signal*—adds images with opposite signals ($Z = X_n + \text{Inv}Y_n$). The images are normalized and image Y is inverted. This is used to add two images when one image has black bands and a white background and the second image has white bands on a black background.
- ✓ *Subtract images*—subtracts two images ($Z = aX - bY$) where a and b are constants that can be used to scale the image. By default, these values are 1.
- ✓ *Subtract normalized images*—normalizes and subtracts two images ($Z = X_n - Y_n$).
- ✓ *Subtract a constant*—subtracts a constant value from an image ($Z = X - a$). In this case, input a value for the constant a.
- ✓ *Absolute difference between two images*—calculates the absolute value difference between two scaled images ($Z = \text{abs}[aX - bY]$), where a and b are constants that can be used for scaling. By default a and b are 1, however, you can input new constants.
- ✓ *Divide two images*—divides two images ($Z = aX / bY$) where a and b are user-defined.
- ✓ *Divide two normalized images*—divides two normalized images ($Z = X_n / Y_n$). See the above descriptions on how the images are normalized.



- ✓ *Threshold, image less than constant*—replaces any pixels that is less than or equal to a designated intensity value and replaces it with assigned constant value.
- ✓ *Threshold, image greater than constant*—replaces any pixels that are greater than or equal to a designated intensity value and replaces it with assigned constant value.

The Formula Options

The Formula options allows you to select a mathematical formula you want to apply. The formula's categories include unary and binary operations with and without constants and logical operations.

The Image Processing Filters Options

Carestream MI Software offers two basic types of image filtering—Single Pixel Filtering and Neighboring Pixel Filtering. These filters are designed to improve the appearance, enhance a particular feature, or add special effects to your image. Different to using the filtering option in the Image Display window, the Image Processing Filters in Image Math generates a new project with changes in the image data.

The options include:

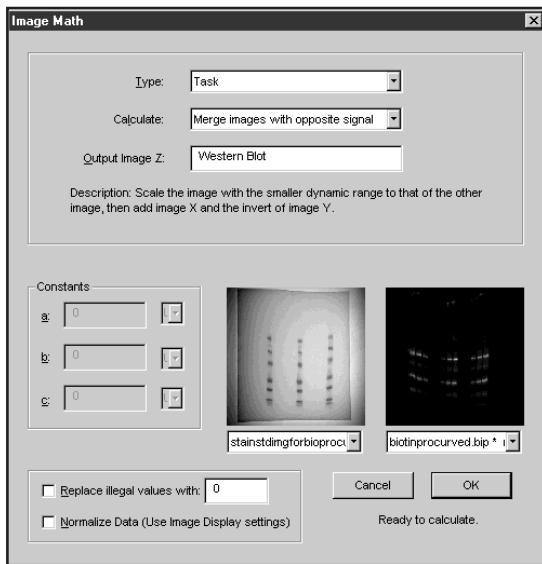
- ✓ *Average*—filter determines the average pixel intensity within a neighborhood and assigns the averaged value to the pixel, resulting in a smoother image.
- ✓ *Row and Column Average*—filters average a row or column of pixels within the neighborhood and assigns the averaged value to the pixel smoothing the image.
- ✓ *Low Pass*—filter smooths the appearance of the image by removing the high contrast edges (blurring the edges). This filter is useful for reducing noise in the image.
- ✓ *Gaussian*—filter applies a weighted average to the pixels; adding low frequency detail.
- ✓ *High Pass*—filter enhances the high contrast edges in an image; high frequency values in the image brighten, while lower frequency portions darken resulting in a sharper image. This filter may also introduce more noise in your image.
- ✓ *Mean*—filter calculates the mean pixel value within a neighborhood and assigns the mean value to the pixel.
- ✓ *Unsharp Mask*—filter gives added emphasis to the edge detail. This filter can help improve an image that appears out of focus.
- ✓ *Laplacian*—filter enhances all edges within the image and is well suited for looking at the noise of an image.

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- ✓ *Horizontal, Vertical, or Diagonal Edge*—filters look for horizontal or vertical edges. The filter takes an image and shifts it by one pixel and subtracts it from the original.
- ✓ *Gradient North, NE, East, SE, South, SW, W, or NW*—filters are designed to highlight one of eight compass directions—creating edge line drawing.
- ✓ *Emboss North, NE, East, SE, South, SW, W, or NW*—filters enhance the edge, making the image features appear as raised 3D objects. The eight different directions indicate of angle for embossing.
- ✓ *Median*—filter determines the median value within a neighborhood and assigns the median value to the pixel. The median filter despeckles an image and is good for reducing noise but also causes some blurring of the image.
- ✓ *Minimum*—filter determines the darkest pixel value within a neighborhood and assigns the darkest value to the pixel.
- ✓ *Maximum*—filter determines the brightness pixel value within a neighborhood and assigns the brightest value to the pixel.

Using Image Math

- 1 Open the input image(s).
- 2 Choose Image Math from Imaging panel. The Image Math dialog box appears.



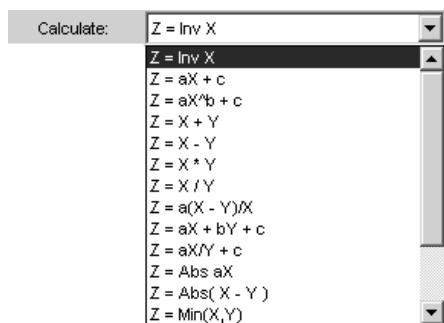
3 Choose Formula, Task, or Image Processing Filters using the Type pop-up menu.



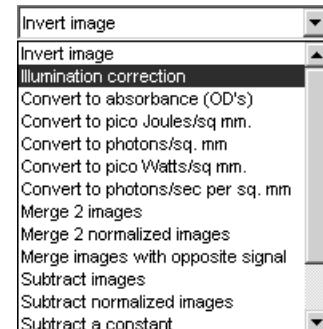
- ✓ *Formula*—displays a list of formulas in the Calculate menu.
- ✓ *Task*—displays a list of common tasks that show in the Calculate menu (i.e., average two images, add two images).
- ✓ *Image Processing Filters*—provides a list of filters that can be permanently applied to the image.

4 Using the pop-up menu choose the appropriate formula or task you'd like to perform.

- ✓ For Formulas, choose one of the following options:



- ✓ For Task, choose one of the following option:



NOTE: When Image Processing Filters is selected, a dialog box appears which allows you to choose the filter type and size.

5 Define the Input Image(s) X and Y (depending on your operation) using the pop-up menus. The files must be open prior to opening the Image Math dialog box to be available for use. When input images are selected, a thumbnail of each appears in the Image Math dialog box.

6 Name the new image using the Output Image Z text edit box.

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- 7** If required, enter constant values for a, b, and c or use the pop-up menu to use a preset option.
- 8** Check the *Replace illegal values with* option if you want to enter your own illegal value. When left unchecked, the program uses internal defaults. These values are used when an illegal value is produced during a calculation.

To normalize the dynamic range between two input images, check Normalize Data. When checked, the software:

- ✓ Determines the dynamic range of each image (*Image Display Max–Image Display Min*).
- ✓ Uses the maximum of these two values to scale the other image (this normalizes the images so they have the same dynamic range). Images are normalized by using the following formula:

$$\text{Intensity}_{\text{Out}} = \text{DynamicRange} \times \left(\frac{\text{PixelDisplay} - \text{Min}}{\text{Max} - \text{Min}} \right) + \text{Min}$$

where:

Dynamic Range = Image Display Max – Image Display Min of the largest dynamic range image.

Pixel value = Pixel intensity value for each pixel in the image.

Min = Image Display Min for the image with the smallest dynamic range.

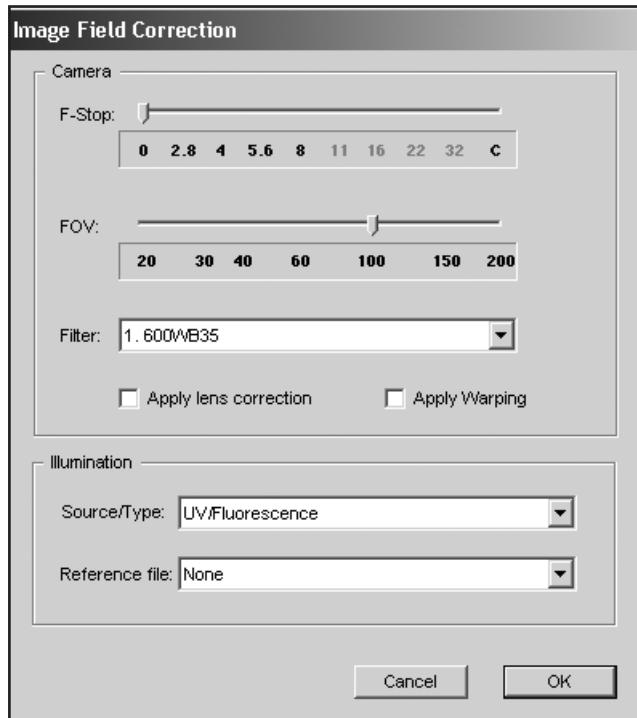
Max = Image Display Max for the image with the smallest dynamic range.

 NOTE: Only one of the two images is scaled prior to performing calculations on the image.

- 9** Click OK. Upon completion the resultant image appears as a new project. The history records how the image was generated.
- 10** Choose Save from the File menu. Depending on your Database Preferences, you may be asked if you want to add this image to the database prior to the Save dialog box appearing.
- 11** The File Information window appears. Enter project information in the fields provided in the Project tab. Click OK.
- 12** The Save As dialog box appears. Enter a destination drive, folder, and a filename. Click Save.

Using Image Correction (Image Station and In-Vivo Imaging System Users)

When you generate an Image Station or In-Vivo Imaging System image, you can choose to apply a lens and/or illumination correction to the image at the time of capture. You can also choose to apply the corrections later, using the Image Capture Settings dialog box as described in your system's User's Guide.



- 1 Choose Image Field Correction from the Edit menu.
- 2 Choose the corrections you want to apply to the image.
 - ✓ The *Apply Lens Correction* adjust for lens curvature. The checkbox applies lens correction to the image.
 - ✓ *Apply Warping* check box turns image warping on or off. Image warping is especially useful for correcting for lens distortion at low magnifications—i.e., 96 well plates, macroarrays.

3 Optimizing Images

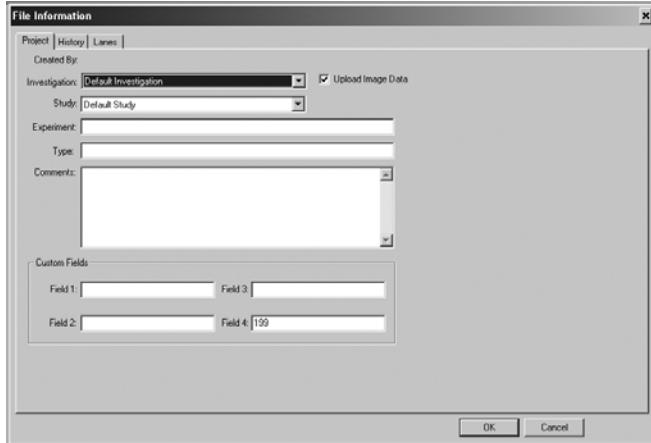
- ✓ Use the *Illumination Correction* to check box to apply illumination correction or select a saved Illumination Reference File. The Illumination Correction improves the quality of your data by applying field illumination correction to images captured. The illumination non-uniformity is highly reproducible and is corrected by dividing the sample image by an illumination reference image. You can choose to create a library of illumination correction files (see *Generating an Illumination Reference File Library* in your Carestream imaging system user's guide)
- ☞ NOTE: You can only apply these corrections as long as the image has not been altered. Please note that cropping, rotating, or flipping alters the image and data file.

Using the File Information Window

The File Information window is divided into three tabs—Project, History and Lanes. The Projects tab stores information concerning the project. The History tab records capture information as well as tracks any changes to the file that are destructive to the image, e.g., cropping. This file is not editable. The Lanes tab records lane assignments if you have performed Lane Analysis on the image.

The Projects Tab

- 1 View the File Information by choosing Info/History from the Image panel or by clicking the Info History on the Quick Access bar.

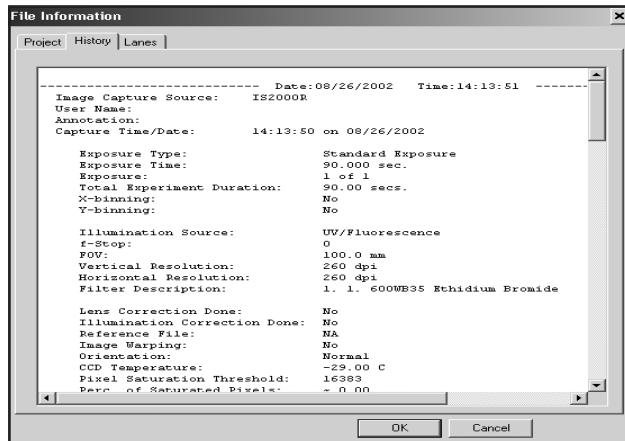


- 2 Click on the Projects tab.
 - 3 Enter the project information in the Name, Date, Project, Samples, Comments, and Custom text edit boxes in the Projects tab.
- NOTE:** If you use the database feature, the information can be used to retrieve or sort saved projects.
- 4 Click OK to save changes.

3 Optimizing Images

The History Tab

- 1 View the File Information by choosing Info/History from the Image panel or by clicking the Info History on the Quick Access bar.



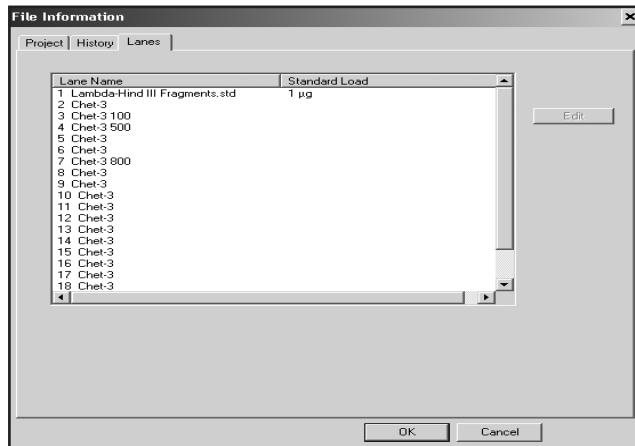
- 2 Click History tab to review the history of the image.

NOTE: The History records image capture conditions. In addition, it tracks any changes that are destructive to the image, i.e., cropping, rotating. This dialog box cannot be edited.

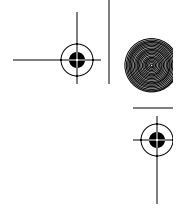
- 3 Click OK.

Lanes Tab

- 1 View the File Information by choosing Info/History from the Image panel or by clicking the Info History on the Quick Access bar.



- 2 Click Lanes tab to review designation of lanes, or use the Edit button in this tab to designate or rename lanes.
- 3 Click OK. The file information is saved.



Defining Lanes



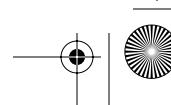
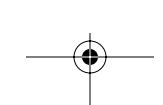
Lanes are automatically found on an image after a selection area is defined using the Selection Rectangle tool. The automatic lane finder uses a multiple pass algorithm to determine the lanes on the image. The image is broken into segments and each segment is searched for bands. The algorithm searches for the left and right edges of bands. A list is built of the location of candidate bands. The band list is used to determine a rough estimate of the width of the lanes and number of lanes in the selected region. Based on the number of calculated lanes and the locations from the band list, the program places each band in a lane. The center of each lane is then calculated. The slanted and straight algorithms use linear regression to determine the lane center, while the curved algorithm uses a third-order polynomial. The lane location is identified and displayed on the image.

The sensitivity can be adjusted using the Adjust Lanes dialog box. To fine tune lane placement, the lane finding sensitivity has seven levels, ranging from -3 (least sensitive) to +3 (most sensitive), with a default setting of 0. This Sensitivity slider is dynamically linked to the Image Display window. Use the Image Display window to adjust the contrast to best represent the image prior to using the automatic lane finder.

If the lanes are straight, the default sensitivity is usually best. If the lanes are slanted or curved, run the appropriate algorithm by clicking Adjust Lanes and adjusting the sensitivity to correctly find lanes.

When the sensitivity is increased, the software finds fainter bands. As a result, image artifacts, spots, etc., are also found and may adversely affect the lane finding. However, if you are working with a gel with faint bands, this may be necessary.

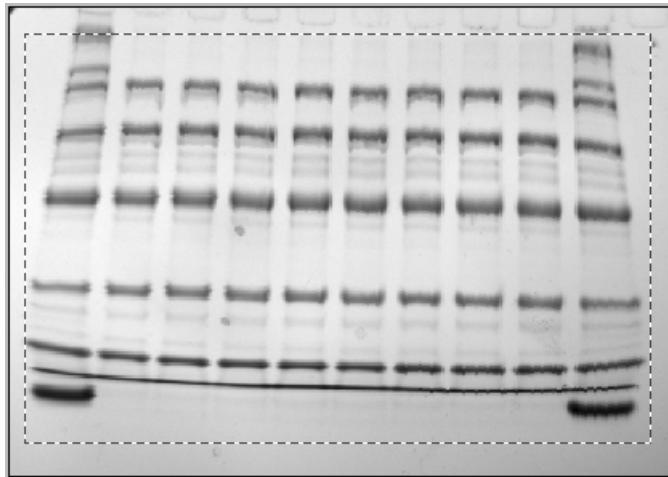
Decreasing the sensitivity does not always reduce the number of lanes found. As the sensitivity is decreased, the threshold that determines a band increases. If your image has bands that are darker or larger on the edges than in the center, the algorithm may misinterpret one lane as two separate lanes. A manual method of marking lanes is also available for use with problem images, and is described later in this chapter.



Finding Lanes

When an image is captured with a Carestream Imaging System, your image capture selections are used by the software to determine whether the bands have a white or black signal. If an image is captured with a TWAIN device or is a TIFF or JPEG image file, the program reviews the image histogram to determine signal type. If the image is not well contrasted, the software may have difficulty identifying the signal type. Use the Find buttons on the Lanes panel to manually define the signal type.

- 1** Select Lanes from the Navigation panel.
- 2** Choose the Set Search Area from the Lanes panel. The Selection Rectangle tool is selected and a default selection rectangle is displayed.
- 3** Adjust the selection rectangle or redraw the rectangle by clicking and dragging to select the area of the image to be analyzed.

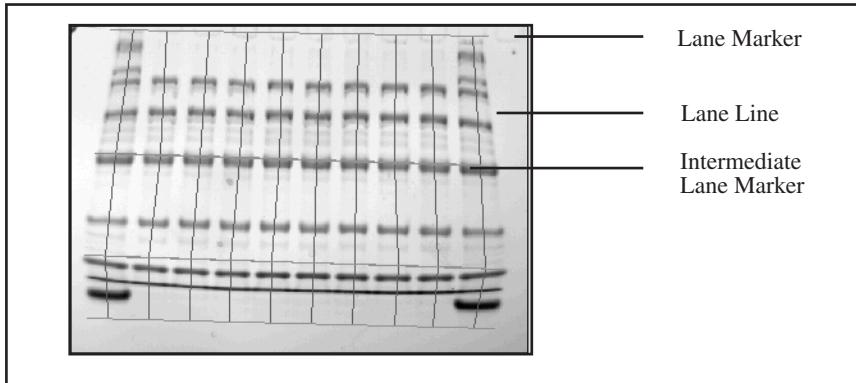


 NOTE: Be sure to select an area close to the edges of the bands in the outer lanes. Be careful when selecting the top and bottom of the region of interest. These areas are used to calculate the background level. Therefore, do not place the edge of the selection on or through bands, wells, or any image artifacts.

4

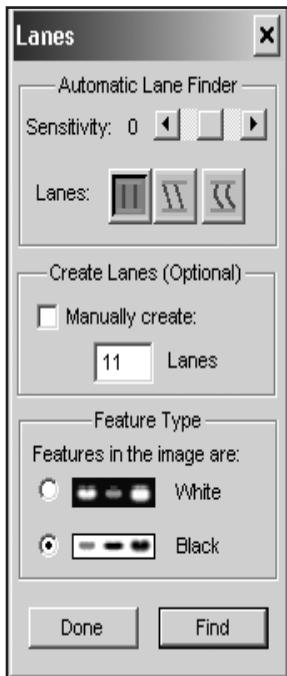
Defining Lanes

- 4** Click New Lane Set from the Lanes panel. A Lane Marker with Lane Lines appear on the image. A Lane Marker consists of at least two horizontal Lane Markers that are connected with vertical Lane Lines designating the lanes.



NOTE: To ensure accurate analysis, make sure that the Lane Lines pass directly through the center of the bands.

- 5** Click Adjust Lanes from the Lanes panel. The Lanes dialog box appears.



- ✓ *Automatic Lane Finder*—uses a multiple pass algorithm to determine the lanes on the image.
- ✓ *Sensitivity*—changes the lane finding sensitivity. Clicking the right arrow increases the sensitivity of the lane finding process, while clicking the left arrow decreases the sensitivity of the lane finding process.
- ✓ *Lanes*—chooses between three methods of finding the centers of the lanes: straight lanes, slanted lanes, and curved lanes.
- ✓ *Create Lanes (Optional)*—provides the tools to manually create lanes.
- ✓ Click the *Manually Create Checkbox*—to ignore the Automatic Lane Finder and allow you to manually enter the desired number of lanes. Enter the number of lanes in the *Manually Create text edit box*.

- ✓ Use the *Reference Band tool* to draw an ROI that bounds a typical band within the gel. The location, width, height, and intensity of the band are automatically used in the Find Lanes algorithms. Once a reference box is drawn, the box can be edited using any of the four corners to resize prior to choosing Find Bands. This is especially useful when the automated finding tools are not producing good results

 NOTE: This number is used by both manual and automatic lane finding. For manual, this sets the number of lanes and spaces them equally across the selection. For Automatic, the program uses this value to determine the best placement of Lane Lines.

- ✓ The *Feature Type buttons*—are used to define the band type as either white or black. This preference affects the lane finding and band finding algorithms.

 NOTE: If you are using a Carestream Imaging System, the selection you make is used by the software to determine signal orientation.

 NOTE: Brightness/contrast adjustments using the Image Display window are dynamically linked to the lane-finding algorithm. To increase sensitivity, adjust the image to best visualize the bands of interest.

- ✓ *Find*—initiates the lane finding process using the defined settings.

- ✓ *Done*—exits the Lanes dialog box and returns to the Project window without any changes.

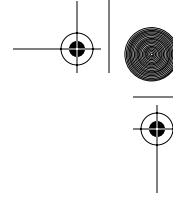
- 6 Adjust the parameters to best fit your image. Click Find again and the new parameters are used to automatically redefine the lanes.

 NOTE: Individual Lane Lines can be removed by selecting them and pressing the Delete key. All lanes can be removed by shift-clicking on multiple lanes and pressing the Delete key.

- 7 To adjust Lane Markers to correct for skews or smiles in your image, proceed to *Adjusting Lane Markers and Creating Iso Molecular Weight Lines* later in this chapter.

 NOTE: Intermediate Lane Markers are automatically placed on your image. These Intermediate Lane Markers are important in adjusting for the skew or curvature of your image.

 NOTE: Multiple Lane Sets can be used on a single image. These lane sets can overlap lanes and are unlimited in number, see *Adding Multiple Lane Sets* later in this chapter.



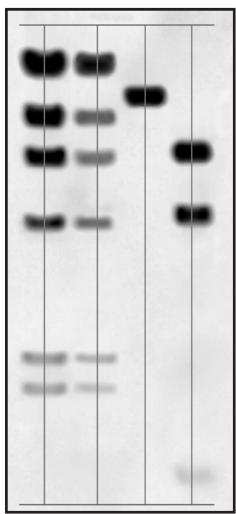
4

Defining Lanes

Manual Lane Finding

You have the option of using the Lane Marker tool to manually define the number of lanes and adjust the lane centers.

Correct placement of the Lane Markers is important in determining accurate molecular weight and band mass estimates.

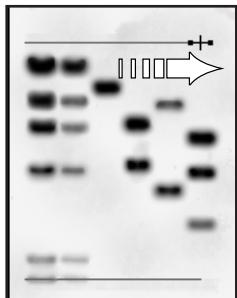


- ✓ The width of the top Lane Marker determines the placement of the vertical Lane Lines. The software distributes the number of lanes you specified in the Make Lanes dialog box equally between the two ends of each Lane Marker. The Lane Lines should run through the center of each lane.
- ✓ If the bands are warped or bowed, adjust the angle of the Lane Markers so that they are parallel to the bands. Band mobility is measured relative to the placement of the top and bottom Lane Markers. It is important that the Lane Markers accurately reflect the skew of the gel. This is accomplished by editing existing Lane Markers or adding new Intermediate Lane Markers.

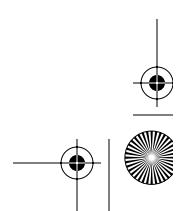
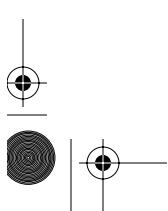
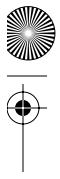
Drawing Lane Markers



- 1 Choose the Lane Marker tool from the Lanes panel.
- 2 Drag from the left edge of the first lane to the right edge of the last lane to create the Lane Marker. Try to draw the Lane Marker so it runs parallel to the bands.



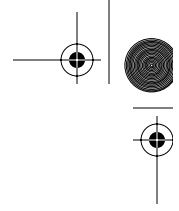
NOTE: Do not draw the first Lane Marker through the bands, wells or artifact, since the top of the Lane Marker designates the first background reading.



- 3 Once you have drawn the first Lane Marker, the Make Lanes dialog box appears. Enter the desired number of lanes in the Number of lanes to create text edit box.



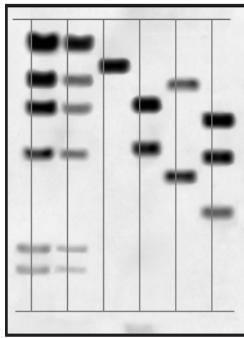
4 Click OK.



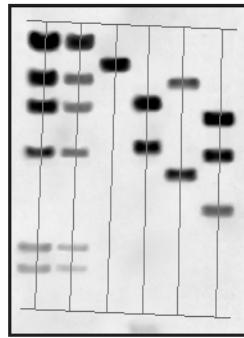
4 Defining Lanes

Lane Markers and Iso Molecular Weight Lines

Once the Lane Markers have been drawn, you can fine tune them to best fit any irregularities in your image—both horizontally and vertically. For best results, position vertical Lane Lines through the center of each lane.



Improperly Placed
Horizontal Lane Markers



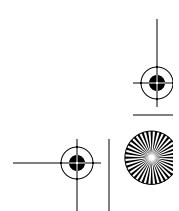
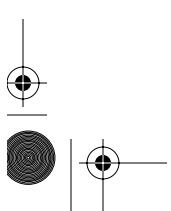
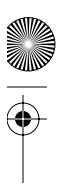
Correctly Placed
Horizontal Lane Markers

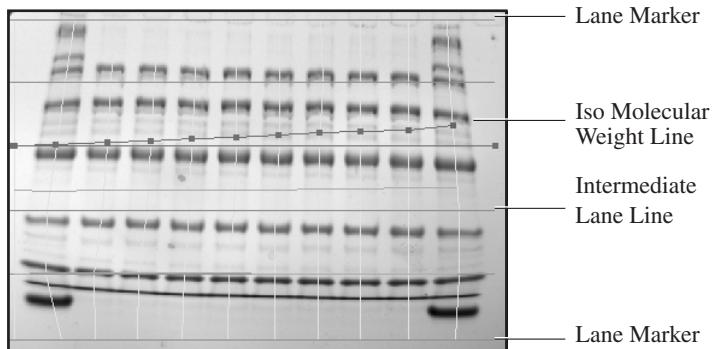
You can move, angle, stretch, and shrink the horizontal Lane Markers. Try to make adjustments that mimic the width of the image and the curve of the lanes.

The top and bottom Lane Markers identify the original selection and have limited movement. For especially warped or skewed gels, the Intermediate Lane Markers can be curved using the Iso Molecular Weight Line function. When you define a custom Iso Molecular Weight Line, the program ignores the placement and slope of any Lane Markers and only uses information from the Iso Molecular Weight Lines to measure mobility.

 NOTE: Lane Markers should still be used to adjust the location of the vertical Lane Lines. By changing the horizontal length of the Lane Marker you can control the placement of the vertical Lane Lines. You can also adjust the Lane Lines to fine tune their position.

Consider the Iso Molecular Weight Lines as an extension of the functionality of Lane Markers. By default, Lane Markers are restricted to straight lines, while Iso Molecular Weight Lines offer control points (on each lane) that can be used to accurately adjust for any localized mobility variations.





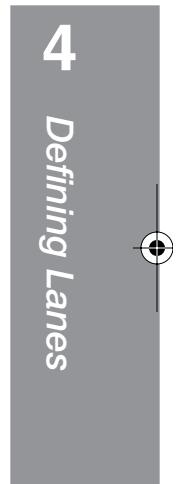
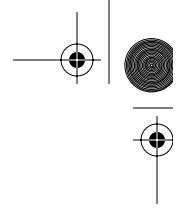
There are a number of different ways Iso Molecular Weight Lines can be used. For example:

- ✓ When you know that a number of your unknown bands are of the same molecular weight; running an Iso Molecular Weight Line through these bands ensures that they all have exactly the same molecular weight in the Lane Analysis Data window.
- ✓ When you run the same standard in different lanes across the gel, you can run Iso Molecular Weight Lines through corresponding bands in the standard lanes. This accurately determines molecular weight for intermediate lanes.

 NOTE: While the addition of a single Iso Molecular Weight Line can significantly improve the accuracy of your results, you may typically want to place two to three Iso Molecular Weight Lines for best results.

When an Intermediate Lane Marker is selected, a control point appears at every Lane Line intersection. By clicking and dragging a control point a new Iso Molecular Weight Line is drawn.

Once an Iso Molecular Weight Line has been created from an Intermediate Lane Marker, the Intermediate Lane Marker is ignored for molecular weight calculations. Both the Intermediate and the Iso Molecular Weight Lines can be displayed on the image. Once bands are found, additional Intermediate Lane Markers or Iso Molecular Weight Lines cannot be added. However, Iso Molecular Weight Lines can still be adjusted.

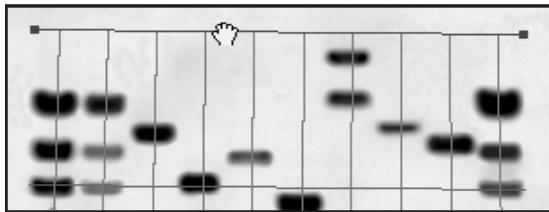


Adjusting Lane Markers and Creating Iso Molecular Weight Lines

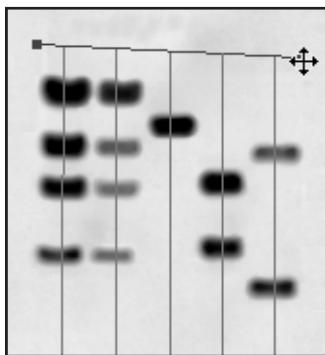


1 Choose the Pointer tool from the Lanes panel.

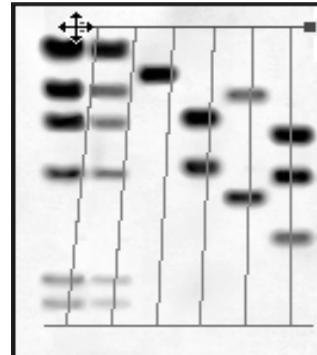
2 Select the top horizontal Lane Marker. The Pointer tool changes to a hand cursor and grab handles appear on each end of the Lane Marker.



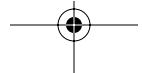
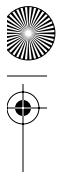
- 3** To adjust the location or skew the Lane Marker in any direction to better fit your image, click the Lane Marker and drag.
- 4** To adjust the width of a Lane Marker, drag the grab handles. Stretch the horizontal Lane Markers so that the Lane Lines come as close as possible to bisecting the bands.



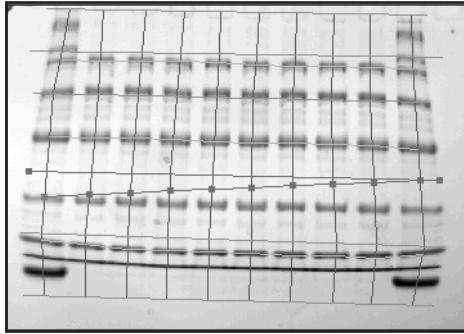
Angling a Horizontal Lane Marker



Resizing a Horizontal Lane Marker



- 5** To compensate for smiling, locate the area of the image where the gel is skewed or the band lane migration angle changes, and select an adjacent Intermediate Lane Marker. The control points are highlighted in red. Click and drag one of the control points to create an Iso Molecular Weight Line.

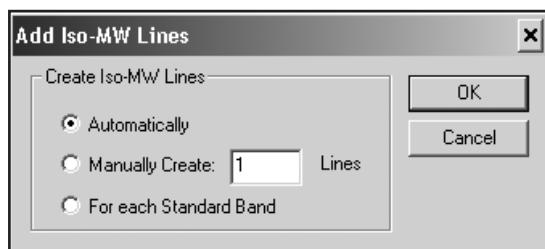


NOTE: Intermediate Lane Markers may be drawn by the program based on the automatic lane finding.

The Lane Markers are designated in Magenta, when selected the Lane Marker becomes red. Notice that as you create an Iso Molecular Weight Line it defaults to green when not selected. As each control point has been edited, the control point turns blue.

NOTE: Prior to finding bands, both the Lane Markers and Isolines can be displayed independently. You can use the Lane Marker or Iso Molecular Weight Line options in the View Options on the Lanes panel to show or hide these items.

- 6** If you need to adjust the number of Intermediate Lane Markers, add a new Lane Marker(s) using the Add Iso MW lines from the Lanes panel. The Create Iso MW Lines dialog box appears.



- ✓ *Automatically*—tries to determine the optimal number of Lane Lines.
- ✓ *Manually Create*—provides you a text edit box to enter the number of lines you would like to create.
- ✓ *For each Standard Band*—places an Intermediate Lane Marker for each standard band in your image

You can also manually draw Intermediate Lane Marker(s) using the Lane Marker tool by dragging from the left edge of the first lane to right edge of the last lane. A new horizontal Lane Marker is added.

 NOTE: Adding Intermediate Lane Markers can only be done prior to finding bands.

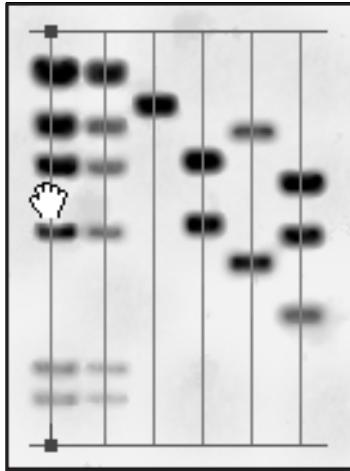
4

Defining Lanes

Adjusting Vertical Lane Lines

You may also need to adjust individual Lane Lines to center them on the bands.

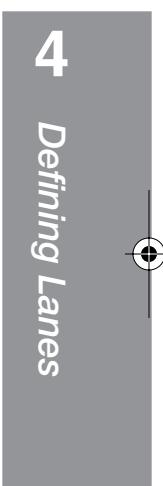
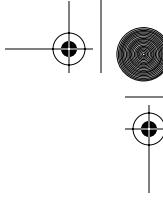
-  1 Choose the Pointer tool from the Lanes panel.
- 2 Select a vertical Lane Line. The Pointer tool changes to a hand cursor and grab handles appear at the intersection of each Lane Marker.



- 3 To move the Lane Line horizontally, click and drag the Lane Line with the hand cursor left or right.
- 4 To adjust the angle of the Lane Line, drag one of the grab handles on either end of the line. Try to angle the Lane Lines so that it runs through the center of the lane. If this is not possible, add another horizontal Lane Marker to compensate for the Lane Line angle. (See *Adjusting Lane Markers and Creating Iso Molecular Weight Lines* earlier in this chapter.)

 NOTE: Centering Lane Markers is particularly important when calculating band mass since the software uses the Lane Marker to define the analysis area.

 NOTE: To learn how to label the created lanes, see *Labeling Lanes* later in this chapter.



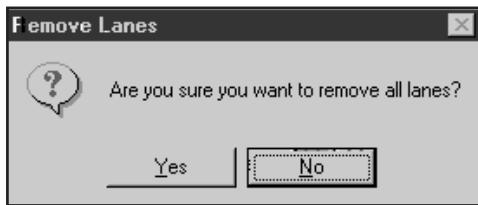
Deleting Lane Markers and Lane Lines

If you did not select the right number of lanes on your image or you are having trouble adjusting Lane Markers, you may want to start over.

Deleting All Lane Markers and Lane Lines

If you did not select the right number of lanes on your image, you need to delete all of the Lane Markers.

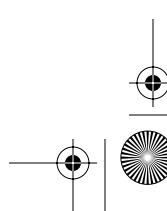
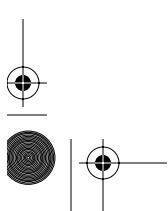
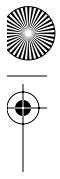
- 1 Select the Lane Set you want to delete using the Pointer tool from the Lanes panel.
- 2 Click Delete Lane Set from the Lanes panel.



- 3 The Remove Lanes confirmation box appears and asks you confirm that you want to remove all lanes.
- 4 Click Yes to remove the active lane set.

Deleting Horizontal Lane Lines

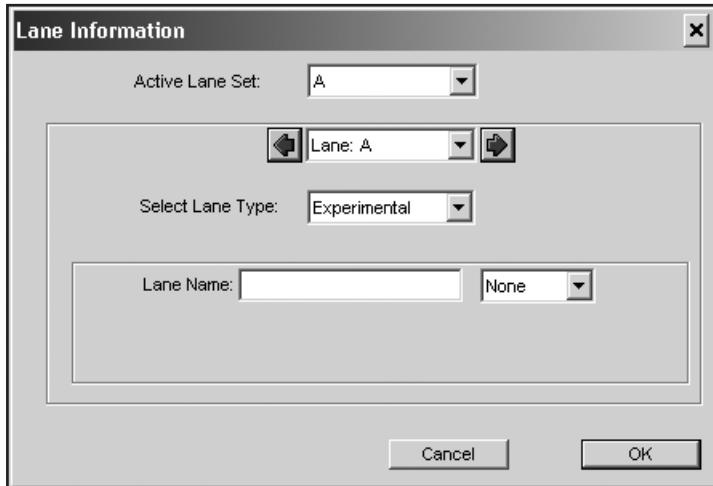
- 1 Choose Pointer tool from the Lanes panel.
- 1 Point and click to select the horizontal Lane Line. Shift-click to select more than one Lane Line.
- 2 Press the Delete key.



Labeling Lanes

You can define lanes as standard, experimental, or inactive for subsequent analysis. This process can occur before or after finding bands.

-  1 Click the Set Standards button from the Lanes panel or double-click a vertical Lane Line with the Pointer tool.
- 2 The Lane Information dialog box appears.

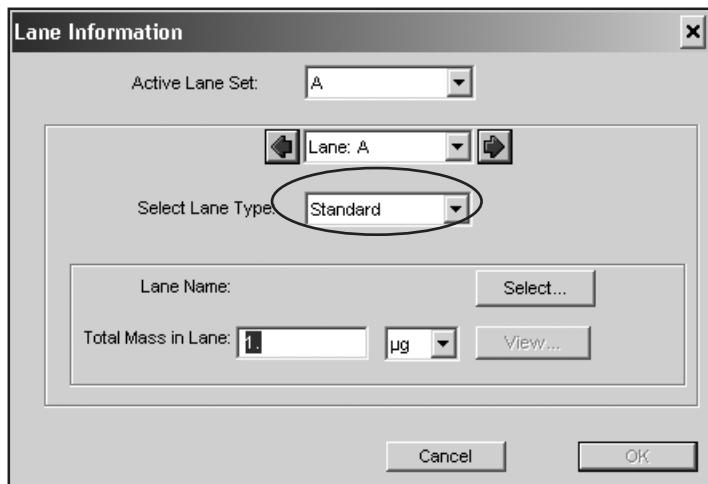


- 3 Choose the Active Lane Set in which you would like to label.
- 4 Use the Lane pop-up menu or the arrows to navigate through the lanes.
- 5 Choose Standard, Experimental or Inactive from the Select Lane Type pop-up menu.
- 6 Enter the name of the lane in the Lane Name text edit box. Choose None for the Lane Name pop-up menu unless you are labeling multiple sequential lanes. See *Labeling Multiple Lanes—Sequentially*, later in this chapter.

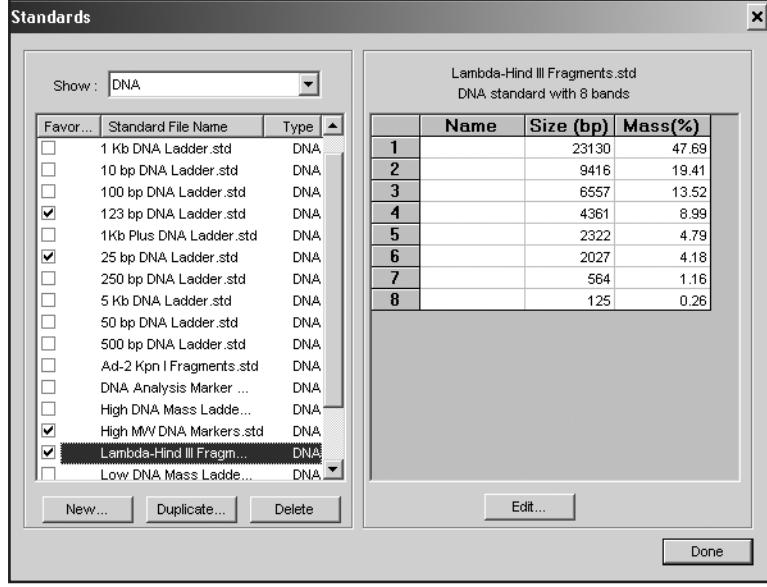
Standard Lanes

For a Standard Lane, you can select a standard for the analysis, set the amount of the standard loaded, and view previously selected standard statistics.

-  1 Click the Set Standards from the Lanes panel or double-click a vertical Lane Line with the Pointer tool.
- 2 The Lane Information dialog box appears.
- 3 Choose the Active Lane Set in which you would like to label.
- 4 Use the Lane pop-up menu or the arrows to navigate to the lane you want to label.
- 5 Choose Standard from the Select Lane Type pop-up menu.



- 6** Click the Select button to access the Standards dialog box.



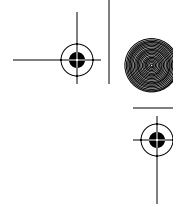
- 7** Use the Show pop-up menu to view standards. You can sort standards by type:

- ✓ *All Standards*—displays all the standards that were pre-loaded with the software and any standards that you have added.
- ✓ *Favorite Standards Only*—displays your commonly used standards. You can designate a Favorite by clicking the standard in the Favorite column.
- ✓ *DNA*—only DNA standards are displayed.
- ✓ *RNA*—only RNA standards are displayed.
- ✓ *Proteins*—only protein standards are displayed.

NOTE: For more information about entering your own standards, see *Creating a New Standard* later in this chapter.

- 8** Select the standard and click Done. The data for the standard you selected is now associated with the lane. Click View to check the standard data. Click OK when done.
- 9** Enter the amount of standard loaded into the Total Mass in Lane text edit box. This is only necessary if you use a mass standard and if you want to calculate band mass.
- 10** Click OK or use the arrows to navigate through the lanes.

NOTE: You can change the color for Standard Lanes in the Preferences.

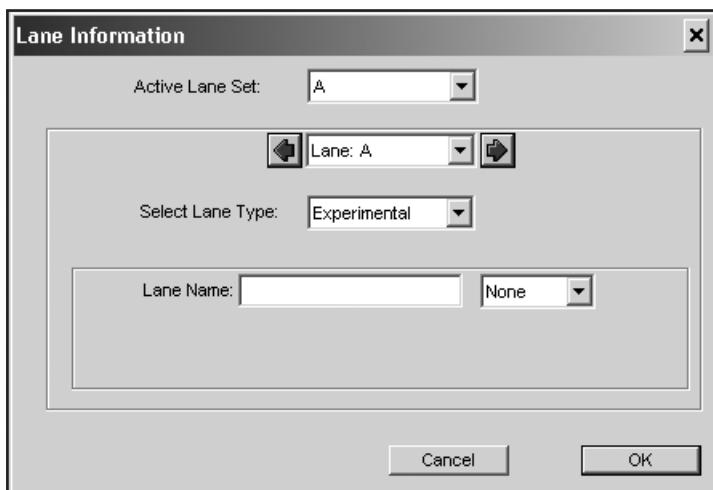


Experimental Lanes

To define an experimental lane, choose Experimental from the Select Lane Type pop-up menu. The Lane Information dialog box becomes specific for an experimental lane. You may label an individual lane or multiple lanes at one time.

Labeling Individual Lanes

- 1 Click the Set Standards from the Lanes panel or double-click a vertical Lane Line with the Pointer tool.
 - 2 The Lane Information dialog box appears.
 - 3 Choose the Active Lane Set in which you would like to label.
 - 4 Use the Lane pop-up menu or the arrows to navigate to the lane you want to label.
 - 5 Choose Experimental from the Select Lane Type pop-up menu.



- 6** Enter the name of the lane in the Lane Name text edit box.
 - 7** Choose None in the Lane Name pop-up menu to the right of the text edit box.
 - 8** Click OK or use the arrows to navigate to the next lane you want to label.

 NOTE: You can change the color for Experimental Lanes in the Preferences.



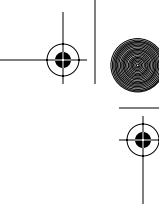
Labeling Multiple Lanes—Sequentially

You can label multiple lanes sequentially.

-  **1** Use the Pointer tool to select a horizontal Lane Line. Shift-click to select multiple Lane Lines or by clicking and drag the mouse over an area of the image containing more than one Lane Line.
- 2** Click Set Standards from the Lanes panel. The Lane Information dialog box appears.
- 3** Choose Experimental from the Select Lane Type pop-up menu.

 NOTE: The Select Lane Type pop-up displays *Mixed* if the selected lanes are different types (standard, experimental and inactive). Check your lane selection for accuracy before changing the lane type.
- 4** Enter a general name in the Lane Name text edit box.
- 5** Choose the type of multiple labeling from the Lane Name pop-up menu, alphabetical or numerical. If you entered “Sample” as the general lane name and choose 1,2,3... from the Lane Name pop-up menu. The lanes are labeled as follows:

Sample 1, Sample 2, Sample 3, etc.
- 6** Click OK. The lanes are labeled sequentially.



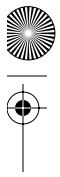
Inactive Lanes

An inactive lane is one that is not used in the analysis. Inactive lanes may include empty lanes, lanes with artifacts or loading errors, or lanes with samples from a different experiment.

-  1 Click the Set Standards from the Lanes panel or double-click a vertical Lane Line with the Pointer tool.
- 2 The Lane Information dialog box appears.
- 3 Choose the Active Lane Set in which you would like to label.
- 4 Use the Lane pop-up menu or the arrows to navigate to the lane you want to label.
- 5 Choose Inactive in the Select Lane Type pop-up menu. The selected lane is not used in any subsequent analyses.
- 6 Click OK or use the arrows to navigate through the lanes. Lanes designated as inactive for analysis are denoted by a change in line color.

 NOTE: You cannot change the color designation of Inactive Lane.

 NOTE: To activate an inactive lane, double-click the vertical Lane Line or choose Lane Information from the Analysis menu and the Lanes submenu. Redefine the lane as experimental or as a standard.

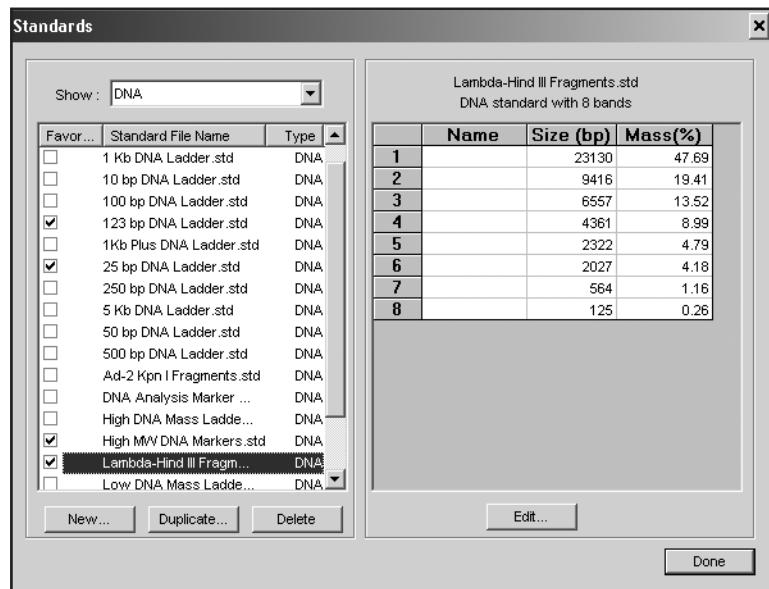


Editing Existing Standards

You may modify existing standard files in the Standards dialog box. Once you edit the standard file, the file is permanently changed. We suggest that you duplicate the standard before editing.

After finding bands, you can assign a specific band a standard value (See *Reassigning a Band a Standard Value* in Chapter 5: *Generating Band Data* or merge unresolved standard bands for a specific project (See *Merging Standard Bands* in Chapter 5: *Generating Band Data*).

- 1 Choose Set Standards from the Lanes panel, double-click on a Lane Line or choose Standards from the Edit menu. The Standards dialog box appears.



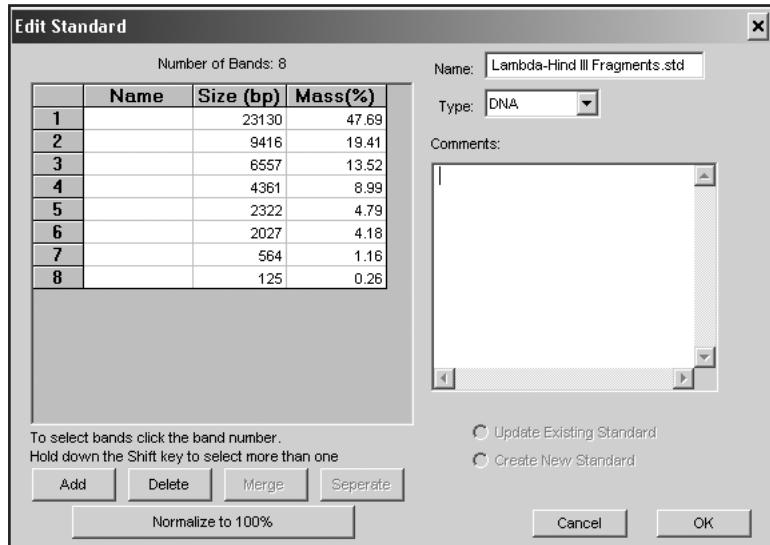
- 2 Select the standard file that you want to edit.

NOTE: We recommend that you duplicate the file before modifying an existing file using the Duplicate button.

4

Defining Lanes

- 3** Click Edit and the Edit Standards dialog box appears.

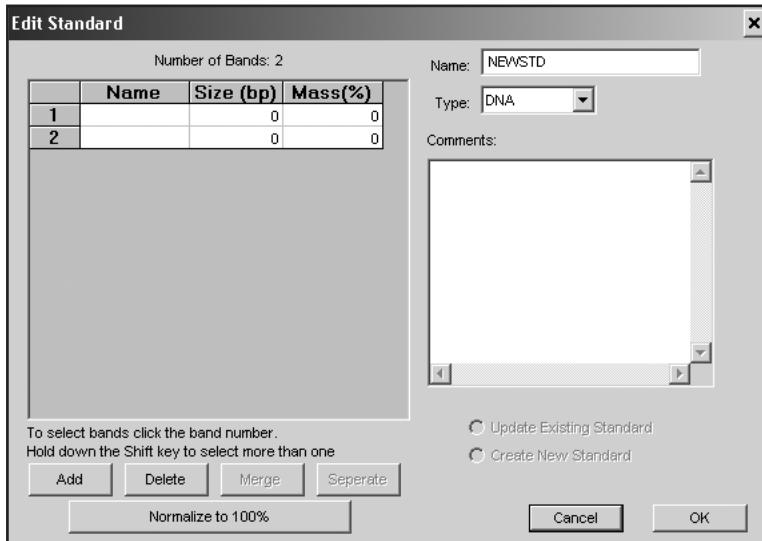


- 4** Edit the cells as you would any spreadsheet.
- ✓ To add rows, press the Enter key (Return key for Macintosh) on the keyboard.
 - ✓ To delete a row, click the row number and press the Delete key.
- 5** Enter an unique name for the new standard. Use the Comments section to record information about the new standard.
- 6** Click OK. The standard file is now available for selection.

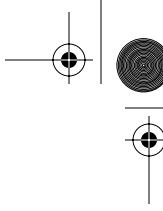
Creating a New Standard

To create a new standard follow these steps:

- 1** Choose Set Standards from the Lanes panel, double-click on a Lane Line or choose Standards from the Edit menu. The Standards dialog box appears.
- 2** Click New and a blank Edit Standard dialog box appears.



- 3** Enter a unique standard name in the Name text edit box.
- 4** Select the standard type using the Type pop-up menu.
- 5** Type each band size in the Size cell. Each row includes the information for one band.
 - ✓ Size is defined in base pairs (bp) for DNA, bases (b) for RNA, and daltons (d) for proteins.
 - ✓ To add rows, press the Enter key (Return key for Macintosh) on the keyboard.
 - ✓ To delete a row, click the row number and press the Delete key.
- 6** Type each band mass in the Band Mass cell. Each Row includes the information for one band.
 - ✓ Band mass is defined as a percentage. To calculate the band mass for the standard file, assume a total mass of 100 units.



✓ You can enter the amount mass loaded in each band and then click Normalize to calculate % mass in each band.

- 7** Continue entering bands until the standard is defined.
- 8** Use the Comments section to record information about the new standard.
- 9** If you have entered data as band mass and not% band mass, click Normalize.
- 10** Click OK. The new standard is now available for selection.

NOTE: The standards shipped with Carestream MI Software are listed in Appendix D.

Calculating Percent Band Mass

A DNA standard usually has both size and mass data; the size in base pairs of each fragment and the percent band mass is the percentage of each band of the total mass loaded. For example, the % band mass is calculated:

EXAMPLE: For Hind III Lambda Standard, band 1 is 23130 base pairs. This is $23130/48502$ (total length of Lambda), or 47.69%.

Protein standards are different, the size of each band is given in daltons. If each protein is combined in an equimolar solution, the percent band mass in each band is based on the total of all the bands.

EXAMPLE: For the low protein mass standard which has 6 proteins added in equal amounts, the mass would be calculated as follows, $100/6$ is equal to 16.67. Each band is assigned as 16.67%.

4

Defining Lanes

Applying Lane Set Templates

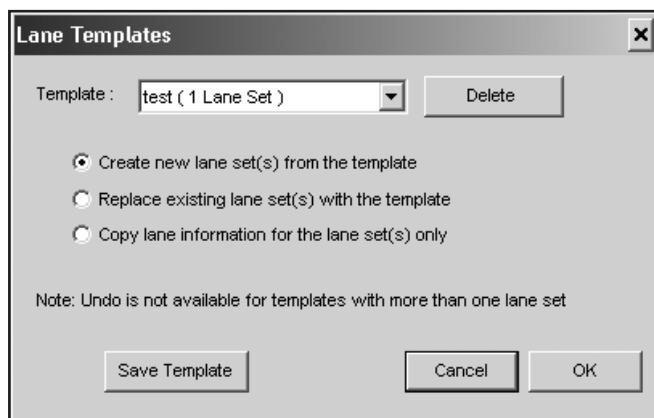
If you routinely run similar loads, save the defined lanes as a template. When you apply a template to an image, an exact copy of the previously defined lanes, lane designations (standard, experimental, inactive), and/or load amounts is applied.

Saving a Lane Set Template

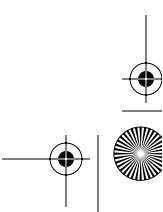
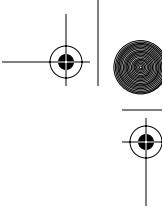
- 1 Define and label lanes as described earlier in this chapter.
 - 2 Choose Templates from the Lanes panel. The Lane Set Template dialog box appears.
 - 3 Enter a name for the template and click OK. The Lane Lines and Lane Labels are saved as a template.
- NOTE:** When naming a template, avoid using characters like a slash, colon, or semicolon.

Using a Lane Set Template

- 1 Acquire or open an image in the Project window.
- 2 Choose Templates from the Lanes panel. The Lane Template dialog box appears.



- 3 Choose the template you wish to apply using the Template pop-up menu.



4

Defining Lanes

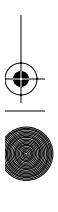
4 Select one of the following options:

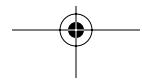
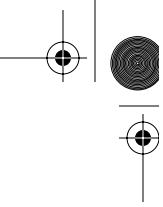
- Create a new lane set(s) from the template*—applies the selected template.
- Replace existing lane set(s) with the template*—replaces both the Lane Markers and the Lane Label information.
- Copy lane information for the lane set(s) only*—applies Lane Labels.
- Delete*—removes the template from the pop-up menu.

NOTE: *Replace existing lane set(s) with the template* and *Copy lane information for the lane set(s) only* are available only if you have already found lanes.

- 5** Click *OK* to apply changes or *Cancel* to exit the dialog box without making any changes.
- 6** Verify that the template was applied properly.

NOTE: Each Lane Line and Lane Marker can be adjusted individually by selecting it using the Pointer tool or as a group by Shift-clicking on multiple Lane Lines and moving them simultaneously.





Generating Band Data

Carestream Molecular Imaging Software performs the analysis using floating point values which is important in maintaining the accuracy of the data. The advantage of floating point is that data is not truncated or clipped when mathematical functions are performed.

Once the lanes have been marked and labeled, you are ready to find bands on the image. When you initiate the Find Bands command, the analysis process begins; the software creates a median profile for each standard and experimental lane, identifies bands, and generates statistics for each band. If you have defined standard lanes, the molecular weight and mass for each experimental band is also determined.

After performing initial band finding, you can adjust the parameters of the band finding algorithm. These parameters are Band Sensitivity and Profile Width.

The Band Sensitivity has seven levels, ranging from -3 (least sensitive) to +3 (most sensitive), with a default setting of 0. If the bands of interest are well resolved and stand out clearly from the background, a less sensitive setting is needed. If the bands of interest are not clearly resolved or do not clearly stand out from the background, a more sensitive setting is necessary. However, the more you increase the sensitivity, the more likely it is that extraneous artifacts in the image are identified as bands. The optimal sensitivity setting is one that finds all the bands of interest and minimizes the number of extraneous bands.

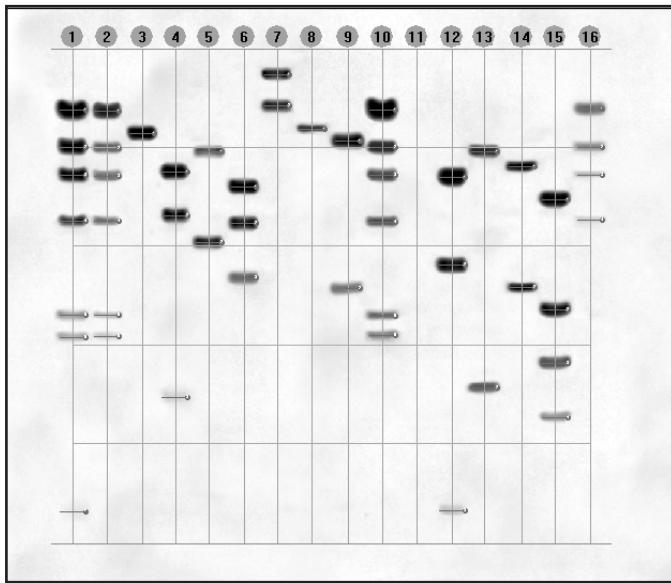
 NOTE: Brightness/contrast adjustments using the Image Display window are dynamically linked to the lane-finding algorithm. To increase sensitivity, adjust the image to best visualize the bands of interest.

The Profile Width defines the width of the lane used for the analysis (centered on the Lane Line). The range is from 1% to 99% and is based on the average distance from lane center to lane center. The optimum Profile Width is one that encompasses the average band without including any background. In the automatic lane finding mode, the Profile Width is automatically calculated. In the manual lane finding mode, the default setting is 70%.

Finding Bands

Now that you have defined the Lane Markers, finding bands is the next step. Let's get started.

- 1 Click the Find Bands button on the Lanes panel. Band Labels appear on the image when the analysis is complete. Each band is numbered sequentially from the top of the image.

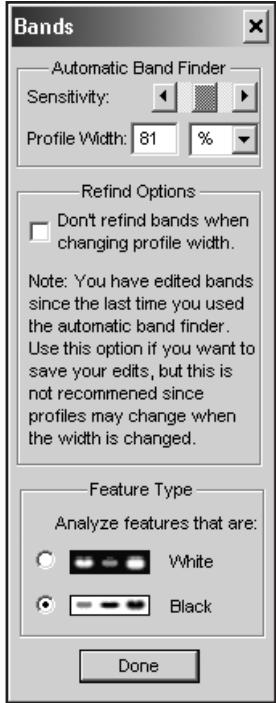


NOTE: When an image is low contrast, Carestream MI Software may have difficulty determining whether the band signal is black or white. The correct signal can be manually selected using the Adjust Bands on the Lanes panel.

- 2 Review the image to determine if the parameters for band finding are set correctly. Look for extraneous bands or bands that have not been defined.

NOTE: If the band finding on the image is accurate, proceed to *Calculating Molecular Weight* later in this chapter.

- 3 Click the Adjust Bands on the Lanes panel. The Bands (Adjust Bands for Macintosh) dialog box appears.



- ✓ The *Sensitivity arrows*—adjusts the band finding sensitivity. The highest sensitivity (+3) finds the most bands, while the lowest sensitivity (-3) finds only well resolved bands. As you adjust the sensitivity, Carestream MI Software updates the number of bands on the image.
- ✓ Adjust the percentage of the *Profile Width* to encompass the band width. Ideally, you want to encompass the entire band with little or no background within the band rectangle. Click on a band and view the Profile window to see the band rectangle. If the *Refind Options* checkbox is checked bands, only the profile would be adjusted but no new bands will be found.
- ☞ NOTE:** A setting of 99% may be necessary for bands which are bleeding into the next lane. A smaller setting may improve accuracy of bands that are curved or nonuniform. Consider using ROI analysis for bands of this type.
- ✓ The *Feature Type buttons*—are used to define the band type as either white or black. This preference affects both the lane finding and band finding algorithms.



- ✓ Use the Reference Band tool from the Tool to draw an ROI that bounds a typical band within the gel. The location, width, height, and intensity of the band are automatically used in the Find Lanes and Find Bands algorithms. Once a reference box is drawn, the box can be edited using any of the four corners to resize prior to choosing Find Bands. This is especially useful when the automated finding tools are not producing good results

☞ NOTE: Brightness/contrast adjustments using the Image Display window are dynamically linked to the lane-finding algorithm. To increase sensitivity, adjust the image to best visualize the bands of interest.

- 4 When all the bands on the image have been found, click Done.

☞ NOTE: You may also add bands using the Band tool to designate bands that Carestream MI Software failed to find. See *Editing Bands*, later in this chapter.

5 Band Analysis Data



Editing Bands

After analysis, you may need to add, delete, or adjust the position or boundaries of bands.

Deleting Selected Band Label(s)

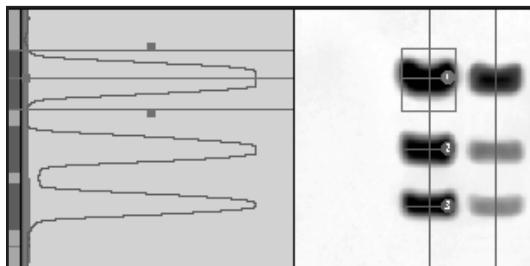
- 1 Select the Pointer tool from the Lanes panel.
- 2 Click on a Band Label. If more than one band needs to be deleted, Shift-click to select multiple objects with the Pointer tool or click and drag the mouse over an area of the image containing more than one object.
- 3 Press the Delete key to remove the band information from the Image section and the Lane Analysis Data window. The remaining bands are automatically renumbered sequentially.

NOTE: To delete all the bands, click Delete Bands from the Lanes panel.

Adding a Band Label

You may need to add Band Labels if all bands of interest were not found automatically. You can add Band Labels with the Band Label tool.

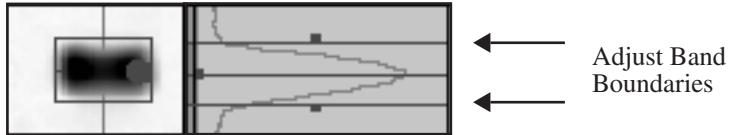
- 1 Choose the Band Label tool from the Lanes panel.
- 2 Click on the image where you want to place a band. The profile for the lane automatically appears in the window.



A Band Label appears and all subsequent Band Labels are renumbered. The band is selected and the band editing lines in the Profile window are active.

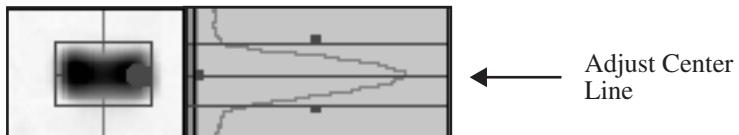


- 3** In the Profile window, drag the top and bottom lines to adjust the band boundaries.



This defines the band rectangle used to determine net intensity, and therefore, band mass.

- 4** In the Profile window, drag the center line to position it at the point of peak intensity. Alternatively, drag the Band Label in the Image section into position.



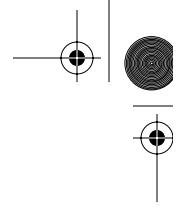
The center line defines the band mobility, which is used to calculate molecular weight if a standard is used.

5 Band Analysis Data

Viewing Lane Analysis Data

The Lane Analysis Data window can display the following information for each band:

- ✓ *Molecular Weight*—for an experimental band, is the mobility relative to one or more standards.
- ✓ *Mobility*—measured location of the band from the top Lane Marker/Iso Molecular Weight Line relative to the length of the lane. Mobility is reported in pixels, inches, or centimeters. You can select units in the Preferences dialog box.
- ✓ *Mass*—for an experimental band, is determined by comparing the sum of the background subtracted intensities (Net Intensity) of all the pixels in the band with the background subtracted intensities of all pixels in the standards.
- ✓ *Band Area*—the area of the band in pixels², inches², or cm². You can select units for area in the Preferences dialog box.
- ✓ *Band Name*—displays the name of the band, if assigned.
- ✓ *Band Peak Intensity*—provides the intensity value at the peak of the profile.
- ✓ *Net Intensity*—the sum of the background-subtracted pixel values in the band rectangle.
- ✓ *Sum Intensity*—the sum of all the pixel intensities in the band rectangle.
- ✓ *Relative to*—offers two options:
 - other bands in the same lane* which calculates the percent intensity contribution of a band within a lane.
 - in the same bands* in which the same band number across lanes is compared. The reference band is entered in the text edit box.
- ✓ *Mean Background Intensity*—the average background intensity in the band rectangle.
- ✓ *Mean Intensity*—the average intensity of the pixels in the band rectangle.
- ✓ *Maximum Intensity*—the maximum pixel intensity in the band rectangle.
- ✓ *Model Net Intensity* (Only available for Gaussian Analysis)—the mathematical approximation of the Net Intensity using a Gaussian or asymmetric Gaussian model.
- ✓ *Model Mass* (Only available for Gaussian Analysis)—the mathematical approximation of the mass using a Gaussian or asymmetric Gaussian model.



To view the lane analysis data:

- 1 Click Analysis on the Quick Access bar. The Lane Analysis Data window appears.

The Lane Analysis Data window displays molecular weight (MW) and mass (ng) for three lanes: 1, 6, and 7. The data is presented in a grid format with two header rows and seven data rows. Lane 1 has MW values of 23130, 9416, 6557, 4361, 2322, 2027, and 564, and mass values of 476.9, 194.1, 135.2, 89.9, 47.9, 41.8, and 11.6 respectively. Lane 6 has MW values of 6000.3, 4305.5, 3126.5, and 101.9, and mass values of 257, 202.5, and 160.7. Lane 7 has MW values of 36603.4, 24814.2, and 159.1, and mass values of 160.7, 159.1, and 159.1.

Lane Analysis Data- tutorial.bip						
Lane Set A		Lanes	Display			
	1: Lambda-Hi...	6: Chet.6	7: Chet.7			
1	23130	476.9	6000.3	257	36603.4	160.7
2	9416	194.1	4305.5	202.5	24814.2	159.1
3	6557	135.2	3126.5	101.9		
4	4361	89.9				
5	2322	47.9				
6	2027	41.8				
7	564	11.6				

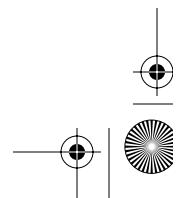
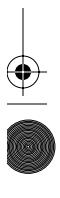
NOTE: If the Profile window is displayed, only the data associated with the Profile window is displayed in the Lane Analysis Data window. If the Lane Profile window is not open, the Lane Analysis Data window displays the results for all active lanes in the Image section.

- 2 Use the Lane Set pop-up menu to choose the Lane Set data you want to view. The active lane set is the default display.

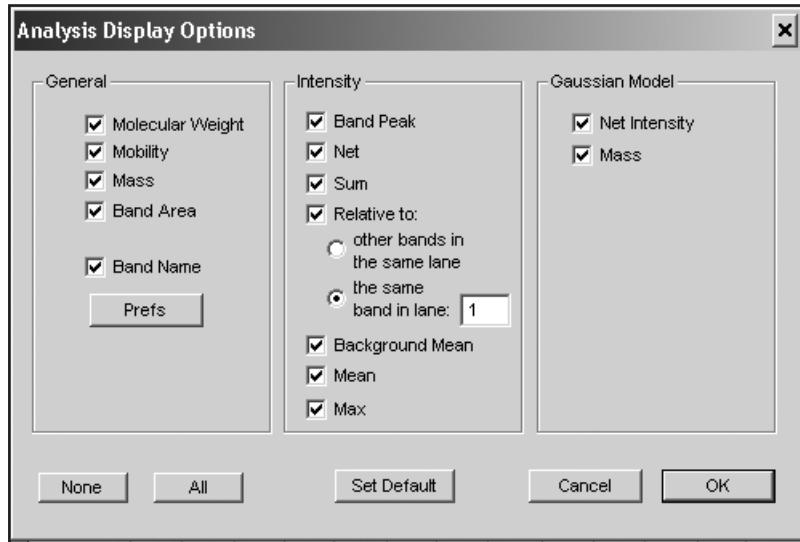
- 3 Select the lanes you want displayed using the Lanes pop-up menu in the Lane Analysis Data window. Choose All, None, or select the lanes you want displayed.



NOTE: The defaults selection is All. When you want to display a single lane, choose None to deselect all lanes, and then select the lane you want displayed.

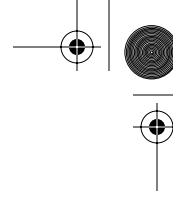


- 4** Choose Display to select the analysis variables you want shown in the Lane Analysis Data window.



 NOTE: The units of your data are defined in the Preferences. Access the Preferences by choosing Preferences from the Quick Access bar.

- 5** Click OK. The Lane Analysis Data window updates to reflect the variables you have selected.



Viewing the Lane Profile

The first step in analyzing the image is to create a median lane profile of pixel intensities of each lane. A profile is generated from the median value along each row of pixels perpendicular to the Lane Line. The lane profile is then analyzed to identify band positions. The bands in each lane correspond to the peaks of the lane profile and is displayed on the right side of the Project window.

Use the Profile window to compare band positions between a standard and an experimental lane or to add, delete, and adjust Band Labels. Also, use the profile to adjust the position of any band (molecular weight calculation) and the analysis boundaries of any band in a lane (mass calculation).

- 1** Click the Profile on the Quick Access bar located across the top of the Project window.
The Profile window appears on the right side of the Project window.
- 2** Click the Pointer tool on the Lanes panel.
- 3** Select a lanes to view by clicking on the appropriate Lane Line or choosing the lane from the Exp pop-up menu at the bottom of the Profile window. To display a Standard, select the Standard (Std) pop-up menu at the bottom of the Profile window.
- 4** Click a band in the active lane. A rectangle appears around the band in the Image section.



This rectangle represents the image data Carestream MI Software uses during the analysis process. Three red lines appear in the profile to indicate the position of the band's vertical center and upper and lower boundaries.

NOTE: Increase the image magnification to better view the lane median profile.

Choosing the Background

Background Methods Overview

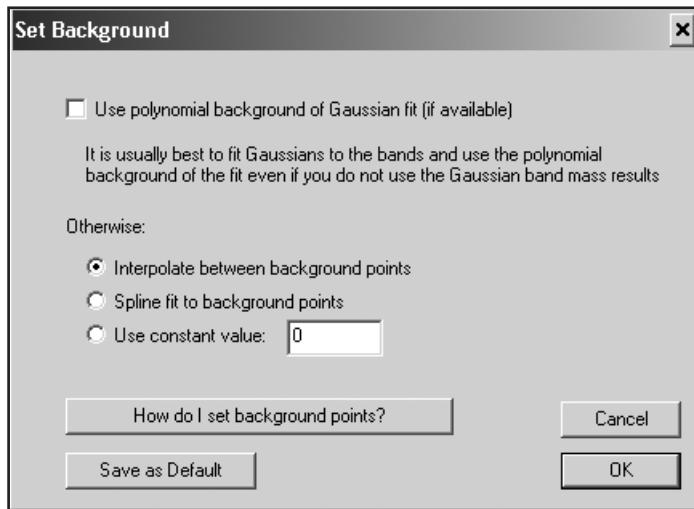
You can choose how background is calculated. You have several options

- ✓ A *Polynomial Background*—best represents the background intensity in your lane profiles because a Gaussian fit considers the profile as a composite of the background and the emission from all the bands at every point in the profile.
- ✓ *Interpolate between background points*—useful when you have well separated bands. Each background is estimated by interpolating between background points along the background.
- ✓ The *Spline*—provides more “manual” control over the shape of the background. A spline is a mathematical function that will go exactly through each of the background control points that you specify while at the same time generating a background curve varies smoothly between all the other points that are not marked as background control points. When you add background control points to a spline curve, it is generally best not to add too many and be careful not to add them too close to one another as this can cause the spline to behave erratically as it tries to go through each point in the profile.
- ✓ *Constant Value*—you can enter any value in the text edit box. The number (gray levels) that you enter is subtracted from each pixel within the band rectangle as background.

You can edit how the background is defined within the lane profile by adding background control points. Backgrounds can be further improved by combining the manual adjustments with fitting bands (which applies Gaussian or asymmetrical Gaussian fitting to the band data) or with splines.

Setting the Background Method

- 1** Click Set Background on the Lanes panel. The Set Background appears.



- 2** Choose the Background method you'd like to use.

- 3** Click OK.

Adjusting Background Points

You can edit how the background is defined within the lane profile by adding background control points. Backgrounds can be further improved by combining the manual adjustments with fitting bands (which applies Gaussian or asymmetrical Gaussian fitting to the band data).

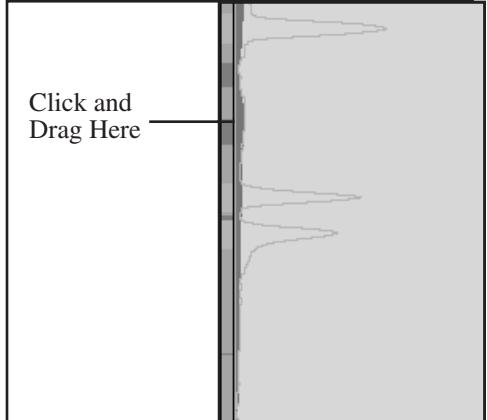
Adding these points forces the software to adjust the background to:

- ✓ Go through local profile points for non-Gaussian fitted bands.
- ✓ Refit the Gaussian model in the localized region using a 10X weight for a single point and a 1X weight for a region of control points.

To add background points along the profile display:

1 Click the Profile on the Quick Access bar located across the top of the Project window. The Profile window appears on the right side of the Project window.

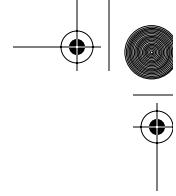
2  Click the Pointer tool in the background portion of the lane profile. The cursor changes to a red line.



3 Click and drag the cursor over the area in which you would like to call the background, or simply click to add individual points.

4 The control points are added and the background is refitted (represented in the window in gray). Next to the lane profile, a solid red line denotes the area in which background measurements are taken. You cannot, however, add background points where the signal is saturated.

 NOTE: To remove, repeat the click and drag over area.



Calculating Molecular Weight

The molecular weight is based on the relative mobility of each experimental band as compared to one or more standard lanes. Since bands of equi-molecular weight do not always uniformly migrate across the width of the gel, the software uses the locations of horizontal Lane Markers or Iso Molecular Weight Lines to compensate for these variations—where the intersection of the Lane Marker or Iso Molecular Weight Line with each lane indicate locations of equal molecular weight. This normalizes the migration data for each of the standard and experimental lanes.

To accurately determine the molecular weight of unknown bands, the normalized migration distance versus molecular weight for each standard lane is plotted. The software uses the intensity information for each band to calculate the center of gravity of the band (3rd moment of intensity). The molecular weight versus mobility curve is created for each standard lane.

The molecular weight for unknown bands is determined using the normalized migration distance for the band. If there is only a single standard lane, the normalized mobility of each experimental band is plotted against the standard curve to determine molecular weight.

When more than one standard lane is used, the software uses a sophisticated algorithm to determine the molecular weight of the experimental bands. If multiple standard lanes fit the criteria, the unknown molecular weight become the average of the most reliable estimates obtained from each of the standards.

The molecular weight of any bands that fall within the molecular weight range of any standard is displayed in black text in the Lane Analysis Data window. For experimental bands that fall outside the molecular weight range of all standards, the molecular weight is estimated and displayed in red. Red values are considered less reliable.

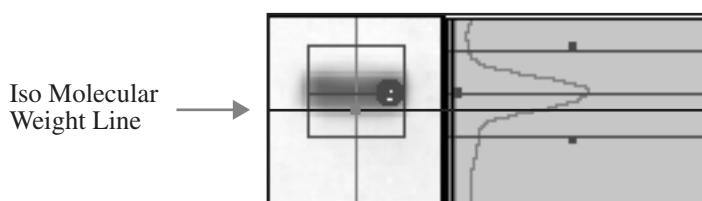
The only difference between the molecular weight calculation from Lane Markers and Iso Molecular Weight Lines is in the determination of the normalized migration distance. With Lane Markers, the software uses information from the top and bottom Lane Marker to determine migration distance. When you create an Iso Molecular Weight Line it internally refines (these are not visible to you) the top and bottom markers to reflect the curvature of the Intermediate Iso Molecular Weight Lines (when you have a curved Iso Molecular Weight Line the program assumes that the top and bottom Lane Markers should have a similar curvature). The top Lane Marker is refined to be parallel to the top-most Iso Molecular Weight Line and similarly, the bottom Lane Marker is redefined to be parallel to the bottom-most Iso Molecular Weight Line.

Adjusting Iso Molecular Weight Lines

Some of the anomalies you will encounter include smiles in the bands, curved lanes, etc. You can compensate for mobility problems through the accurate placement of Lane Markers. See Chapter 4: *Defining Lanes*. The Iso Molecular Weight Lines indicate points of equivalent band migration. These lines can be adjusted at any time during the analysis process (both before and after bands have been found); however, it is typically recommended to adjust Iso Molecular Weight Lines after bands have been found.

With Carestream MI Software, you can view the mobility of the bands in the image after finding bands. The mobility is critical in determining the molecular weight.

- 1 Click the Profile on the Quick Access bar located across the top of the Project window. The Profile window appears on the right side of the Project window.
-  2 Click the Pointer tool in the Lanes panel.
- 3 Click on the band label of interest.



The black line that appears in the Profile window indicates your position relative to the lane shown in the profile. The Iso Molecular Weight Lines appear in green.

 NOTE: It is important to place the desired number of Lane Markers prior to finding bands. After bands have been found you can no longer add any additional Lane Markers or Iso Molecular Weight Lines.

- 4 Select the Pointer tool and click on a horizontal Iso Molecular Weight Line. The line becomes highlighted in red and control points appear on each of the intersections with the vertical Lane Lines.
-  Prior to the editing of any control point, the Iso Molecular Weight Line and Lane Markers function identically. Once any control point is edited, the Lane Marker no longer is used in the analysis process.

- 5** Click and drag one of the control points up or down a vertical Lane Line. Since any of the control points can be edited, you have unlimited control over the shape of the Iso Molecular Weight Line.

✓ To simplify the process of editing Iso Molecular Weight Lines, the software automatically interpolates the curve between edited control points and/or the end points. The appearance of the control points in the Iso Molecular Weight Line indicates whether or not the control point has been edited. Blue control points have been edited.

- 6** As you drag a control point near a Band Label, the program automatically locks the control point. By locking an Iso Molecular Weight Line to the band call point, you get the most accurate molecular weight calculation possible.

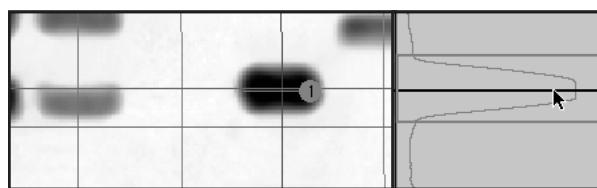
 NOTE: If the control point has locked to a Band Label, you can continue to drag the cursor to unlock the control point.

Iso Molecular Weight Lines have the same restrictions as Lane Markers. No part of an Iso Molecular Weight Line can cross the bottom or top Lane Marker or another Iso Molecular Weight Line.

Adjusting Molecular Weight

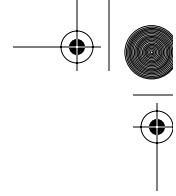
The position of a Band Label is used to measure the mobility of a band and calculate its molecular weight. When bands are found automatically, the Band Label position is determined by finding the vertical center of the signal (that is, the center of gravity) in the band rectangle. If you disagree with the band position, or if you add a band manually, you can adjust the band position as follows:

- 1** Click the Profile on the Quick Access bar located across the top of the Project window. The Profile window appears on the right side of the Project window.
- 2** Click the Pointer tool in the Lanes panel.
- 3** Select the band label of interest.



Three red lines with handles appear in the Profile window, identifying the band area.

- 4** Drag the handle on the center line. Position the line in the center of the band peak, or at the leading edge (bottom) depending upon where you normally designate molecular weight.
- 5** The mobility and molecular weight measurements are automatically updated.

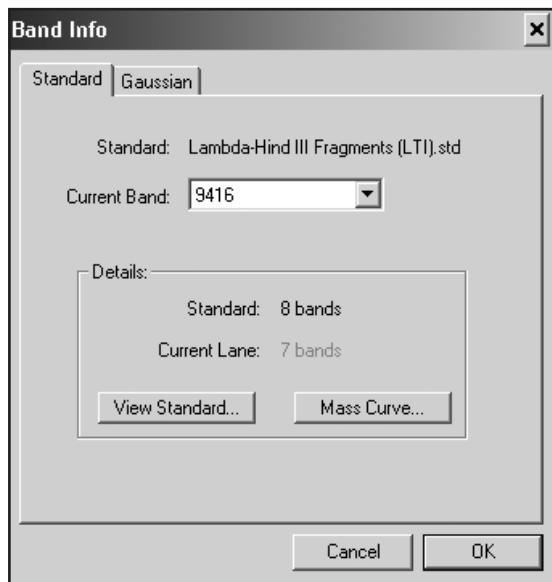


Reassigning Molecular Weight Values to Standard Bands

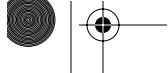
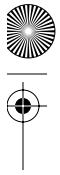
When assigning standard bands a molecular weight, it is assumed that the first band found is the first band in the standards band list. If some of the bands are unresolved, you can assign molecular weights to these bands. You can also merge two bands together—averages their molecular weight and sums their mass values.

Reassigning Molecular Weight Values

- 1 Click the Pointer tool in the Lanes panel.
- 2 Double-click the standard band to be reassigned. The Band Info dialog box appears. Choose the Standard tab.
- 3 The current band assignment appears in the Current Band pop-up menu. Use the pop-up menu to reassign the band molecular weight if it is incorrect.



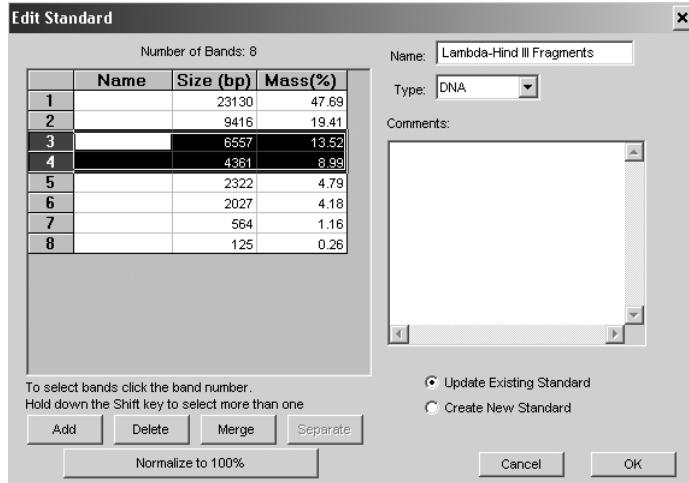
- 4 Click OK. The band is reassigned and the data in the Lane Analysis Data window is updated.



Merging Unresolved Band Values

Sometimes standard bands do not resolve. You can merge these bands' standard data for analysis. This speeds up the analysis process by eliminating the need to edit or create standards files. When merged their molecular weight is averaged and their respective mass values are summed.

- 1** Click the Pointer tool in the Lanes panel.
- 2** Double-click the standard band to be reassigned. The Band Info dialog box appears.
- 3** Click View Standard, the Edit Standard dialog box appears.



- 4** Select the bands you want to merge by Shift-clicking on the band numbers.
- 5** Click Merge Unresolved Bands.
- 6** Click OK. The bands are merged—the merged bands are represented by the same number and the data text color changes in the Edit Standard dialog box. The molecular weight values in the Bands Info dialog box and the Current Bands pop-up menu updates. You can then assign their averaged molecular weight to the standard band.

NOTE: You can separate merged bands by selecting any band number of the merged band set in the Edit Standards dialog box, and then clicking Separate Bands.

Calculating Band Mass

Band mass calculations (for non-Gaussian fits) use the intensity information for each band. The program first determines the leading and trailing edge for each band (shown in the Profile window). Next, Carestream MI Software sums the intensity of each pixel within the band rectangle. During these calculations, the software uses the background method you choose to calculate the true band intensity. Using the band intensity measured for each standard band, the data is modeled using different functional forms: power, exponential, 2nd and 3rd order polynomial. A fit is generated from the mass information in the standard file and the measured signal of the bands in the standard lane(s). This fitted curve and the background adjusted signal of the unknown bands are used to determine the mass.

Using Mass Curve, you can view the standard data, choose the standards points to use and select a function that best fits your standard data.

When more than one standard lane is used, all the data is used. The resulting curve is used to determine the mass of experimental bands. For very low mass experimental bands the curve may have a negative mass value. In these cases the band mass cell appears as “---.” When the unknown signal is within the standard range, the mass is displayed in black in the Analysis window. When the unknown signal is outside the standard range, the mass is displayed in red and is considered less reliable.

The quality of the mass calculation is dependent on the quality of the original image and on the bands chosen as standards. Images with a low dynamic range where the bands of interest are very close to the background level gives poor results. Bands that are saturated or poorly resolved also skew the results. In these cases, the data should be Gaussian fit for more accurate results as explained later in this chapter.

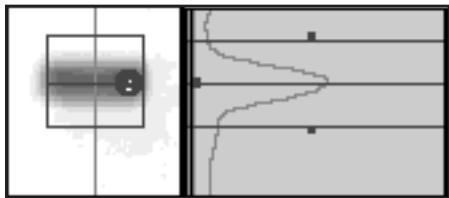
Adjusting Band Mass

The band rectangle is used to estimate the band mass and to measure the sum intensity and mean intensity of the band. When a band is added, the top and the bottom boundaries of the band rectangle are set to an arbitrary default. You must adjust these arbitrary boundaries. Occasionally, you may also need to adjust bands found automatically.

- 1 Click the Profile on the Quick Access bar located across the top of the Project window. The Profile window appears on the right side of the Project window.

-  2 Click the Pointer tool in the Lanes panel.

- 3 Select the band to be adjusted. Three red lines with handles appear in the Profile window, identifying the band area.

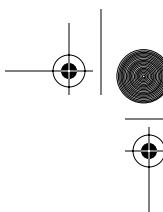


- 4 Drag the handle on the top line. Drag up or down to adjust the band boundary. Position the line at the tip edge of the band.

- 5 Drag the handle on the bottom line. Drag up or down to adjust the band boundary. Position the line at the bottom edge of the band.

 NOTE: The edge of the band is determined mathematically. However, you can manually adjust the band edge.

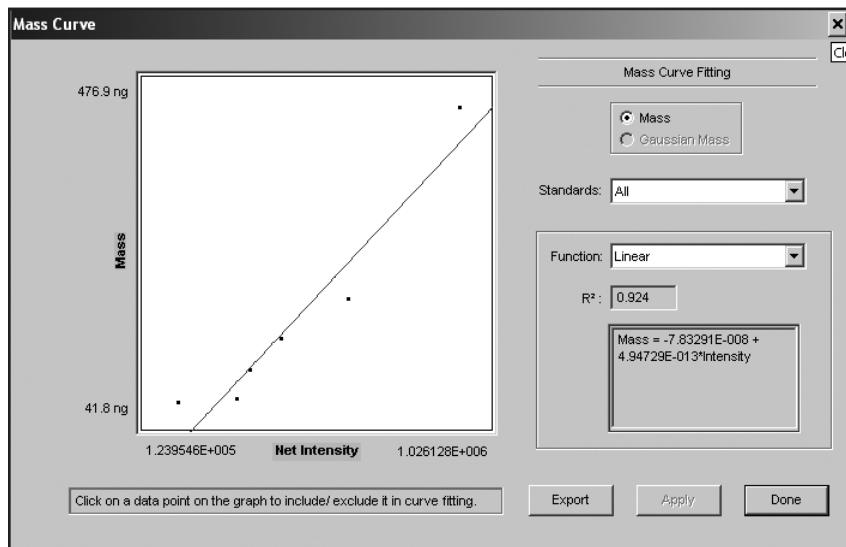
- 6 The measurement of the sum intensity and mean intensity of the band rectangle, and the mass estimate for the band are automatically updated in the Lane Analysis window.



Reviewing the Mass Standard Curve

The Standard data is plotted on a graph which is used to calculate the experimental mass values (net intensity versus standard band mass). You can optimize the fit function to the data and choose which bands to be included in the determination of mass.

- 1 Access the Mass Curve dialog box by selecting Mass Curve from Lanes panel or by double-clicking on a standard band and selecting Mass Curve from the Band Info dialog box. The Mass Curve dialog box appears.
- 2 Use the Mass buttons to review the mass standard curve or the Gaussian mass curve (if done).

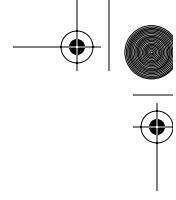


To maximize the accuracy of your mass determination, you may want to review both curves to make sure that the appropriate points are included, and the correct functional form is selected.

- 3 Select which standards to use in the mass curve. You can view the mass curve for each standard to evaluate whether or not they should be used in the standard curve, i.e., R^2 is not recalculated as you choose different view of the standards data.
- 4 Choose the fitting function that best represents the data using the Function pop-up menu. The R^2 value aids you in determining the best fit.

- 5 Remove any obvious outliers by clicking points in the graph. The points appears with an X and is not be used in the mass calculations. Reactivate a point by clicking on the X.
- 6 Click Apply when the graph best represents the data.

 NOTE: If you want to print a copy of the mass curve, you can place the mass curve on an Annotation page. When printing, click the Annotations checkbox.



Redefining a Standard

In your experimental design you may decide to use more than one standard. You may also choose to use different standard lanes for analysis of different regions in a gel. The software compiles all standard lane information to generate the most accurate standard curve.

Inactivating a Standard

- 1 Click the Set Standards button from the Lanes panel or by double-clicking a vertical Lane Line with the Pointer tool.
- 2 The Lane Information dialog box appears. Change the Lane Type pop-up menu to Inactive.
- 3 Click OK. The Lane Analysis Data window automatically updates the data using only the remaining standard(s).

Reactivating a Standard

- 1 Click the Set Standards button from the Lanes panel or by double-clicking a vertical Lane Line with the Pointer tool.
 - 2 Change the Lane Type pop-up menu to Standard.
 - 3 Click OK. The Lane Analysis Data window automatically updates the data using the additional standard.
- NOTE: If you edit a standard file after finding bands, you must refind bands to update the Lane Analysis Data window.

Removing Bands

If the situation arises where you need to begin again, remove all of the bands and start over.

- 1 Choose Delete Bands from the Lanes panel. A dialog box appears asking if you want to remove all bands.
- 2 Click Yes to delete all bands.

Using Gaussian Fit Bands

You may want to fit your bands to more accurately predict their mass. Data modeling may be particularly applicable in determining values for unresolved bands, over-saturated bands, and for images with an uneven or high background.

Quantitative analysis of overlapping bands on an image is best approximated by fitting the data to a Gaussian or asymmetric Gaussian curve (Vohradsky J and Panek J, Electrophoresis, 1993; 14:601-12). Fitting Gaussians to bands is appropriate since the migration of the molecules through the matrix is a random process and is usually well-modeled using Gaussians.

The Gaussian curve was named after the mathematician Karl F. Gauss in the early 1800's. It is a normal "bell" shaped distribution curve, described mathematically as:

$$y = ae^{-\left(\frac{(x-b)^2}{c^2}\right)}$$

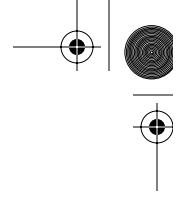
where:

- a* is the amplitude of the peak
- b* is the x value of the peak
- c* is a characteristic width

A modified function is referred to as an "asymmetrical" Gaussian which has a different width for either side of the peak. Most bands are well described by a Gaussian fitting function, but some bands, particularly those with sharp leading edges and long tails, are better described by an asymmetric Gaussian.

When you Gaussian fit bands, you are creating a mathematical function that best approximates your data. The software performs this "fitting" using a sophisticated nonlinear least squares technique first developed by Levenberg and later modified by Marquardt (Marquardt DW. Journal of the Society of Industrial and Applied Mathematics, 1963; 11:431-41).

Each lane is fit with a fifth order polynomial, plus a separate Gaussian or asymmetric Gaussian function for each band in the lane. This fitting is an iterative process that starts with an initial guess for all the fit parameters using the current background and the band information in the Lane Analysis Data window. The fitting process adjusts the coefficients of the background polynomial and the parameters of each Gaussian as it attempts to minimize the chi-square of the difference between the model and the lane profile. This difference is referred to as the residual.



When to Fit the Data

Use the Profile window to examine each lane profile. Modeling your data is particularly valuable if:

- ✓ One or more bands are saturated (flat-peaked).
- ✓ Bands are unresolved (closely spaced).
- ✓ Faint bands lie near very bright bands.
- ✓ The background is uneven and highly variable.

Even if none of these cases hold true for your data, you may want to try fitting in order to optimize the estimation of the background. Without fitting, the default background is calculated using point-to-point interpolation to estimate the background in each lane. If you see that the background for a particular lane does not appear to follow the “true” background after adjusting, you may want to try fitting because the polynomial background of the model usually produces a better estimate.

Remember that modeling your data is a nondestructive process. You can choose to ignore the model's estimated parameters for any or all of the bands in a lane. Net intensity and mass is calculated by simply integrating the pixel values in the band rectangle. The only difference is that after modeling, it uses the new more accurate polynomial background.

Along with the mass derived from simple integration, the software reports a model estimated mass and net intensity. Keep in mind that these estimates are based on the one-dimensional profile. If your bands have uneven brightness variations perpendicular to the Lane Lines or if your bands have pronounced “smiles,” the model estimated values may not be valid.

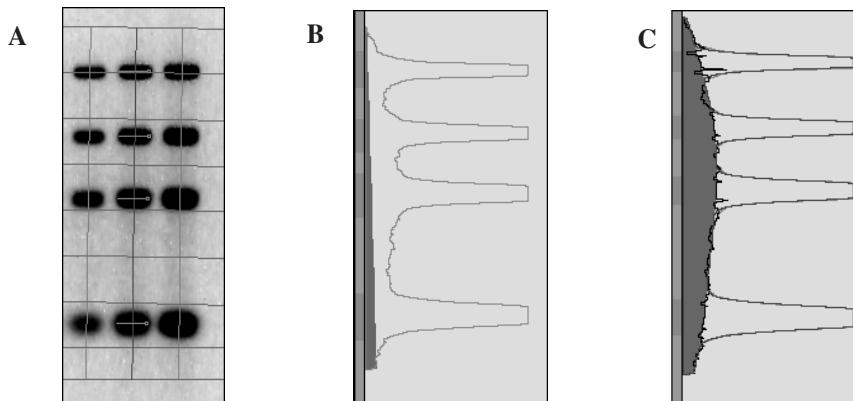
Use the profile to choose whether the bands are best fit with Gaussian or asymmetric Gaussian function. The profile and model curves are displayed, in addition to the residual of the curve. The residual of the curve is the difference between the model and the original data. In modeling, the software calculates a fit which minimizes the residuals.

For saturated bands, the Gaussian technique is able to use the information on the shoulders of the band's profile to determine the shape of the Gaussian that best models the data. Therefore, the Gaussian technique better estimates bands that are beyond the dynamic range of the capture device.





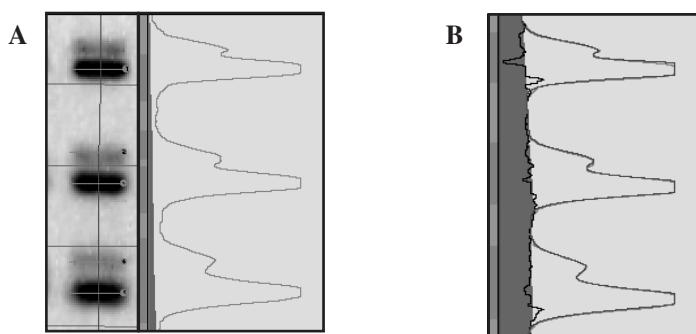
This image demonstrates the use of a Gaussian for saturated bands.



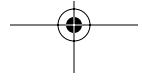
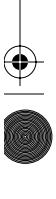
- ✓ In Diagram A, the gel image has well resolved bands.
- ✓ After finding bands, the profile can be displayed as in Diagram B.
- ✓ Diagram C shows the profile after modeling with the Gaussian curve.

When a profile has uneven background as depicted in B and/or saturated bands, a Gaussian curve should be used.

This gel image and corresponding profiles show how Gaussians can be useful for estimating poorly resolved bands.



- ✓ Diagram A shows a gel image with unresolved bands which can be seen in the profile.
- ✓ Diagram B shows the profile after modeling with the asymmetric Gaussian curve. Also notice the difference in the background before and after modeling the data.



Assessing the Profile for Modeling

Now that the bands are identified, it's time to determine whether or not the data should be adjusted by fitting the results to a Gaussian curve.



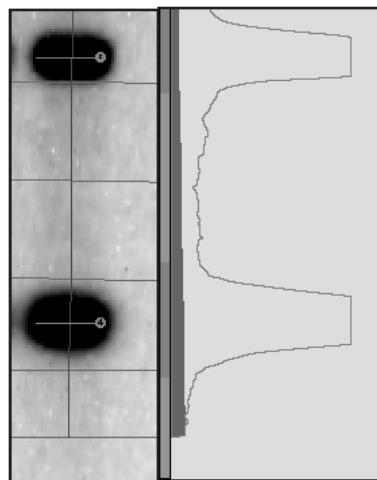
- 1 Open the Profile by clicking on selecting the Profile on the Quick Access bar.

The profile appears for the first lane in the image or the lane selected along with a standard lane profile, if one has been designated.

- 2 Review the profile for background, band saturation, and the presence of unresolved bands.

Background

In this example, the background has not been calculated accurately (as shown in dark gray in the profile). Fitting with a Gaussian improves the saturated band data, while also providing a better background. The new background values are used to calculate the modeled net intensity and mass, as well as to update the traditional mass results.

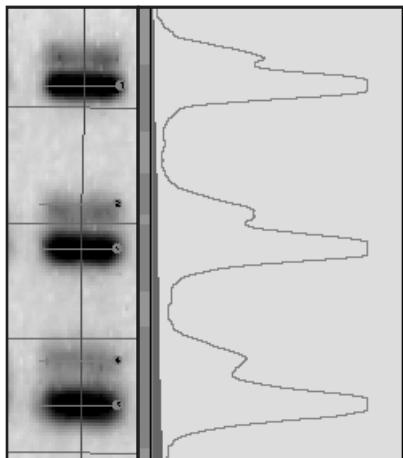


NOTE: The background can also be adjusted by adding control points. For more information, see *Adjusting the Background*, earlier in this chapter.



Poorly Resolved Bands

Notice the curve of the profile in relation to the bands on the right. The small upper blip indicates a possible second band before the found band. Add the upper band using the Band tool before fitting the data. Fitting improves the data for this image.

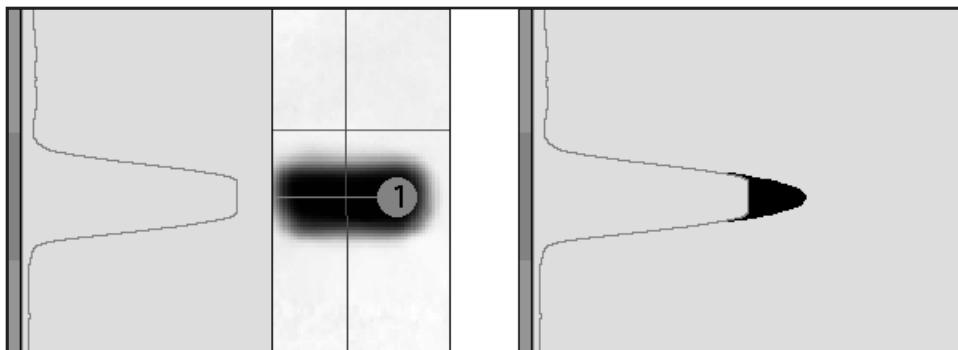


NOTE: If you have trouble inserting a band in the precise location, add a band in the blank area above or below the location or use the grab handles to reduce the size of the found band before adding the new band.



Over-Saturation

Notice the flat peak of the band profile, indicating the band is over-saturated. As stated previously, over-saturated bands are ideally suited for Gaussian band fitting. In the example shown, notice that a significant part of the peak is missed when you do not model the data.



Fitting the Bands

Gaussian Fit Bands

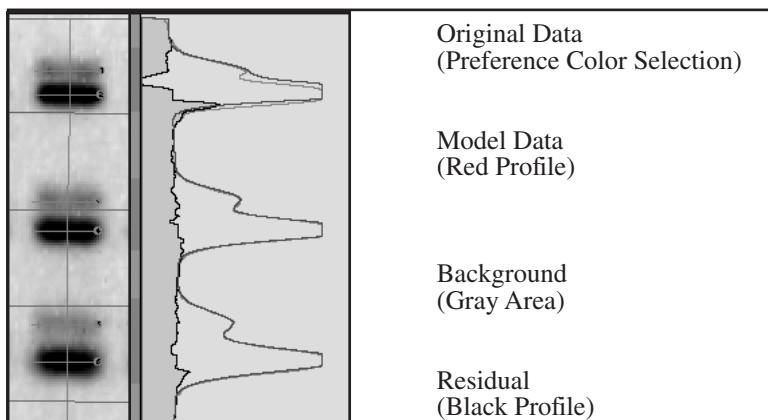
- 1 Click Gaussian Fit Bands button on the Lanes panel. The software initiates the default Gaussian algorithm.
 - 2 The default can be set to either Gaussian or asymmetric Gaussian using the Band Information dialog box which can be accessed by clicking Adjust Gaussian Fit. A progress indicator appears showing the progress of the algorithm.
- NOTE:** If a dialog box appears stating that a number of bands need to be deleted or adjusted. See *Optimizing the Band Fit*, later in this chapter.

Profile

Std:1 Exp:2 ▾

- 3 View the Profile by choosing Profile from the Quick Access bar or Lanes from the Mode. The profile appears displaying the first lane in the image.
- 4 Select a lanes to view by clicking on the appropriate Lane Line or choosing the lane from the Exp pop-up menu at the bottom of the Profile window.

- 5 To display a Standard by selecting the Std pop-up menu at the bottom of the Profile window.



5
Band Analysis Data

- 6 The original data, the model, background, and the residual can be shown by using the pop-up menu at the bottom of the Profile window.
- 7 Look at the original profile. Has the program selected the correct number and placement of bands?
 - ✓ If yes, review the residual and assess the Gaussian type.
 - ✓ If no, add or delete the bands and refit the lanes.

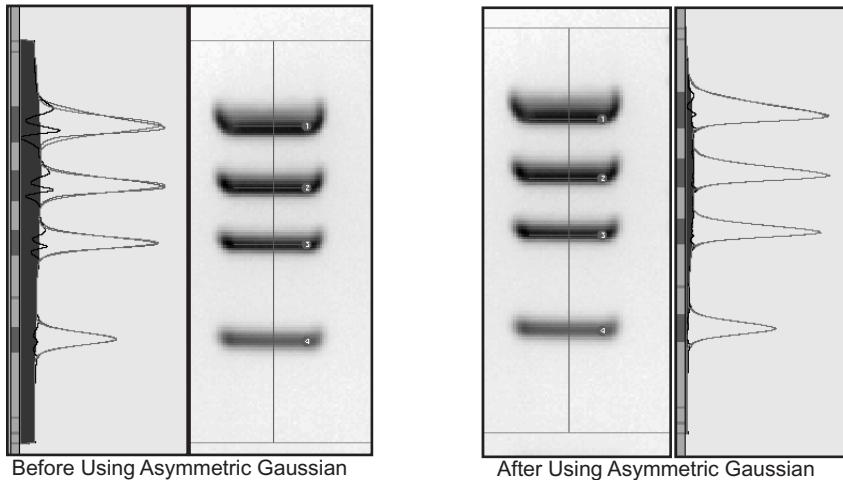
Assessing Gaussian versus Asymmetric Gaussian

Review the residual, for the band. Is it an asymmetric residual?

- ✓ If no, the analysis is complete.
- ✓ If yes, change the fit parameters as follows.

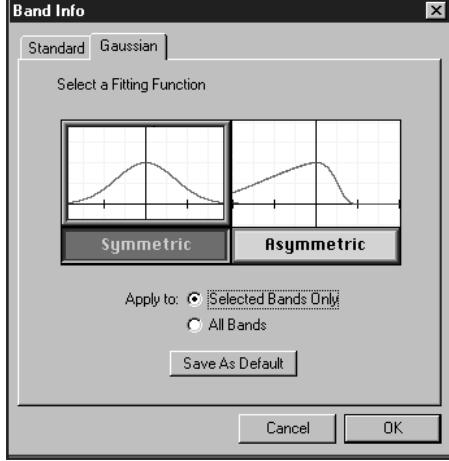
Selecting Gaussian Type

-  1 Select the band to be fit to the asymmetric Gaussian curve with the Pointer tool. Use Shift-click to select multiple bands.
- 2 Double-click on the band of interest or choose Band Information from the Analysis menu and the Bands submenu. The Band Info dialog box appears.



- 3 Select Adjust Gaussian Fit.

- 4** Click the Asymmetric Gaussian button.



- 5** Choose the Selected Bands Only or all Bands to apply.
6 Click OK. The band(s) is refit to a asymmetric Gaussian curve.
7 View the Profile window by clicking Profile from the Quick Access bar.

The model represents the fit data and is displayed in the Lane Analysis Data window with the data before the fitting.

Optimizing the Band Fit

Understanding how the data is modeled aids you in optimizing your experimental results. When the software fits your data it starts with a set of initial guesses for the fit parameters. Changing the initial guesses for the fit parameters can help find a much better final fit.

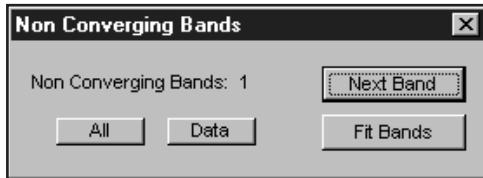
The first time you fit a lane, the software automatically generates the initial guesses for the fit parameters and proceeds to iterate toward a minimum in chi-square. If this initial fit fails to converge (i.e., arrive at an optimum fit), you are prompted to review the profile for the lane and make changes. You might also want to edit the fit for the lane if the initial fit does not produce the results you want.

After you edit the model for a lane, click Fit Bands Again and the software starts with your initial guesses for the fit parameters and “ tweaks” them as it tries to minimize the chi-square of the fit. Feel free to edit the model and refit it until you get the results you want.

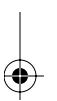




To review the bands that do not converge, choose Non Converging Bands. The Non Converging Bands window displays the number of bands that do not converge.



- ✓ *All*—displays all non converging bands in the image and the Lane Analysis Data dialog box.
- ✓ *Data*—opens the Lane Analysis Data dialog box displaying only lanes with bands that did not converge. Bands are identified by a bullet following the modeled value.
- ✓ *Next Band*—selects the next non converging band—highlighting one band at a time
- Fit Bands* performs the fit command.
- ✓ *Fit Bands*—executes the fitting function on the bands that you have edited.





Troubleshooting Gaussian Fitting

You will be prompted if the software is unable to optimize the fit for a lane when one or more bands failed to converge correctly. You can usually get better results if you follow these hints:

Remove “False” Bands

Examine the profile and remove any obviously false bands in the data. If you really want to fit a band at that position, see *Problems Fitting Very Faint Bands* below.

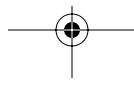
Problems Fitting Very Faint Bands

Very faint bands can be difficult to fit because their signal is not much higher than the background. If a very faint band is not fitting correctly, try the following procedure:

-  1 Select the faintest bands with the Pointer tool and Shift-clicking on each band to select and then press the Delete key.
- 2 Click the Gaussian Fit Bands Button on the Lanes panel or Fit Bands button on the Non Converging Bands window. The fit is calculated again. A better background level is obtained, and therefore, better calculation of the band mass.
-  3 Add the faint bands back into the lane with the Band tool.

 NOTE: Anytime you add, delete, or adjust bands during a Gaussian fitting, refit the bands by clicking the Gaussian Fit Bands Button on the Lanes panel or Fit Bands button on the Non Converging Bands window.

- 4 If needed, adjust the band boundaries (three red lines) in the Profile window.
- 5 Click the Gaussian Fit Bands Button on the Lanes panel or Fit Bands button on the Non Converging Bands window. The software uses the calculations in the previous iteration without the faint bands to determine the values for the faint bands.
- 6 Review the results.



Singular Matrix

Sometimes, as the software attempts to invert the matrix of equations it uses to compare the model to the data, it finds that the matrix is “singular.” This means that the software could not continue because it has reached a situation where it would have to divide by zero. Usually this happens if the model is a very poor representation of the data. It can also happen if the data does not constrain to the model.

If a singular matrix results for a lane, use the following procedure:

- 1** Examine the profile for the lane. Are many of the bands saturated (flat topped)? Does the background show unusual variations? If so, it may be difficult to obtain a satisfactory fit.
- 2** Delete all the bands in the lane by selecting with the Pointer tool.
- 3** Click the Gaussian Fit Bands Button on the Lanes panel or Fit Bands button on the Non Converging Bands window to fit just the background.
- 4** Use the Band tool to add a band for the very brightest band (the peak in the residual).
- 5** Adjust the fit parameters for that band to minimize its residual, then click the Fit Bands Again button.
- 6** Repeat steps 4 & 5 adding in the next brightest band until you have added all the bands back into the fit.

Remember that if chi-square minimization fails to produce the desired fit, you don't have to click Fit Bands Again button after you have optimized the fit by hand. You can still use the model parameters shown in the Lane Analysis Data window and refer to this as a “fit by eye” in your publication.

6**Manual ROIs**

Manual ROIs

You can perform measurements by creating regions of interest (ROIs) on the image. The Manual ROIs panel guides you through the manual ROI process. To manually draw ROIs, tools are provided to make both area and volume measurements. Once drawn, these ROIs are editable, movable, and can be duplicated.

You can create linear objects for area and intensity measurements, which measure the pixel information of the line. These measurements are useful in measuring distances between objects or sizing objects.

- ✓ *ROI Line* analyzes the pixels along the line and is useful in measuring distances and intensities.

You can also perform volume measurements that use the pixel information within the object. Volume measurements can be performed using:

- ✓ *ROI Ellipse* for elliptically shaped objects.
- ✓ *ROI Rectangle* for rectangular-shaped objects.
- ✓ *ROI Polygon* for irregular geometric shaped objects.
- ✓ *ROI Free Form* for irregularly shaped objects.
- ✓ *ROI Magic Wand* automatically defines a free form ROI for you.

Once you have drawn an ROI(s), you can choose to automatically center the ROI based on the centroid (center of mass or the 2nd moment of the intensity distribution).

The measurements generated are displayed in both the ROI Info window and the ROI Analysis Data window. The ROI Info window provides the current ROI selection information and is useful for “on the fly” measurements. The ROI Analysis Data window is where you can record a series of measurements that can be sorted, referenced, and exported.

The Manual ROIs Panel

The Manual ROIs Buttons

The Manual ROIs panel guides you through the manual ROI process.



- ✓ **New ROI Set**—defines a series of region of interests as a set. You have the ability to make an unlimited number of ROI sets within an image.
- ✓ **Set Standards**—opens the ROI Analysis Data window where you assign multiple ROIs as standards for the mass curve calculations. Select an ROI and click in the Standard (STD) column in the spreadsheet. The ROI Mass window opens so that you define the name of the standard and the Total Mass in the ROI. Each ROI set must have its own set of standards.
- ✓ **Set Background**—lets you define how background for an ROI is calculated. Options include using the perimeter intensity values (median, mean, minimum, or maximum) around each ROI, using any constant value, or using the median, mean, minimum or maximum intensity of a selection.
- ✓ **Mass Curve**—once you have defined your standards, select Mass Curve to open the ROI Mass Curve dialog box. Mass Curve displays an interactive plot of the standard data which is used to calculate the experimental mass values (net intensity versus mass). You can optimize the fit function to the data and can choose which are ROIs to be included in the determination of mass.
- ✓ **Templates**—accesses the ROI Templates dialog box where you can create and apply a template from a defined ROI set to a new image. The ROI template recalls the number of ROIs, and Standard load amounts. This is especially useful if you routinely run a similar experiments.
- ✓ **Center ROIs**—automatically centers ROIs based on the centroid (center of mass or the 2nd moment of the intensity distribution).

6**Manual ROIs**

- ✓ *Delete ROI Set*—deletes the active ROI set.
- ✓ *View Options*—define what ROI sets and what analysis data are displayed (e.g. ROI Boundaries, ROI IDs). In addition you can choose to show the ROI Analysis window and/or the Active ROI Info window.

The Manual ROIs Tools

Use the ROI Rectangle, ROI Ellipse, ROI Line, ROI Polygon, ROI Free Form and ROI Magic Wand tools to define an area for analysis. Once drawn, the ROI is editable, movable, and can be duplicated.

 NOTE: The line color designating the ROI is defined in the Preferences window.



Zoom Tools

Use the Zoom tools to adjust the size of the image through levels of magnification (0.25X, 0.33X, 0.50X, 1X, 1.5X, 2X, 4X, 6X, 8X, 16X, and 32X). To increase the magnification, click the image with the + Zoom tool. To reduce magnification, click the image with the - Zoom tool.

When you change the magnification with the Zoom tools, the software positions the point where you clicked in the center of the Image section. This differs from the Magnification slider which maintains the center of image in the window.



Pointer Tool

Use the arrow-shaped Pointer tool to select ROIs and to resize selected ROIs. Shift-click on multiple objects with the Pointer tool or click and drag the mouse over an area of the image containing more than one object.



White Point /Black Point Tools

Use the White Point/Black Point tools to select the white or black point values used to adjust the contrast in the image. To use, click the Black Point tool over the pixel in which you want to represent black point. Click the White Point tool over the pixel in which you want the to represent the white point. The image is then remapped using these points.

 NOTE: The white point and black point can also be adjusted using the Image Display window.



Selection Rectangle Tool

Click and drag the Image Selection tool to select a rectangular selection area for printing, exporting as a TIFF or JPEG file, or copying to the clipboard.



ROI Ellipse Tool

The ROI Ellipse tool is used to draw a round or elliptically shaped ROI and is used to analyze objects like dot blots, arrays, or spots.

To draw a circle, select the Ellipse tool from the Manual ROI panel. Position the cursor in the center of the circle you want to draw, click and drag out to increase the size of the circle. Release the mouse and the drawn ROI is represented by moving red and white dashes (active ROI). To record the ROI analysis data, press the Enter key (Return key for Macintosh) or drag and drop the active ROI to a new position to record an additional ROI. This may be done in succession to create a series of the same ROI. Once recorded, the size and shape of the ROI can be edited using the Pointer tool.



NOTE: To draw a perfect circle, press the Shift key while defining the ROI using the ROI Ellipse tool.



By double-clicking on the ROI Ellipse tool, you can choose your preference between drawing from the center or from the corner.



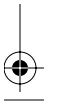
ROI Rectangle Tool

The ROI Rectangle tool is used to draw a box or rectangular shaped ROI and is useful when analyzing bands in a gel or blot.

To draw a rectangle, select the Rectangle tool from the Manual ROI panel. Position the cursor at the starting point, one corner of the rectangle and click and drag diagonally across to the ending location of the rectangle. Release the mouse and the drawn ROI is represented by moving red and white dashes (active ROI). To record the ROI analysis data, press the Enter key (Return key for Macintosh) or drag and drop the active ROI to a new position to record an additional ROI. This may be done in succession to create a series of the same ROI. Once recorded, the size and shape of the ROI can be edited using the Pointer tool.



NOTE: To draw a perfect square, press the Shift key while defining the ROI using the ROI Rectangle tool.





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Manual ROIs



ROI Polygon Tool

The ROI Polygon tool is used to draw a polyshape ROI and is useful for analyzing irregularly shaped objects like tissue sections or tumors. The ROI Polygon tool draws a series of line segments that are connected by points that can be edited.

To draw a Polygon or polyline, select the Polygon tool from the Manual ROI panel. Position the cursor at the starting location of the first segment. Click and drag to the end of the first segment. Release the mouse, then click and drag to draw the next segment. Continue to draw segments. To close the shape, click and drag the last end point to the start point. Release the mouse and the drawn ROI is represented by moving red and white dashes (active ROI). To record the ROI analysis data, press the Enter key (Return key for Macintosh) or drag and drop the active ROI to a new position to record an additional ROI. This may be done in succession to create a series of the same ROI. Once recorded, the size and shape of the ROI can be edited using the Pointer tool.

If the object is closed it becomes a Polygon with volume analysis. If it is not closed, then it is referred to as a polyline and no volume measurements will be generated.



ROI Free Form Tool

The ROI Free Form tool draws a continuous free form object and is useful for analyzing irregularly shaped objects like tissue sections or tumors. The free form ROI is always a closed shaped.

To draw a free form object, select the Free Form tool from the Manual ROI panel. Click and drag the mouse to outline the region of interest. Release the mouse and the drawn ROI is represented by moving red and white dashes (active ROI). To record the ROI analysis data, release the mouse and press the Enter key (Return key for Macintosh) or drag and drop the active ROI to a new position to record an additional ROI. The free form ROI cannot be edited.



ROI Line Tool

The Line tool is used to draw line as ROIs. This tool can be useful when measuring distances between objects.

To draw a line, select the ROI Line tool from the Manual ROI panel. Position the cursor at the starting location of the line, click and drag to the end location of the line. Release the mouse and the drawn ROI is represented by moving red and white dashes. To record the ROI analysis data, press the Enter key (Return key for Macintosh) or drag and drop the active ROI to a new position to record an additional ROI. This may be done in succession to create a series of the same ROI. Once recorded, the size and shape of the ROI can be edited using the Pointer tool.



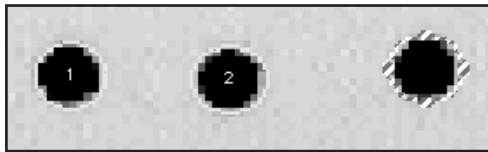


NOTE: To draw a straight line, press down the Shift key while drawing. This constrains lines to nearest 45° angle (0°, 45°, and 90° angles).



Reactivate ROI Tool

The Reactivate ROI tool reactivates an ROI so that it can be used to make multiple recordings of the same shape. Select the ROI with the Reactivate ROI tool from the Manual ROI panel. An active ROI is represented by moving red and white dashes. Drag and drop the active ROI to a new position to record an additional ROI. This may be done in succession to create a series of the same ROI. Once recorded, the size and shape of the ROI can be edited using the Pointer tool.



Rotate Selected ROI Tool

The Rotate Selected ROI tool is used to rotate a selected ROI. Click on the tool and then select a grab handle on an ROI and rotate in any direction.



Magic Wand Tool

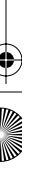
The Magic Wand tool automatically defines an ROI for you. To use, click and drag along a small portion of the edge of the ROI that you want to define. The gray level information from these pixels is used to define the boundary.

Alternately, you can click in the center of an ROI and the finder determines the edge using the threshold level value selected in the Magic Wand options. You can adjust how close the Magic Wand tool sets the threshold level with respect to its estimate of the background. To access the Magic Wand tool options, double-click on the tool.



Separate ROIs Tool

The Separate ROIs tool selects an ROI cutting tool. Click and drag across the boundary of two connected ROIs to cut. The Separate ROIs tool only works with free form ROIs drawn by the ROI Free Form tool, the Magic Wand tool or the Auto ROI methods.





6

Manual ROIs

Drawing an ROI Set



1 Click Manual ROIs on the Navigation panel. The Manual ROI panel opens.

2 Use the Magnification slider or the Zoom tools to maximize the size of the image on-screen.

New ROI Set

3 Click on the New ROI set to record that a new set of ROIs are to be recorded.

4 Draw ROIs using the ROI Ellipse, ROI Rectangle, ROI Polygon, ROI Free Form, ROI Line or ROI Magic Wand tools from the Manual ROI panel. Each tool is described in the previous section titled *Manual ROI Tools*.

- ✓ To draw a circle, select the Ellipse tool from the Manual ROI panel. Position the cursor in the center of the circle you want to draw, click and drag out to increase the size of the circle.
- ✓ To draw a rectangle, select the Rectangle tool from the Manual ROI panel. Position the cursor at the starting point, one corner of the rectangle and click and drag diagonally across to the ending location of the rectangle.
- ✓ To draw a Polygon or polyline, select the Polygon tool from the Manual ROI panel. Position the cursor at the starting location of the first segment. Click and drag to the end of the first segment.
- ✓ To draw a free form object, select the Free Form tool from the Manual ROI panel. Click and drag the mouse to outline the region of interest. Use the Separate ROIs tool to edit the ROI.
- ✓ To draw a line, select the ROI Line tool from the Manual ROI panel. Position the cursor at the starting location of the line, click and drag to the end location.
- ✓ To use the Magic Wand tool to automatically define an ROI shape, click and drag along a small portion of the edge of the ROI that you want to define. The gray level information from these pixels is used to define the boundary. Alternately, you can click in the center of an ROI and the finder determines the edge using the threshold level value selected in the Magic Wand options. Use the Separate ROIs tool to edit the ROI.

5 Release the mouse and the drawn ROI is represented by moving red and white dashes (active ROI).

6 To record the ROI analysis data, press the Enter key (Return key for Macintosh).

NOTE: To create a new ROI Set, click the New ROI Set on the Manual ROI panel. The new ROI set becomes the active set.



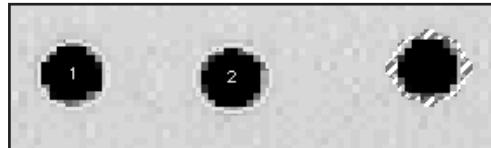
Multiple ROIs by Dragging and Dropping an Active ROI

An active ROI can be used to make multiple recordings of the same shape.

-  **1** If the ROI is not active, choose the Reactivate ROI tool and click on the ROI you want to replicate.

 NOTE: An active ROI is outlined by a moving red and white dashed line.

- 2** Place your cursor in the center of the active ROI; the cursor changes to a hand.
- 3** Click and drag the ROI to a new location. A new ROI is created. This may be done in succession to create a series of the same ROI.



- 4** To record the last ROI analysis data, press the Enter key (Return key for Macintosh).



Copying a Single ROI

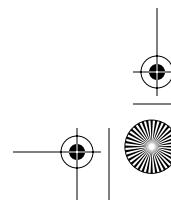
You can also duplicate a selected ROI using the Copy and Paste menu options.

-  **1** Select the ROI you want to duplicate with the Pointer tool.
- 2** Choose Copy from the Edit menu, followed by Paste from the Edit menu. The ROI is duplicated and appears as the selected object on top of the original ROI.
- 3** Place your cursor in the center of the selected ROI, the cursor changes to a hand.
- 4** Click and drag the ROI to a new location.

Centering an ROI

Center ROIs automatically centers ROIs based on the centroid (center of mass or the 2nd moment of the intensity distribution).

-  **1** Use the arrow-shaped Pointer tool to select ROIs and to resize selected ROIs. Shift-click on multiple objects with the Pointer tool or click and drag the mouse over an area of the image containing more than one object.
- 2** Click Center ROIs from the Manual ROI panel.



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Editing Manual ROIs

You can resize ROIs using the control points. All manual ROIs, except free form shapes defined with the ROI Free Form tool or Magic Wand tool, can be edited using control points. However Free Form and Magic Wand tool ROIs can be edited using keyboard shortcuts.

- ✓ *Line*—two control points, one at either end
- ✓ *Rectangle*—four control points, one at the four corners
- ✓ *Ellipse*—four control points (2 centered horizontally, 2 centered vertically)
- ✓ *Polygon*—control points at each segment joint
- ✓ *Free Form*—no control points, use Separate ROIs tool

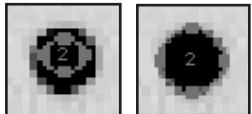
Resizing ROIs Using Control Points

-  1 Select the ROI to be resized with the Pointer tool.

 NOTE: You cannot edit an ROI if more than one ROI is selected.

- 2 Position the Pointer tool over a control point.

- 3 Click and drag the control point in the direction that you want to resize the object.



 NOTE: Magnify the image if you have trouble selecting the control point.

Adding a New Segment to an Open Polygon

-  1 Select the open Polygon with the Pointer tool.

- 2 Click on the first point and drag to draw a new segment.

- 3 To record the last ROI analysis data, press the Enter key (Return key for Macintosh).



Editing Free Form ROIs

You can edit the Free Form ROI objects using the Separate ROI tool and keyboard shortcuts.

- ✓ Using a keyboard shortcut—to increase or decrease the size of an ROI object, select the object you want to edit with the Pointer tool while pressing the Alt key (Option key for Macintosh). The cursor shows a plus sign. Click and drag the area in which you want to include.
- ✓ Using the Separate ROI tool, click on the object and draw across the area that you want to separate.

Closing an Open Polygon

- 1 Select the open Polygon with the Pointer tool.
- 2 Click on the first point and drag to draw a new segment to the last control point.

Adding Control Points to a Polygon

- 1 Select the polygon with the Pointer tool.
- 2 Press down the Shift key, click and drag on a control point.

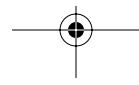
NOTE: Remove control points by dragging a control point over another.

Rotating ROIs

You can rotate ROIs using the control points. All manually drawn ROIs can be rotated except the free form shapes.

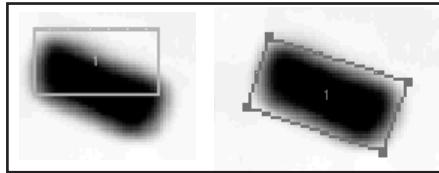
- ✓ *Line*—two control points, one at either end.
- ✓ *Rectangle*—four control points, one at the four corners.
- ✓ *Ellipse*—four control points (2 centered horizontally, 2 centered vertically).
- ✓ *Polygon*—control points at each segment joint.
- ✓ *Free Form*—no control points.

- 1 Select the ROI to be rotated with the Rotate Selected ROI tool.



6*Manual ROIs*

- 2** Position the tool over a control point, click and drag the control point in the direction that you want to rotate the object.



Deleting ROIs

You can also delete a selected ROI.



- ✓ Delete a specific ROI by selecting the ROI with the Pointer tool and then pressing the Delete key.



NOTE: You can select multiple objects by Shift-clicking the ROIs, by dragging the Pointer tool over the ROIs, or by selecting them in the ROI Analysis Data window.



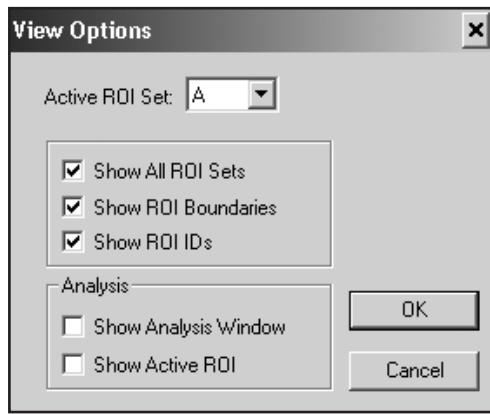
- ✓ Delete all the ROIs in a Set by choosing one of the ROIs in the set and clicking on Delete ROI Set from the Manual ROIs panel.



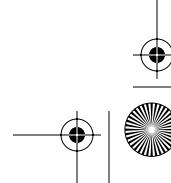
View Options

Define what ROI sets and what analysis data are displayed (e.g., ROI Boundaries, ROI IDs). In addition you can choose to show the ROI Analysis window and/or the Active ROI Info window.

- 1 Click View Options from the Manual ROIs panel or choose ROI from the Show menu. The View Options dialog box appears.



- 2 Choose the features you want to be displayed on-screen.
 - ✓ Select the ROI set that you want active using the Active ROI Set pop-up menu.
NOTE: You can also make an ROI set active by selecting an ROI in the ROI set using the Pointer tool or by selecting the ROI set from the pop-up menu in the ROI Analysis window.
 - ✓ Choose *Show All ROI Sets* to display all ROI Sets.
 - ✓ Choose *Show ROI Boundaries* to display the ROI boundaries on-screen.
 - ✓ Choose *Show ROI IDs* to display the ROI ID numbers on-screen.
- 3 Choose the Analysis view options.
 - ✓ Select *Show Analysis Window* to display all the data in the spreadsheet format.
 - ✓ Select *Show Active ROI* to display data for only a selected ROI.
- 4 Click OK to save your selections. These selections are remembered when opening the project.

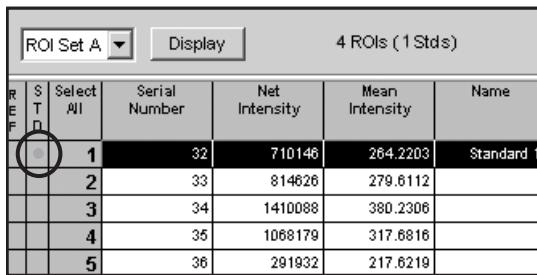


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Setting Standards

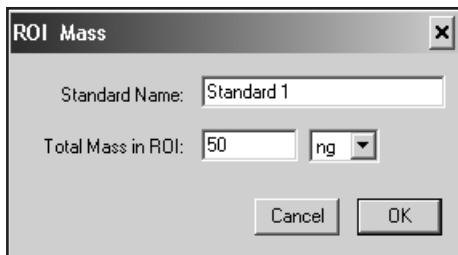
You can designate ROIs as standards and use these standards to generate a mass curve to quantitate unknown ROIs.

- 1** Select Set Standards from the Manual ROI panel. The ROI Analysis window opens.
- 2** Click in the Standard (STD) column to select an ROI as a standard.



R	S	Select	Serial Number	Net Intensity	Mean Intensity	Name
E	E	All				
		<input checked="" type="checkbox"/>	1	32	710146	264.2203
			2	33	814626	279.6112
			3	34	1410088	380.2306
			4	35	1068179	317.6816
			5	36	291932	217.6219

- 3** The selected ROI is added to the standards list and ROI Mass dialog box appears.



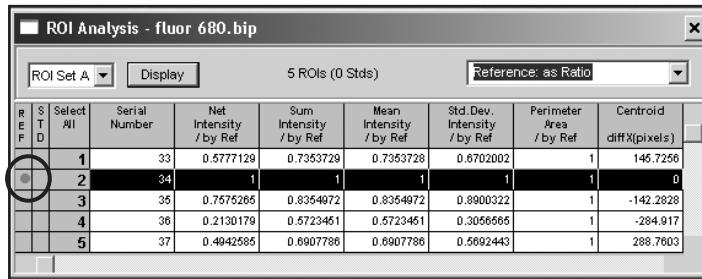
- 4** Enter the standard name and the total mass in the Standard Name and the Total Mass in ROI text edit boxes, respectively.
 - ✓ The maximum number of characters in a standard name is 32.
 - ✓ The units in the Total Mass in ROI pop-up menu corresponds to the options in the Preferences menu.
 - ✓ Each ROI set, must have its own mass curve and set of standards.
- 5** Click OK. The ROI is assigned a mass value and is used in the mass calculation.



Using References

The Reference Selector in the ROI Analysis Data window designates a single ROI as a reference to measure against all other ROIs.

- Select a row in the ROI Analysis Data window as a reference by clicking in the adjacent Reference (REF) column.



R	S	Select	Serial Number	Net Intensity / by Ref	Sum Intensity / by Ref	Mean Intensity / by Ref	Std.Dev. Intensity / by Ref	Perimeter Area / by Ref	Centroid diffX(pixels)
E	T	All							
F	D								
		<input checked="" type="checkbox"/>	1	33	0.5777129	0.7353729	0.7353728	0.6702002	1 146.7256
		<input type="checkbox"/>	2	34	1	1	1	1	0
		<input type="checkbox"/>	3	35	0.7575265	0.8354972	0.8354972	0.8900322	1 -142.2828
		<input type="checkbox"/>	4	36	0.2130179	0.5723451	0.5723451	0.3056655	1 -284.917
		<input type="checkbox"/>	5	37	0.4942685	0.6907786	0.6907786	0.5692443	1 288.7603

- Choose how you want your data to be displayed using the Reference pop-up menu.



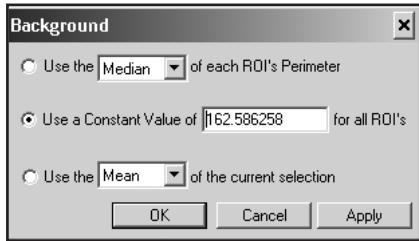
- ✓ *Reference—Don't Use* is the default and will not compare the data against the reference set.
- ✓ *Reference—as% Difference* displays the ROI data as a percent difference between the reference and the experimental ROIs.
- ✓ *Reference—as Ratio* displays the ROI data as a ratio between the reference and the experimental ROIs.

Setting Background

You can define how background for an ROI is calculated. Options include using the perimeter intensity values (median, mean, minimum, or maximum) around each ROI, using any constant value, or using the median, mean, minimum or maximum intensity of a selection. The default is median of each ROI of the perimeter since it is less susceptible to single pixel noise.

- Set Background** 1 Select Set Background from the Manual ROIs panel. The Background dialog box appears.

- 2 Select from one of the three options:



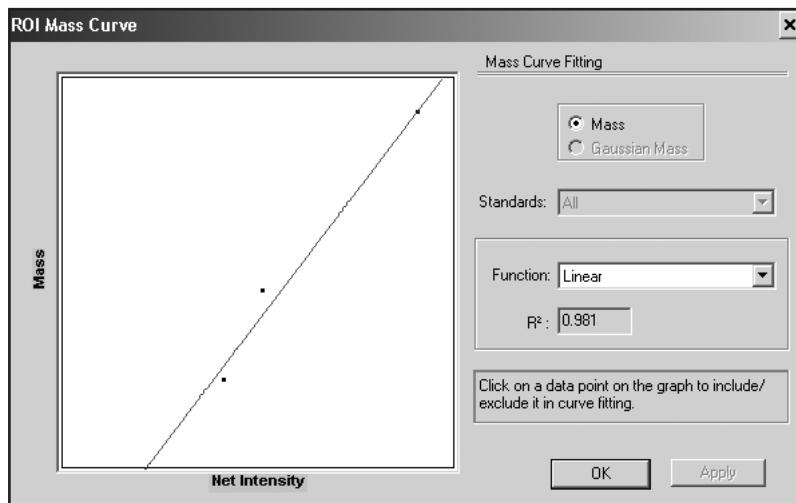
- ✓ Use the perimeter of each ROI—each ROI uses perimeter intensity information to calculate a local background. Use the pop-up menu to choose Median, Mean, Minimum, or Maximum of the each ROI's perimeter.
- ✓ Use a constant value for all ROIs—you can enter any value in the text edit box. The number (gray levels) that you enter are subtracted from each pixel within the ROI as background.
- ✓ Use the current selection to generate a background for all the ROIs—allows you to define a selection using the Selection Rectangle tool. The intensity value from this selection are used to calculate the background. Use the pop-up menu to choose Median, Mean, Minimum, or Maximum of the selection as the background.

- 3 Click Apply to update the background.

Reviewing the ROI Mass Standard Curve

Once you have defined standards. The standard data is plotted on a graph which is used to calculate the experimental mass values (net intensity versus standard mass). You can optimize the fit function to the data and choose which ROIs to include in the determination of mass.

- 1 Access the Mass Curve dialog box by selecting Mass Curve from Manual ROIs panel. The Mass Curve dialog box appears.



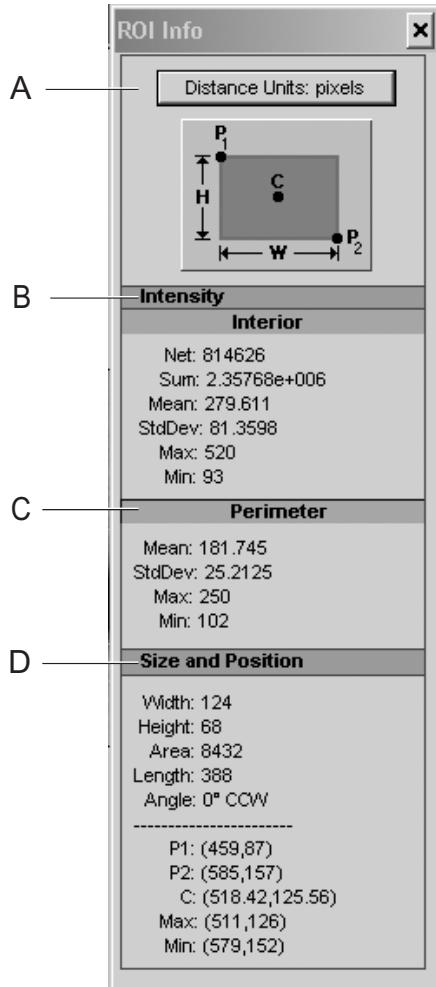
- 2 To maximize the accuracy of your mass determination, review the curves to make sure that the appropriate points are included.
- 3 Choose the fitting function that best represents the data using the Function pop-up menu. The R^2 value aids you in determining the best fit.

The ROI Info Window

Once you have defined a region of interest, view the analysis data for the current selection using the ROI Info window.

To display the window:

- ✓ Choose to display the Active ROI Info window by selecting Show Active ROI in the ROI Options dialog box or click Active ROI Info from the Show menu. The Active ROI Info window appears.

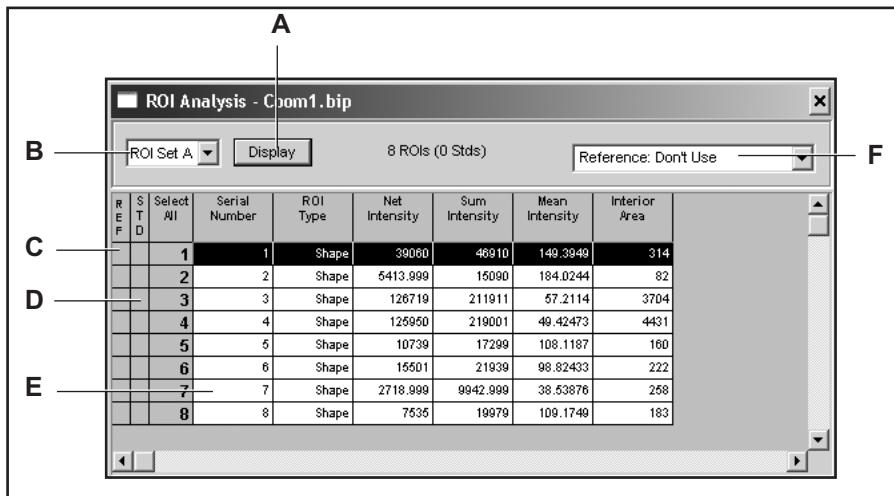


- A** *Distance Units* pop-up menu allows you to define the units for position and area measurements. Use the pop-up to choose pixels, centimeters, or inches.
- B** *Interior* provides net, sum, mean, standard deviation, maximum, and minimum intensity values of the interior of the object.
- C** *Perimeter* provides mean, standard deviation, maximum, and minimum intensity values of the perimeter of the object.
- D** *Size and Position* provides width, height, area, length, and rotation angle of the object. The data provides information on positioning of the centroid and minimum and maximum intensities.

The ROI Analysis Data Window

The ROI Analysis Data window shows all the ROI data in a spreadsheet format. You can select cells in the ROI Analysis Data window, copy them, and then paste them into other programs or export a tab-delimited text file.

Although all of the statistics should be familiar, a few statistics need to be defined in more detail in the context of digital imaging. Not all of the statistics apply to all ROIs. For example, the area of an ROI can only be calculated if the ROI has interior pixels. Other statistics like width and height have different meanings for different ROI types.



- A The *Display button* accesses the Analysis Display dialog box where you can choose the type of data you want displayed in the ROI Analysis Data window. You can also choose how the background is calculated.
- B The *ROI Set pop-up menu* allows you to choose the ROI set you want to display. The active ROI set is the default.
- C The *Reference Selector* designates a single ROI as a reference to measure against all other ROI's. Select a row as a reference by clicking in the adjacent Reference (REF) column.
- D The *Standard Selector* designates an ROI as a standard point. Select by clicking in the adjacent Standard (STD) column. An ROI Mass window opens allowing you to assign an ROI name and assign a mass load. At least two standard points are required to generate a mass curve.
- E *Data Fields* contain the measurement values and calculated data for each ROI.
- F The *Reference pop-up menu* allows you to display the referenced values (if a reference has been designated) as either a ratio or a % difference. The option default is *Don't Use*.

Viewing the ROI Analysis Data

The ROI Analysis Data can display the following information for each ROI.

ROI General Data

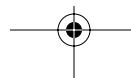
- ✓ *Serial Number*—is a unique identifier for ROIs. If the ROI is deleted, the serial number is never reused. To reset the serial numbers, all ROIs need to be deleted.
- ✓ *ROI Type*—specifies the type of ROI (Rectangle, Ellipse, Shape, or Line).
- ✓ *Comments*—annotate ROI with information about the ROI (up to 255 characters).
- ✓ *Name*—assign a name to the ROI (up to 255 characters).
- ✓ *Mass*—for an experimental ROI, is determined by comparing the sum of the background subtracted intensities (Net Intensity) of all the pixels in the ROI with the background subtracted intensities of all pixels in the standard ROIs.

ROI Intensity Data

- ✓ *Net*—is the sum of the background-subtracted pixel values within the ROI.
- ✓ *Sum*—adds together all the pixel intensities within the ROI (includes background).
- ✓ *Mean*—is the average intensity of the pixels within the ROI.
- ✓ *Background*—provides the background value. This is defined within the ROI Analysis Display dialog box.
- ✓ *Maximum*—provides the maximum pixel intensity within the ROI.
- ✓ *Minimum*—provides the minimum pixel intensity within the ROI.
- ✓ *Standard Deviation*—is the square root of the sum of the squared deviation of each pixel value from the mean pixel value. The standard deviation is a useful measure of the statistical error or noise in your data. To find the noise level of your image, create an ROI that includes only background pixels typical of the image. The standard deviation for this ROI is a good indicator of the random variations you can expect for other pixels in the image.

ROI Geometry Data

- ✓ *Width/Height*—for all ROIs except the rectangle and oval, are the horizontal and vertical distances between the centers of the *top left* and *bottom right* pixels described above. For rectangular ROIs, width and height are the number of interior pixels in the horizontal and vertical direction when the ROI is in its unrotated position. The software makes this special case so that area equals width x height for unrotated rectangles, as expected. For ellipses the width and height are always the width and height of the major and minor axes of the interior pixels in the unrotated ellipse. The width and height values of rectangles and ovals do not change when they are rotated. Width and height may change for other ROIs when rotated, due to changes in the bounding rectangle.



- ✓ *Area*—for each ROI is calculated by counting the number of interior pixels. This is done so you know exactly how many pixels were used to calculate other statistics, such as the mean and r.m.s. or the interior pixels. If an ROI has no interior pixels (e.g., a line or an open polygon) the area is zero. Note that the area is not calculated from geometry (e.g., $W \times H$ for a rectangle) because when ROIs are rotated the number of interior pixels may change due to the nature of a digital imaging.
 - ✓ *Angle*—(for all ROIs except the line ROI) is the current counter clockwise rotation angle of the ROI. For the line ROI the angle is reported as the angle from the +x axis to the line. Note that it makes a difference which end of the line is the starting point and which is the end point of the line. If you click and draw a line ROI up, the angle is $+90^\circ$. If you click and draw the line down the angle is -90° although the two line ROIs appear the same on the image.
 - ✓ *Perimeter Length*—for all ROIs, except the Polygon and ellipse ROI, the perimeter length is calculated by summing the Pythagorean distances between the centers of the vertices (nodes) of the ROI and/or the centers of the end points. For the free form ROI, which has no control points, the perimeter length is the length of the line that connects the centers of the perimeter pixels in the original order that the perimeter points were drawn. When ROIs are rotated their perimeter lengths may change as a result of the new locations of the perimeter pixels, end points or vertices. For oval ROIs, a formula is used to calculate the perimeter of the ellipse that passes through the centers of the perimeter pixels. This is more accurate than using the techniques for arbitrary shapes and polygons because continuous curves and like ovals are not as well represented in digital imaging.
-  NOTE: The centers of the perimeter pixels is used to calculate the perimeter lengths because this is less ambiguous for very complicated shapes or unclosed figures like open polygons and lines that have no “inside” or “outside.”
- ✓ *Perimeter Area*—is the area that is designated by the perimeter.

ROI Position Data

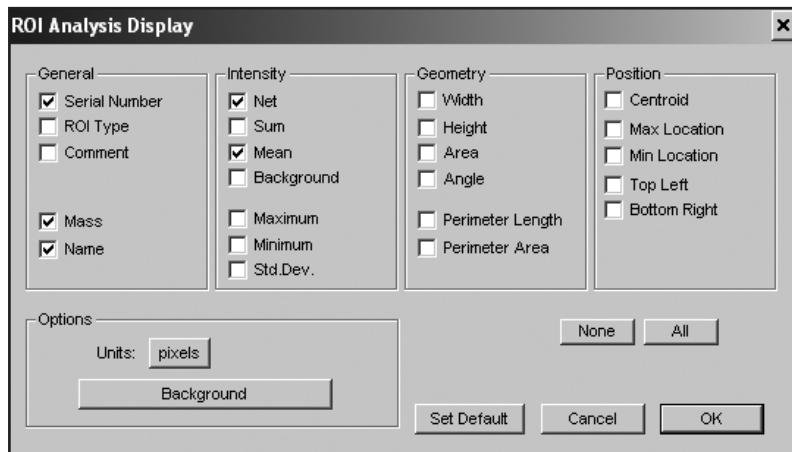
- ✓ *Centroid*—for all ROIs, the (x,y) location of the “center of mass” or the 2nd moment of the intensity distribution. You can calculate the geometrical center of the ROI from the *top left* and *bottom right* values. The centroid is calculated because it is a better indicator of the position of the ROI; the 2nd moment finds the position of the feature inside the boundary of the ROI even if the boundary is not well centered on the object.
- ✓ *Max Location*—defines the pixel that has the highest intensity value.
- ✓ *Min Location*—defines the pixel that has the lowest intensity value.

6**Manual ROIs**

- ✓ *Top Left/Bottom Right*—are determined by finding the smallest rectangle that completely encloses the perimeter pixels of the ROI. The (x,y) coordinates of the pixels in the *top left* and *bottom right* corners of the bounding rectangle are what is reported for *top left* and *bottom right*. When you rotate an ROI, its bounding rectangle may change, therefore, the *top left* and *bottom right* values for the ROI may change after it is rotated.

You can choose the ROI set and the analysis data type to be displayed.

- 1** Click Analysis from the Quick Access bar or choose ROI Analysis Data from the Show menu. The ROI Analysis window appears.
- 2** Choose the ROI Set you want to display using the ROI Set pop-up menu.
- 3** Select Display button. The ROI Analysis Display box appears.



- 4** Select the variables that you want to display for all ROIs.
 - 5** Click OK. The data appears in the ROI Analysis Data window.
- ☞ NOTE:** As you click on an ROI analysis data field, that ROI is selected in the Image section. As you select an ROI in the Image section, the data for that ROI is highlighted.

Sorting the ROI Analysis Data

As ROIs are created, ROIs are added to the bottom of the list. You can, however, sort the data by any column.

- ✓ Click any column heading, this sorts the data in descending order.
- ✓ Double-click any column heading to sort in ascending order.
- ✓ To sort in original order, click on the Serial Number heading.

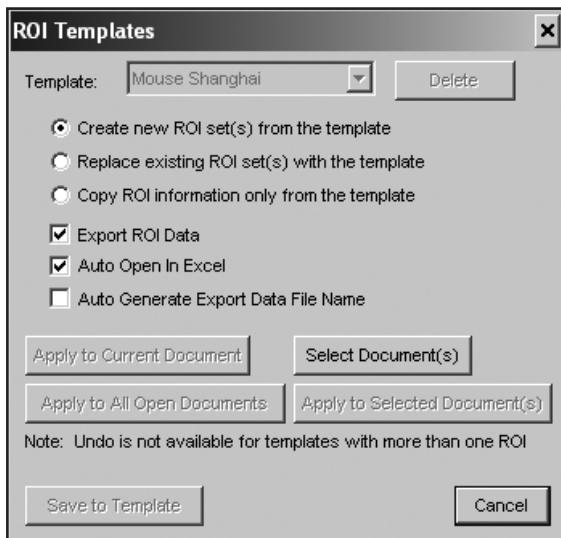
Using ROI Templates

You can save ROIs and define them as a template. When you apply a template to an image, an exact copy of the previously defined ROIs is applied.

Saving an ROI Template

- 1 Define manual ROIs.

- 2 Select Templates from the Manual ROIs panel. The Templates dialog box appears.



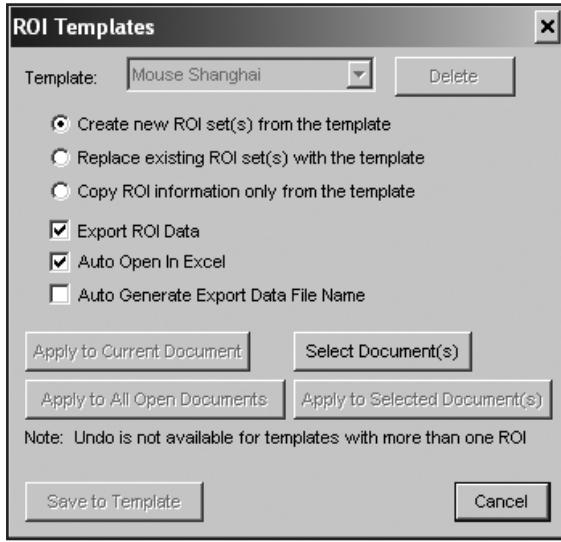
NOTE: If you have not previously saved an ROI template, a warning may appear stating that there are no saved templates files. Click OK to access the ROI Template dialog box.

- 3 Click the Save Template button. The ROI Set Template dialog box appears.
- 4 Enter a name for the template in the New Template File Name text edit box.
- 5 Click OK
- 6 The ROIs in a template format is saved.

NOTE: When naming a template, avoid using characters like a slash, colon, or semicolon.

Using a Saved ROI Template

- 1** Acquire or open an image.
- 2** Select Templates from the Manual ROIs panel. The ROI Templates dialog box appears.



- 3** Choose the template you wish to apply using the Template pop-up menu and select any options that you want to apply.
 - NOTE:** Some export options are not active until the Export ROI checkbox is selected.
 - Create new ROI sets(s) from the template* applies the template selected in the template pop-up list to the current project.
 - Replace existing ROI set(s) with the template* replaces the current ROIs on the current project with the saved template that is selected in the template pop-up list.
 - Copy ROI information only from the template* copies the names information from the selected template and applies it to the current set of ROIs
 - Export ROI Data* checkbox - exports the ROI data exported to a pre-named tab delimited text file once the selected ROI template has been applied to a file.
 - Auto open in Excel*—formats the data as an Excel file. This check box is only activated if Export ROI Data is checked.
 - Auto Generate Export Data File Name* checkbox —if checked, MI will use a date time stamp to auto generate a file name where ROI export data will be saved. This check box is only activated if Export ROI Data is checked.

6**Manual ROIs**

- 4** Click the appropriate button to apply the template.
 - ✓ *Apply to Current Document* button—applies selected ROI Template to currently active document in MI.
 - ✓ *Apply to All Open Documents*—applies selected ROI template to all currently active documents in MI. In order to apply a template, the documents must be saved.
 - ✓ *Select Document(s)* button—launches a default file selection dialog that allows you to select MI Project, *.bip, document(s) from a directory/folder of previously saved documents.
 - ✓ *Apply to Selected Document(s)* button— applies selected the ROI Template to documents you have selected. This option is only available after you have selected at least one MI Project using the Select Documents button.
 - ✓ *Save to Template* button—saves ROI set(s) of a currently opened document to a ROI template file. This option is disabled if there are currently no documents opened in MI.
- 5** Verify that the template was applied properly.

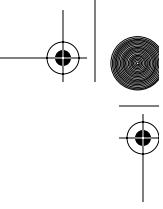
 NOTE: Click Center ROIs from the Manual ROIs panel to automatically center the ROI based on the centroid (center of mass or the 2nd moment of the intensity distribution).

Deleting a Saved ROI Template

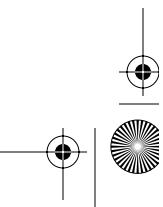
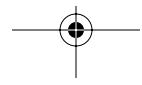
- 1** Select Templates from the Manual ROIs panel. The Templates dialog box appears
- 2** Choose the ROI Template Set that you wish to delete using the Template pop-up menu.
- 3** Click Delete. A confirmation text box appears. Click Yes to delete the selected ROI Template Set.



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7

Auto ROIs



Auto ROIs

Auto ROIs automatically defines ROIs using four different methods:

- ✓ *Edge Detection* searches for the edges of features. This method looks for gradient edges and is usually not biased by intensity variations or background in the image (relative to the background).

The rate of intensity changes in the boundary is calculated by searching for gradient or slope variations. The number of ROIs found is controlled by the gradient setting (0.1%–200%). This value is used by the algorithm to determine how steep of a slope the edge must have (rate of change of intensity across the edge) before it is defined as a new ROI object. The higher the percentage chosen, the sharper the edges that are searched for and the more intense the ROI.

- ✓ *Threshold* uses a predefined signal threshold value to segregate image objects from the background. This method is useful when your image contains objects that are well separated and has an even background (i.e., dot and slot blots).

In this method, groups of pixels above or below the threshold value are grouped together as ROIs (the current state of the Above and Below options depends on the Black/White button on the ROIs tab). This technique will not work well for images with a large variation in the intensity of the background.

- ✓ *Density Slice* segments the image into three discrete density ranges. Like the Threshold technique, this technique is also sensitive to background variations in the image.

ROIs are formed from those groups of pixels that are in the middle range. Using this technique you can set the maximum and minimum signal value to find and analyze only lighter or darker objects, e.g., counting blue/white colony assays or gels that are stained with different dyes.

- ✓ The *Peak Finder* differs from the other automatic ROI finders used in the program in that it creates oval or rectangular ROIs, not arbitrary shapes, around peaks of intensity. The peak finder can separate peaks that the other finders might group together as one. Also, the peak finder is much less sensitive to variations in the image background than the other auto find ROI methods. You find the peak finder most useful if you are counting, e.g., colony plates or plaque assays.

The measurements generated are displayed in both the ROI Info window and the ROI Analysis Data window. The ROI Info window provides the current ROI selection information and is useful for “on the fly” measurements. The ROI Analysis Data window is where you can record a series of measurements that can be sorted, referenced, and exported.



The Auto ROIs Panel

The Auto ROIs panel guides you through the auto ROI process.

The Auto ROIs Buttons



- ✓ The *Set Search Area*—defines the search area for a new ROI set. When this button is selected, a selection rectangle appears on-screen that can be edited to define the area to be analyzed. Use the Rectangle Selection tool or Ellipse Selection tool to draw a new search area.
- ✓ *New ROI Set*—launches the Auto Find panel where you can use various automatic boundary detection methods.
- ✓ *Set Standards*—opens the ROI Analysis Data window where you assign multiple ROIs as standards for the mass curve calculations. Select an ROI and click in the Standards (STD) column in the spreadsheet. The ROI Mass window opens so that you define the name of the standard and the total mass in the ROI. Each ROI set must have its own mass curve and set of standards.
- ✓ *Set Background*—lets you define how background for an ROI is calculated. Options include using the perimeter intensity values (median, mean, minimum, or maximum) around each ROI, using any constant value, or using the median, mean, minimum or maximum intensity of a selection.
- ✓ *Mass Curve*—once you have defined your standards you can select Mass Curve to open the ROI Mass Curve dialog box. An interactive plot of the standard data is generated to calculate the experimental mass values (net intensity versus standard band mass). You can optimize the fit function to the data and to choose which ROIs are included in the determination of mass.
- ✓ *Templates*—accesses the ROI Templates dialog box where you can create and apply a template from a defined ROI set to a new image. The ROI template recalls the number of ROIs and standard load amounts. This is especially useful if you routinely run similar experiments.
- ✓ *Delete ROI Set*—deletes the active ROI set.



7

Auto ROIs

- ✓ **View Options**—defines what ROI sets and what analysis date are displayed (e.g., ROI Boundaries, ROI IDs). In addition you can choose to show the ROI Analysis window and/or the Active ROI Info window.

The Auto ROI Tools



Zoom Tools

Use the Zoom tools to adjust the size of the image through levels of magnification (0.25X, 0.33X, 0.50X, 1X, 1.5X, 2X, 4X, 6X, 8X, 16X, and 32X). To increase the magnification, click the image with the + Zoom tool. To reduce magnification, click the image with the - Zoom tool.

When you change the magnification with the Zoom tools, the software positions the point where you clicked in the center of the Image section. This differs from the Magnification slider, which maintains the center of image in the window.



Pointer Tool

Use the arrow-shaped Pointer tool to select ROIs and to resize selected ROIs. Shift-click on multiple objects with the Pointer tool or click and drag the mouse over an area of the image containing more than one object.



White Point /Black Point Tools

Use the White Point/Black Point tools to select the white or black point values used to adjust the contrast in the image. To use, click the Black Point tool over the pixel in which you want to represent black point. Click the White Point tool over the pixel in which you want the to represent the white point. The image is then remapped using these points.



NOTE: The white point and black point can also be adjusted using the Image Display window.



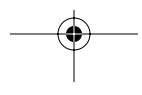
Selection Rectangle Tool

Click and drag the Image Selection tool to select a rectangular selection area for printing, exporting as a TIFF or JPEG file, or copying to the clipboard.



Reference ROI Tool

Use the Reference ROI tool to draw an ROI that bounds a typical ROI within the image. The location, width, height, and intensity of the ROI will automatically be used in the Find ROIs algorithms. Select the shape of the reference ROI (Ellipse or Rectangle) using the





Reference ROI tool options. To access the options, double-click on the Reference ROI tool.

To draw a rectangle, select the Rectangle tool from the Auto ROI panel. Position the cursor at the starting point, one corner of the rectangle and click and drag diagonally across to the ending location of the rectangle.

To draw a circle, select the Ellipse tool from the Auto ROI panel. Position the cursor in the center of the circle you want to draw, click and drag out to increase the size of the circle.

Release the mouse and the drawn ROI is represented by moving red and white dashes (active ROI). Do not record the ROI, the ROI must be active to be a reference. This tool is especially useful when the automated finding tools are not producing good results.



Separate ROIs Tool

The Separate ROIs tool selects an ROI cutting tool. Click and drag across the boundary of two connected ROIs to cut. The Separate ROIs tool only works with free form ROIs drawn by the ROI Free Form tool, the Magic Wand tool or the Auto ROI methods.



Selection Ellipse Tool

Click and drag the Selection Ellipse tool to select the area of the image to be analyzed.



Rotate Selected ROI Tool

The Rotate Selected ROI tool is used to rotate a selected ROI. Click on the tool and then select a grab handle on an ROI and rotate in any direction.



NOTE: Magnify the image if you have trouble selecting the control point.

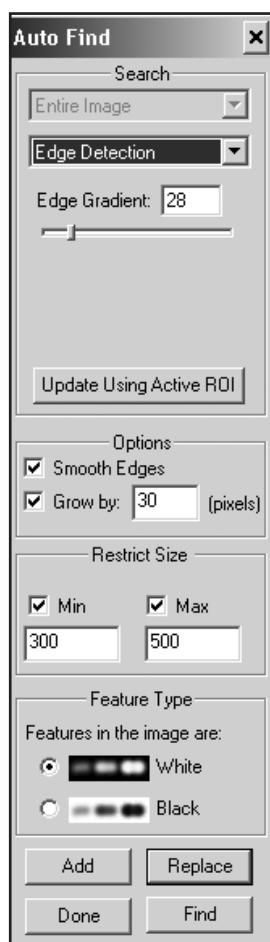
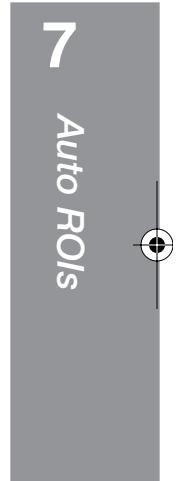
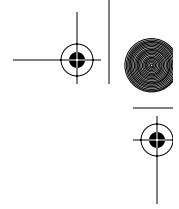


Magic Wand Tool

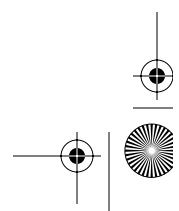
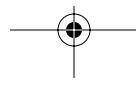
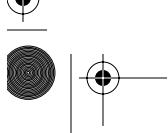
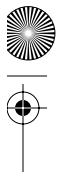
The Magic Wand tool automatically defines an ROI for you. To use, click and drag along a small portion of the edge of the ROI that you want to define. The gray level information from these pixels is used to define the boundary.

Alternately you can click in the center of an ROI and the finder determines the edge using the threshold level value selected in the Magic Wand options. You can adjust how close the Magic Wand tool sets the threshold level with respect to its estimate of the background. To access the Magic Wand tool options, double-click on the tool.



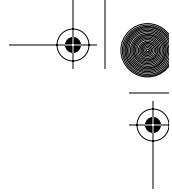


- 1** Click Auto ROIs from the Navigation panel. The Auto ROIs panel opens.
- 2** Use the Magnification slider or the Zoom tools to maximize the size of the image on-screen.
NOTE: You may want to draw a Reference ROI to provide information to the program on what features you are trying to identify. Double-click the Reference ROI tool to draw an ROI that bounds a typical ROI within the image. The location, width, height, and intensity of the ROI is automatically used in the Find ROIs algorithms. Select the shape of the reference ROI (Ellipse or Rectangle) using the Reference ROI tool options. The Reference ROI tool is especially useful when the automated finding tools are not producing good results.
- 3** Click on the New ROI Set to record that a new set of ROIs are to be recorded. The Auto Find dialog box appears.
- 4** Use the Search pop-up menu to define what part of the image you want to find ROIs.
 - ✓ *Entire Image*—searches the entire image for ROIs.
 - ✓ *Selection Only*—searches the interior of the selection. The selection is defined by the Rectangle or Ellipse Selection tools. This option is only available when a selection is defined prior to entering the dialog box.
 - ✓ *Oval Quadrant 1, 2, 3, 4*—uses the selection borders to create a circle within the box (the selection is defined by the Ellipse Selection tool). The circle is then divided into four equal quadrants. This option is only available when a selection is defined prior to entering the dialog box.





MI



5 Choose Edge Detection from the Search Method pop-up menu.

NOTE: *Edge Detection* searches for the edges of features. This method looks for gradient edges and is usually not biased by intensity variations or background in the image.

6 Use the Gradient text edit box or slider to define a gradient setting.



This value is used by the algorithm to determine how steep of a slope the edge must have (rate of change of intensity across the edge) before it is defined as a new ROI object. The higher the percentage, the sharper the edge and more intense the transition.

- ✓ For low contrasted images (low signal to background), lower the gradient value.
- ✓ For well contrasted images, increase the gradient value.

7 Choose the Smooth Edges checkbox to remove noise artifacts that appear around the edge of the ROIs. Boundary detection does not always create smooth edges around the object.

8 Use the Grow ROIs by checkbox and text edit box to define the number of pixels you want to grow the edge off the feature edge. The Grow feature should be used when:

- ✓ The gradient is set too low and large number of artifacts are found. When a larger gradient doesn't find the edge—use the Grow command to find proper edges.
- ✓ Two or more ROIs are connected. In this case setting the gradient higher separates the ROIs.

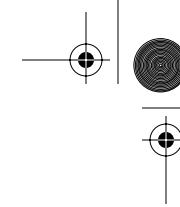
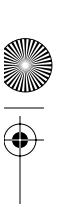
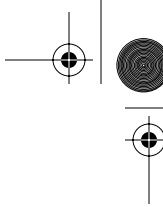
9 Use the Minimum (Min) and Maximum (Max) checkboxes and text edit boxes to define the minimum and maximum sizes of the ROIs found. ROIs found below the minimum or above the maximum values are automatically removed.

NOTE: If you have drawn a Reference ROI, the information from the reference is automatically entered.

10 Verify that the signal of the feature you are searching for is accurately defined as either as either white or black. This preference affects the Auto Find algorithms.

NOTE: If you are using a Carestream Imaging System, the selection you made when acquiring images is used by the software to determine signal orientation.





7

Auto ROIs

11 Click Find. Carestream MI uses these settings to find ROIs.

12 Review the ROIs found and refine the search, as needed.

- ✓ Use the Gradient text edit box or the slider to adjust the Find parameters. Click Replace.
- ✓ Separate closely spaced or touching ROIs using the Separate ROIs tool. See *Editing Automatic ROIs*, later in this chapter. Using ROI tools, you may also manually define ROIs that were not found.
- ✓ The Add button allows you to add selection results together. This can be useful in finding ROI in different regions of the image using different boundary detection techniques. To use this option, make a selection, find ROIs and then exit the Find ROIs dialog box. Define another selection, and begin the Find ROIs process again. Add becomes an available option in the dialog box.

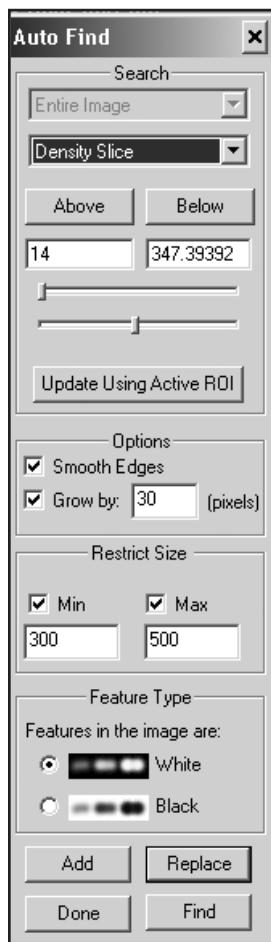
13 When you are satisfied with the results, choose Done to exit the dialog box.

NOTE: You can add additional ROI using the Magic Wand tool or manual defining ROI shapes in the Manual ROI panel.

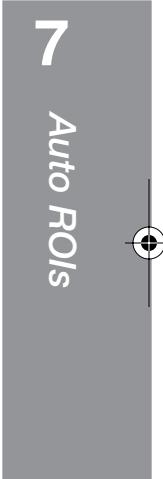
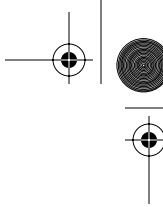
NOTE: You can create an unlimited number of ROI Sets by repeating the above steps.

Finding ROIs Using Density Slice

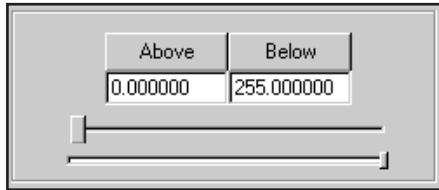
With this method, you can define the minimum and maximum signal values used by Carestream MI Software to find ROIs. The software identifies regions on the image within these two signal values as separate ROIs.



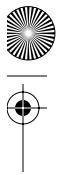
- 1 Click Auto ROIs from the Navigation panel. The Auto ROIs panel opens.
 - 2 Use the Magnification slider or the Zoom tools to maximize the size of the image on-screen.
- ☞ NOTE:** You may want to draw a Reference ROI to provide information to the program on what features you are trying to identify. Double-click the Reference ROI tool to draw an ROI that bounds a typical ROI within the image. The location, width, height, and intensity of the ROI is automatically used in the Find ROIs algorithms. Select the shape of the reference ROI (Ellipse or Rectangle) using the Reference ROI tool options. The Reference ROI tool is especially useful when the automated finding tools are not producing good results.
- 3 Click on the New ROI Set to record that a new set of ROIs are to be recorded. The Auto Find dialog box appears.
 - 4 Use the Search pop-up menu to define what part of the image you want to find ROIs.
 - ✓ *Entire Image*—searches the entire image for ROIs.
 - ✓ *Selection Only*—searches the interior of the selection. The selection is defined by the Rectangle or Ellipse Selection tools. This option is only available when a selection is defined prior to entering the dialog box.
 - ✓ *Oval Quadrant 1, 2, 3, 4*—uses the selection borders to create a circle within the box (the selection is defined by the Ellipse Selection tool). The circle is then divided into four equal quadrants. This option is only available when a selection is defined prior to entering the dialog box.



- 5 Choose Density Slice from the Search Method pop-up menu.
- 6 Use the Above and Below pop-up menus, the text edit boxes, or sliders to define the image minimum or maximum signal values used in the ROI finding algorithm.



- NOTE: If you have drawn a Reference ROI, the information from the reference is automatically entered.
 - NOTE: As you change the minimum and maximum signal value, notice that Carestream MI Software displays the pixels that are below and above the values in color. The regions of the image that appear in gray corresponds to the ROIs created.
 - NOTE: If these values are improperly assigned, you will create donut shaped ROIs.
- 7 Choose the Smooth Edges checkbox to remove noise artifacts that appear around the edge of the ROIs. Density Slice detection does not always create smooth edges around the object.
 - 8 Use the Grow ROIs by checkbox and text edit box to define the number of pixels you want to grow the edge off the feature edge.
 - 9 Use the Minimum (Min) and Maximum (Max) checkboxes and text edit boxes to define the minimum and maximum sizes of the ROIs found. ROIs found below the minimum or above the maximum values are automatically removed.
- NOTE: Use an ROI tool to measure the area of the smallest object of interest on your image. Use this information to when selecting values.
 - 10 Verify that the signal of the feature you are searching for is accurately defined as either white or black. This preference affects the Auto Find algorithms.
 - NOTE: If you are using a Carestream Imaging System, the selection you made when acquiring images is used by the software to determine signal orientation.



11 Click Find. Carestream MI Software uses settings to find ROIs.

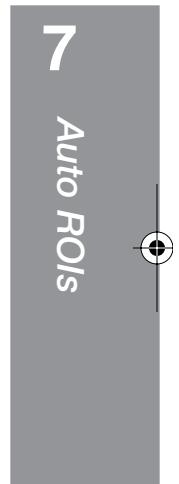
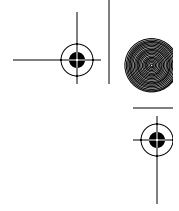
12 Review the ROIs found and refine the search as needed.

- ✓ Use the Above and Below pop-up menus, text edit boxes, or sliders to refine the image minimum and maximum intensity values. Click Replace.
- ✓ KODSK MI Software provides you tools to separate closely spaced or touching ROIs. See *Editing Automatic ROIs*, later in this chapter. You may also manually define ROIs that the software failed to find.
- ✓ The Add button allows you to add selection results together. This can be useful in finding ROI in different regions of the image using different boundary detection techniques. To use this option, return to the Find ROIs dialog box. Define another selection, and begin the Find ROIs process again. Add becomes an available option in the dialog box.

13 When you are satisfied with the results, choose Done to exit the dialog box.

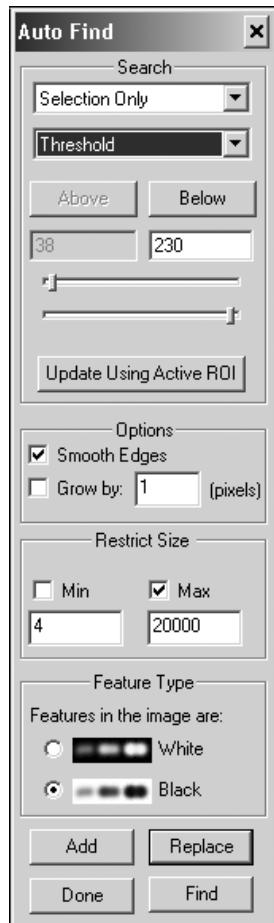
 NOTE: You can add additional ROI using the Magic Wand tool or manual defining ROI shapes in the Manual ROI panel.

 NOTE: You can create an unlimited number of ROI Sets by repeating the above steps.

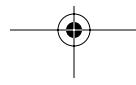
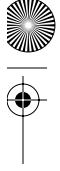


Finding ROIs Using Threshold

With this method, you define the threshold signal value that allows you to segregate image features from the background. The current state of the Above or Below options depends on the black/white points.

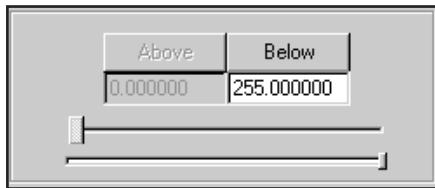


- 1 Click Auto ROIs from the Navigation panel. The Auto ROIs panel opens.
 - 2 Use the Magnification slider or the Zoom tools to maximize the size of the image on-screen.
- NOTE:** You may want to draw a Reference ROI to provide information to the program on what features you are trying to identify. Double-click the Reference ROI tool to draw an ROI that bounds a typical ROI within the image. The location, width, height, and intensity of the ROI is automatically used in the Find ROIs algorithms. Select the shape of the reference ROI (Ellipse or Rectangle) using the Reference ROI tool options. The Reference ROI tool is especially useful when the automated finding tools are not producing good results.
- 3 Click on the New ROI Set to record that a new set of ROIs are to be recorded. The Auto Find dialog box appears.
 - 4 Use the Search pop-up menu to define what part of the image you want to find ROIs.
 - ✓ *Entire Image*—searches the entire image for ROIs.
 - ✓ *Selection Only*—searches the interior of the selection. The selection is defined by the Rectangle or Ellipse Selection tools. This option is only available when a selection is defined prior to entering the dialog box.
 - ✓ *Oval Quadrant 1, 2, 3, 4*—uses the selection borders to create a circle within the box (the selection is defined by the Ellipse Selection tool). The circle is then divided into four equal quadrants. This option is only available when a selection is defined prior to entering the dialog box.
 - 5 Choose Threshold from the Search Method pop-up menu.





- 6** Use the *Above* or *Below* (depending on the “Find” choice) pop-up menus, text edit boxes, or sliders to define the image minimum or maximum signal values. The “Find” choice defines whether the signal is white on black or black on white.



NOTE: If you have drawn a Reference ROI the information from the reference is automatically entered.

NOTE: Notice that Carestream MI Software displays the excluded pixels in color. Choose the Smooth Edges checkbox to remove noise artifacts that appear around the edge of the ROIs. Threshold detection does not always create smooth edges around the object.

- 7** Use the Grow ROIs by checkbox and text edit box to define the number of pixels you want to grow the edge off the feature edge.
- 8** Use the Minimum (Min) and Maximum (Max) checkboxes and text edit boxes to define the minimum and maximum sizes of the ROIs found. ROIs found below the minimum or above the maximum values are automatically removed.

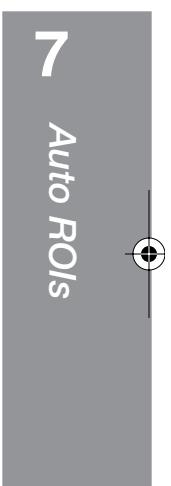
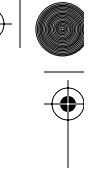
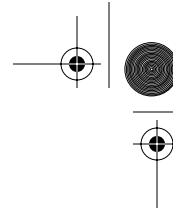
NOTE: Use an ROI tool to measure the area of the smallest object of interest on your image. Use this information when selecting values.

- 9** Verify that the signal of the feature you are searching for is accurately defined as either as either white or black. This preference affects the Auto Find algorithms.

NOTE: If you are using a Carestream Imaging System, the selection you made when acquiring images is used by the software to determine signal orientation.

- 10** Click Find. Carestream MI Software uses these settings to find ROIs.





11 Review the ROIs found and refine the search as needed.

- ✓ Use the Above and Below pop-up menus, text edit boxes, or sliders to refine the image minimum and maximum intensity values. Click Replace.
- ✓ Carestream MI Software provides you tools to separate closely spaced or touching ROIs. See *Editing Automatic ROIs*, in this chapter. You may also manually define ROIs that the software failed to find.
- ✓ The Add button allows you to add selection results together. This can be useful in finding ROI in different regions of the image using different boundary detection techniques. To use this option, return to the Find ROIs dialog box. Define another selection, and begin the Find ROIs process again. Add becomes an available option in the dialog box.

12 When you are satisfied with the results, choose Done to exit the dialog box.

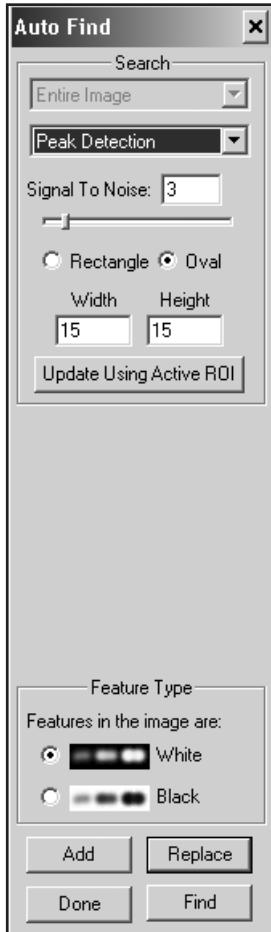
NOTE: You can add additional ROI using the Magic Wand tool or manual defining ROI shapes in the Manual ROI panel.

NOTE: You can create an unlimited number of ROI Sets by repeating the above steps.

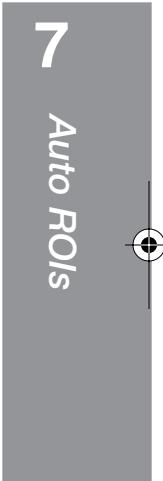
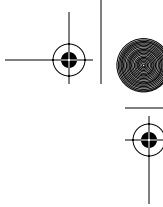


Finding ROIs Using Peak Detection

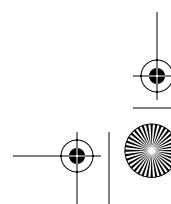
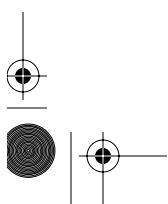
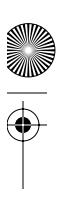
With this method, the program creates oval or rectangular ROIs, not arbitrary shapes, around peaks of intensity. The peak finder can separate peaks that the other finders might group together as one. Also, the peak finder is much less sensitive to variations in the image background than the other auto find ROI methods. You find the peak finder most useful if you are counting, e.g., colony plates or plaque assays.



- 1 Click Auto ROIs from the Navigation panel. The Auto ROIs panel opens.
 - 2 Use the Magnification slider or the Zoom tools to maximize the size of the image on-screen.
- NOTE:** You may want to draw a Reference ROI to provide information to the program on what features you are trying to identify. Double-click the Reference ROI tool to draw an ROI that bounds a typical ROI within the image. The location, width, height, and intensity of the ROI is automatically used in the Find ROIs algorithms. Select the shape of the reference ROI (Ellipse or Rectangle) using the Reference ROI tool options. The Reference ROI tool is especially useful when the automated finding tools are not producing good results.
- 3 Click on the New ROI Set to record that a new set of ROIs are to be recorded. The Auto Find dialog box appears.
 - 4 Use the Search pop-up menu to define what part of the image you want to find ROIs.
 - ✓ *Entire Image* searches the entire image for ROIs.
 - ✓ *Selection Only* searches the interior of the selection. The selection is defined by the Rectangle or Ellipse Selection tools. This option is only available when a selection is defined prior to entering the dialog box.
 - ✓ *Oval Quadrant 1, 2, 3, 4* uses the selection borders to create a circle within the box (the selection is defined by the Ellipse Selection tool). The circle is then divided into four equal quadrants. This option is only available when a selection is defined prior to entering the dialog box.



- 5** Choose Peak Detection from the Search Method pop-up menu.
- 6** Start with the Signal to Noise value at 3.
 ☞ NOTE: If you have drawn a Reference ROI, the information from the reference is automatically entered.
- 7** Select the shape of the Reference ROI. Choose between a Rectangle or Oval.
 ☞ NOTE: If you have previously drawn a Reference ROI, the location, height and weight is automatically entered. You can also manually enter this information into the Width and Height text edit boxes.
- 8** Verify that the signal of the feature you are searching for is accurately defined as either as either white or black. This preference affects the Auto Find algorithms.
 ☞ NOTE: If you are using a Carestream Imaging System, the selection you made when acquiring images is used by the software to determine signal orientation.
- 9** Click Find. Carestream MI Software uses settings to find ROIs.
- 10** To adjust your results, redefine your reference ROI or adjusting the Signal To Noise ratio using the slider or text edit box.
- 11** Review the ROIs found and refine the search as needed.
 - ✓ Use the Above and Below pop-up menus, text edit boxes, or sliders to refine the image minimum and maximum intensity values. Click Replace.
 - ✓ Carestream MI Software provides you tools to separate closely spaced or touching ROIs. See *Editing Automatic ROIs*, in this chapter. You may also manually define ROIs that the software failed to find.
 - ✓ The Add button allows you to add selection results together. This can be useful in finding ROI in different regions of the image using different boundary detection techniques. To use this option, return to the Find ROIs dialog box. Define another selection, and begin the Find ROIs process again. Add becomes an available option in the dialog box.
- 12** When you are satisfied with the results, choose Done to exit the dialog box.
 ☞ NOTE: You can add additional ROI using the Magic Wand tool or manual defining ROI shapes in the Manual ROI panel.
 ☞ NOTE: You can create an unlimited number of ROI Sets by repeating the above steps.





Editing Automatic ROIs

You can edit the Free Form ROI objects using the Separate ROI tool and keyboard shortcuts.

- ✓ Using a keyboard shortcut—to increase or decrease the size of an ROI object, select the object you want to edit with the Pointer tool while pressing the Alt key (Option key for Macintosh). The cursor shows a plus sign. Click and drag the area in which you want to include.
- ✓ Using the Separate ROI tool, click on the object and draw across the area that you want to separate.

Deleting ROIs

You can also delete a selected ROI.

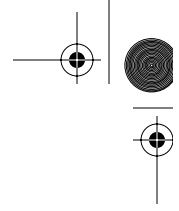


- ✓ Delete a specific ROI by selecting the ROI with the Pointer tool and then pressing the Delete key.

NOTE: You can select multiple objects by Shift-clicking the ROIs, by dragging the Pointer tool over the ROIs, or by selecting them in the ROI Analysis Data window.

- ✓ Delete all the ROIs in a Set by choosing one of the ROIs in the set and clicking on Delete ROI Set from the Auto ROIs panel.

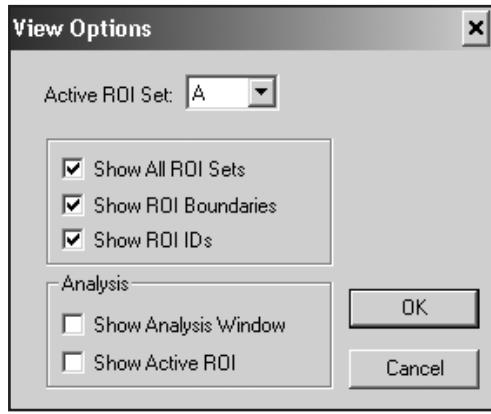




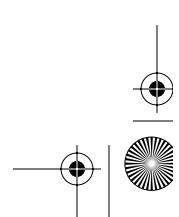
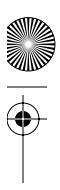
View Options

Define what ROI sets and what analysis data are displayed (e.g., ROI Boundaries, ROI IDs). In addition you can choose to show the ROI Analysis window and/or the Active ROI Info window.

- 1 Click View Options from the Auto ROIs panel or choose ROI from the Show menu. The View Options dialog box appears.



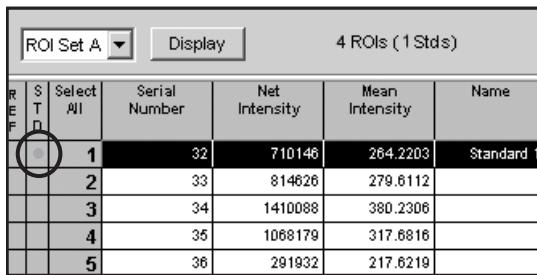
- 2 Choose the features you want to be displayed on-screen.
 - ✓ Select the ROI set that you want active using the Active ROI Set pop-up menu.
 - ☞ NOTE: You can also make an ROI set active by selecting an ROI in the ROI set using the Pointer tool or by selecting the ROI set from the pop-up menu in the ROI Analysis window.
 - ✓ Choose Show All ROI Sets to display all ROI Sets.
 - ✓ Choose Show ROI Boundaries to display the ROI boundaries on-screen.
 - ✓ Choose ROI IDs to display the ROI ID numbers on-screen.
- 3 Choose the Analysis view options.
 - ✓ Select Show Analysis window to display all the data in the spreadsheet format.
 - ✓ Select ROI Info to display data for only a selected ROI.
- 4 Click OK to save your selections. These selections are remembered when opening the project.



Setting Standards

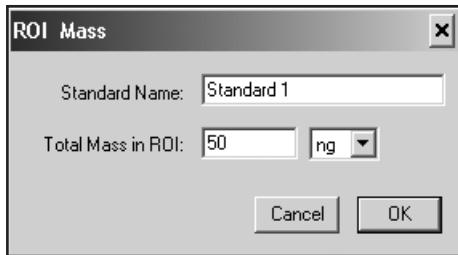
You can designate ROIs as standards and use these standards to generate a mass curve to quantitate unknown ROIs.

- 1** Select Set Standards from the Auto ROI panel. The ROI Analysis window opens.
- 2** Click in the Standard (STD) column to select an ROI as a standard.

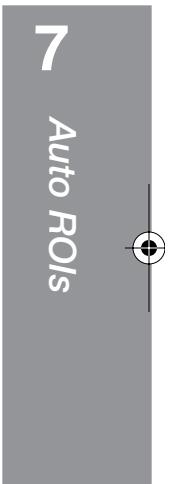
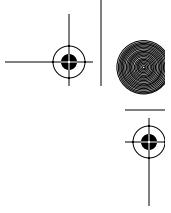


R	S	Select	Serial Number	Net Intensity	Mean Intensity	Name
E	T	All				
		<input checked="" type="checkbox"/>	1	32	710146	264.2203
			2	33	814626	279.6112
			3	34	1410088	380.2306
			4	35	1068179	317.6816
			5	36	291932	217.6219

- 3** The selected ROI is added to the standards list and ROI Mass dialog box appears.



- 4** Enter the standard name and the total mass in the Standard Name and the Total Mass in ROI text edit boxes, respectively.
 - ✓ The maximum number of characters in a standard name is 32.
 - ✓ The units in the Total Mass in ROI pop-up menu corresponds to the options in the Preferences menu.
 - ✓ Each ROI set, must have its own mass curve and set of standards.
- 5** Click OK. The ROI is assigned a mass value and is used in the mass calculation.



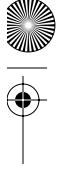
Using References

The Reference Selector in the ROI Analysis Data window designates a single ROI as a reference to measure against all other ROIs.

- 1 Select a row in the ROI Analysis Data window as a reference by clicking in the adjacent Reference (REF) column.

R	S	Select	Serial Number	Net Intensity / by Ref	Sum Intensity / by Ref	Mean Intensity / by Ref	Std.Dev. Intensity / by Ref	Perimeter Area / by Ref	Centroid diffX(pixels)
E	T	All							
F	D								
			1	33	0.5777129	0.7353729	0.7353728	0.6702002	1 146.7256
		<input checked="" type="checkbox"/>	2	34	1	1	1	1	0
			3	35	0.7575265	0.8354972	0.8354972	0.8900322	1 -142.2828
			4	36	0.2130179	0.5723451	0.5723451	0.3056655	1 -284.917
			5	37	0.4942685	0.6907786	0.6907786	0.5692443	1 288.7603

- 2 Choose how you want your data to be displayed using the Reference pop-up menu.
 - ✓ *Don't Use*—is the default and will not compare the data against the reference set.
 - ✓ *as % Difference*—displays the ROI data as a percent difference between the reference and the experimental ROIs.
 - ✓ *as Ratio*—displays the ROI data as a ratio between the reference and the experimental ROIs.



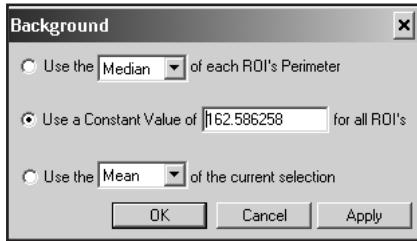
Setting Background

You can define how background for an ROI is calculated. Options include using the perimeter intensity values (median, mean, minimum, or maximum) around each ROI, using any constant value, or using the median, mean, minimum or maximum intensity of a selection. The default is median of each ROI of the perimeter since it is less susceptible to single pixel noise.

Set Background

- 1 Select Set Background from the Auto ROIs panel. The Background dialog box appears.

- 2 Select from one of the three options:



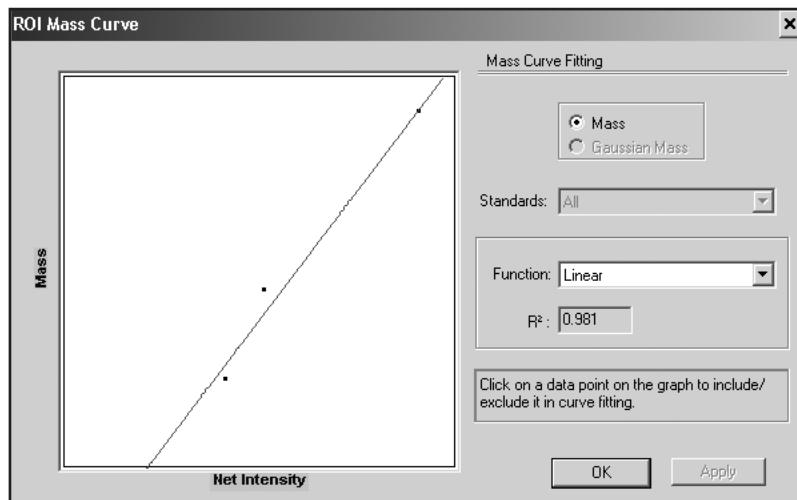
- ✓ *Use the perimeter of each ROI*—each ROI uses perimeter intensity information to calculate a local background. Use the pop-up menu to choose Median, Mean, Minimum, or Maximum of the each ROI's perimeter.
- ✓ *Use a constant value for all ROIs*—you can enter any value in the text edit box. The number (gray levels) that you enter is subtracted from each pixel within the ROI as background.
- ✓ *Use the current selection*—to generate a background for all the ROIs allows you to define a selection using the Selection Rectangle tool. The intensity value from this selection are used to calculate the background. Use the pop-up menu to choose Median, Mean, Minimum, or Maximum of the selection as the background.

- 3 Click Apply to update the background.

Reviewing the ROI Mass Standard Curve

Once you have defined standards. The standard data is plotted on a graph which is used to calculate the experimental mass values (net intensity versus standard mass). You can optimize the fit function to the data and choose which ROIs to be included in the determination of mass.

- 1 Access the Mass Curve dialog box by selecting Mass Curve from Auto ROIs panel. The Mass Curve dialog box appears.



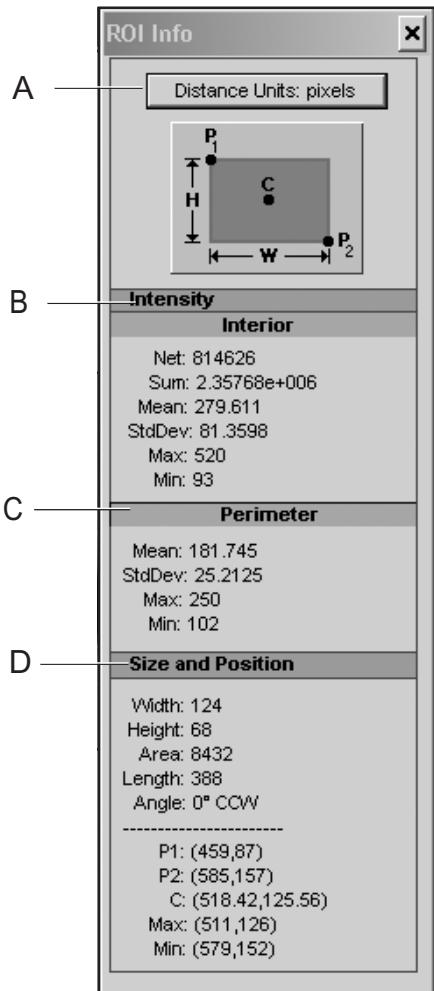
- 2 To maximize the accuracy of your mass determination, review the curves to make sure that the appropriate points are included.
- 3 Choose the fitting function that best represents the data using the Function pop-up menu. The R^2 value aids you in determining the best fit.

The ROI Info Window

Once you have defined a region of interest, view the analysis data for the current selection using the ROI Info window.

To display the window:

- ✓ Choose to display the Active ROI Info window by selecting Show Active ROI in the ROI Options dialog box or click Active ROI Info from the Show menu. The Active ROI Info window appears.

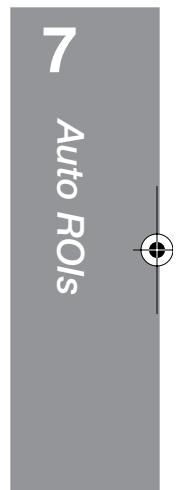
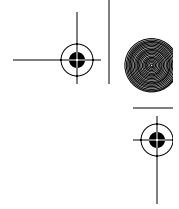


A *Distance Units* pop-up menu allows you to define the units for position and area measurements. Use the pop-up to choose pixels, centimeters, or inches.

B *Interior* provides net, sum, mean, standard deviation, maximum, and minimum intensity values of the interior of the object.

C *Perimeter* provides mean, standard deviation, maximum, and minimum intensity values of the perimeter of the object.

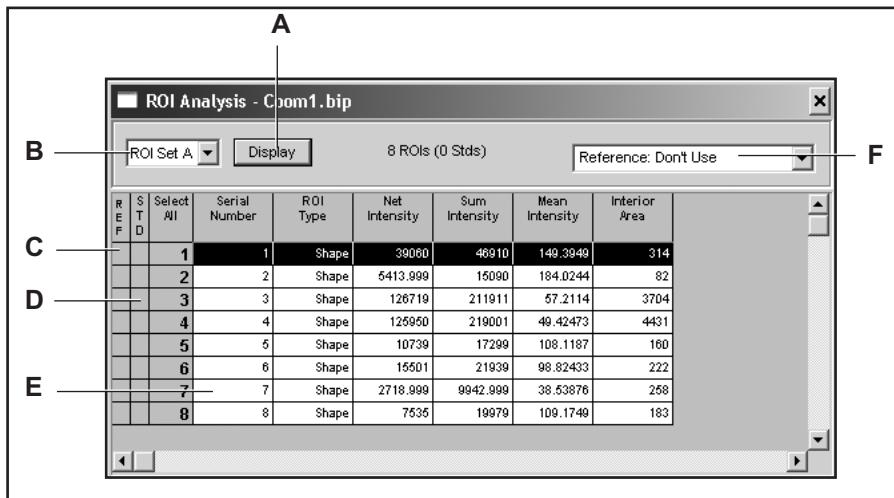
D *Size and Position* provides width, height, area, length, and rotation angle of the object. The data provides information on positioning of the centroid and minimum and maximum intensities.



The ROI Analysis Data Window

The ROI Analysis Data window shows all the ROI data in a spreadsheet format. You can select cells in the ROI Analysis Data window, copy them, and then paste them into other programs or export a tab-delimited text file.

Although all of the statistics should be familiar, a few statistics need to be defined in more detail in the context of digital imaging. Not all of the statistics apply to all ROIs. For example, the area of an ROI can only be calculated if the ROI has interior pixels. Other statistics like width and height have different meanings for different ROI types.



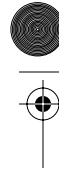
- A The *Display* button accesses the Analysis Display dialog box where you can choose the type of data you want displayed in the ROI Analysis Data window. You can also choose how the background is calculated.
- B The *ROI Set* pop-up menu allows you to choose the ROI set you want to display. The active ROI set is the default.
- C The *Reference Selector* designates a single ROI as a reference to measure against all other ROI's. Select a row as a reference by clicking in the adjacent Reference (REF) column.
- D The *Standard Selector* designates an ROI as a standard point. Select by clicking in the adjacent Standard (STD) column. An ROI Mass window opens allowing you to assign an ROI name and assign a mass load. At least two standard points are required to generate a mass curve.
- E *Data Fields* contain the measurement values and calculated data for each ROI.
- F The *Reference* pop-up menu allows you to display the referenced values (if a reference has been designated) as either a ratio or a % difference. The option default is *Don't Use*.





MI

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Viewing the ROI Analysis Data

The ROI Analysis Data can display the following information for each ROI.

ROI General Data

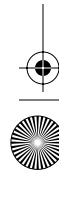
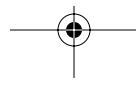
- ✓ *Serial Number*—is a unique identifier for ROIs. If the ROI is deleted, the serial number is never reused. To reset the serial numbers, all ROIs need to be deleted.
- ✓ *ROI Type*—specifies the type of ROI (Rectangle, Ellipse, Shape, or Line).
- ✓ *Comments*—annotate ROI with information about the ROI (up to 255 characters).
- ✓ *Name*—assign a name to the ROI (up to 255 characters).
- ✓ *Mass*—for an experimental ROI, is determined by comparing the sum of the background subtracted intensities (Net Intensity) of all the pixels in the ROI with the background subtracted intensities of all pixels in the standard ROIs.

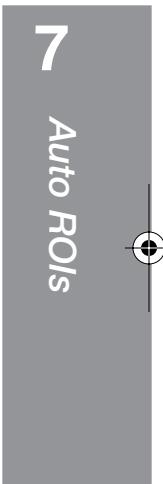
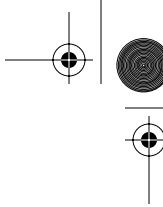
ROI Intensity Data

- ✓ *Net*—is the sum of the background-subtracted pixel values within the ROI.
- ✓ *Sum*—adds together all the pixel intensities within the ROI (includes background).
- ✓ *Mean*—is the average intensity of the pixels within the ROI.
- ✓ *Background*—provides the background value. This is defined within the ROI Analysis Display dialog box.
- ✓ *Maximum*—provides the maximum pixel intensity within the ROI.
- ✓ *Minimum*—provides the minimum pixel intensity within the ROI.
- ✓ *Standard Deviation*—is the square root of the sum of the squared deviation of each pixel value from the mean pixel value. The standard deviation is a useful measure of the statistical error or noise in your data. To find the noise level of your image, create an ROI that includes only background pixels typical of the image. The standard deviation for this ROI is a good indicator of the random variations you can expect for other pixels in the image.

ROI Geometry Data

- ✓ *Width/Height*—for all ROIs except the rectangle and oval, are the horizontal and vertical distances between the centers of the *top left* and *bottom right* pixels described above. For rectangular ROIs, width and height are the number of interior pixels in the horizontal and vertical direction when the ROI is in its unrotated position. Carestream MI Software makes this special case so that area equals width x height for unrotated rectangles, as expected. For ellipses the width and height are always the width and height of the major and minor axes of the interior pixels in the unrotated ellipse. The width and height values of rectangles and ovals do not





change when they are rotated. Width and height may change for other ROIs when rotated, due to changes in the bounding rectangle.

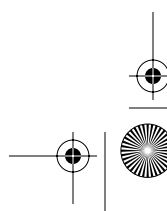
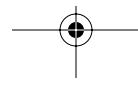
- ✓ *Area*—for each ROI is calculated by counting the number of interior pixels. This is done so you know exactly how many pixels used to calculate other statistics, such as the mean and r.m.s. or the interior pixels. If an ROI has no interior pixels (e.g., a line or an open polygon) the area is zero. Note that the area is not calculated from geometry (e.g., W x H for a rectangle) because when ROIs are rotated the number of interior pixels may change due to the nature of a digital imaging.
- ✓ *Angle*—(for all ROIs except the line ROI) is the current counter clockwise rotation angle of the ROI. For the line ROI the angle is reported as the angle from the +x axis to the line. Note that it makes a difference which end of the line is the starting point and which is the end point of the line. If you click and draw a line ROI up the angle is +90°. If you click and draw the line down the angle is -90° although the two line ROIs appear the same on the image.
- ✓ *Perimeter Length*—for all ROIs, except the Polygon and ellipse ROI, the perimeter length is calculated by summing the Pythagorean distances between the centers of the vertices (nodes) of the ROI and/or the centers of the end points. For the free form ROI which has no control points, the perimeter length is the length of the line that connects the centers of the perimeter pixels in the original order that the perimeter points were drawn. When ROIs are rotated their perimeter lengths may change as a result of the new locations of the perimeter pixels, end points or vertices. For oval ROIs, a formula is used to calculate the perimeter of the ellipse that passes through the centers of the perimeter pixels. This is more accurate than using the techniques for arbitrary shapes and polygons because continuous curves and like ovals are not as well represented in digital imaging.

 NOTE: The centers of the perimeter pixels is used to calculate the perimeter lengths because this is less ambiguous for very complicated shapes or unclosed figures like open polygons and lines that have no “inside” or “outside.”

- ✓ *Perimeter Area*—is the area that is designated as the perimeter.

ROI Position Data

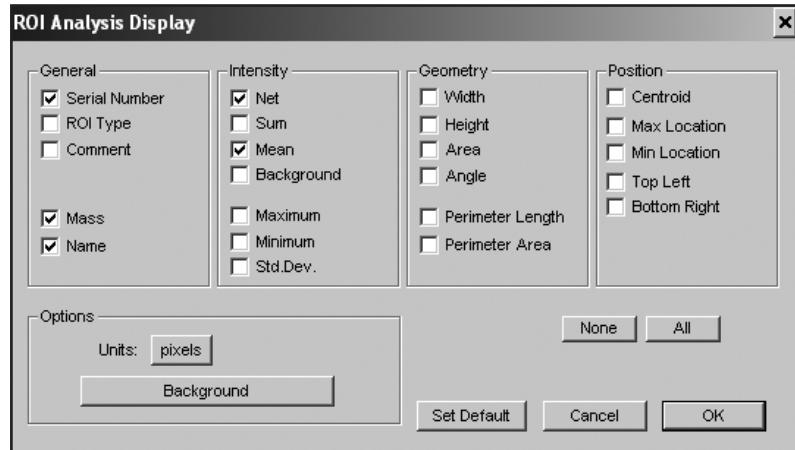
- ✓ *Centroid*—for all ROIs, the (x,y) location of the “center of mass” or the 2nd moment of the intensity distribution. You can calculate the geometrical center of the ROI from the *top left* and *bottom right* values. The centroid is calculated because it is a better indicator of the position of the ROI; the 2nd moment finds the position of the feature inside the boundary of the ROI even if the boundary is not well centered on the object.
- ✓ *Max Location*—defines the pixel that has the highest intensity value.



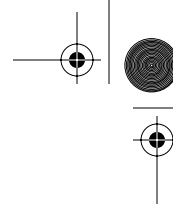
- ✓ *Min Location*—defines the pixel that has the lowest intensity value.
- ✓ *Top Left/Bottom Right*—are determined by finding the smallest rectangle that completely encloses the perimeter pixels of the ROI. The (x,y) coordinates of the pixels in the *top left* and *bottom right* corners of the bounding rectangle are what is reported for *top left* and *bottom right*. When you rotate an ROI, its bounding rectangle may change, therefore, the *top left* and *bottom right* values for the ROI may change after it is rotated.

You can choose the ROI set and the analysis data type to be displayed.

- 1 Click Analysis from the Quick Access bar or choose ROI Analysis Data from the Show menu. The ROI Analysis window appears.
- 2 Choose the ROI Set you want to display using the ROI Set pop-up menu.
- 3 Select Display button. The ROI Analysis Display box appears.



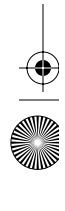
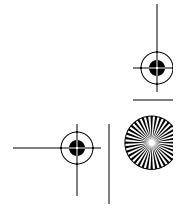
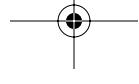
- 4 Select the variables that you want to display for all ROIs.
 - 5 Click OK. The data appears in the ROI Analysis Data window.
- ☞ NOTE:** As you click on an ROI analysis data field, that ROI is selected in the Image section. As you select an ROI in the Image section, the data is field is highlighted.



Sorting the ROI Analysis Data

As ROIs are created, ROIs are added to the bottom of the list. You can, however, sort the data by any column.

- ✓ Click any column heading, this sorts the data in descending order.
- ✓ Double-click any column heading to sort in ascending order.
- ✓ To sort in original order, click on the Serial Number heading.

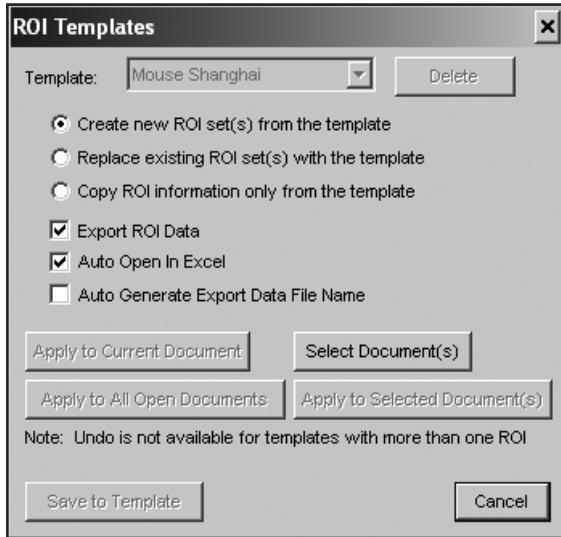


Using ROI Templates

You can save ROIs and define them as a template. When you apply a template to an image, an exact copy of the previously defined ROIs is applied.

Saving an ROI Template

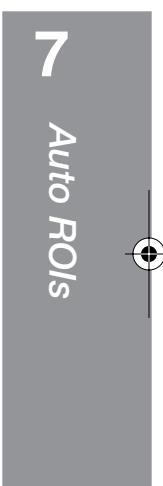
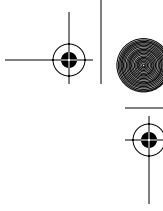
- 1** Define manual ROIs.
- 2** Select Templates from the Auto ROIs panel. The Templates dialog box appears.



☞ NOTE: If you have not previously saved an ROI template, a warning may appear stating that there are no saved templates files. Click OK to access the ROI Template dialog box.

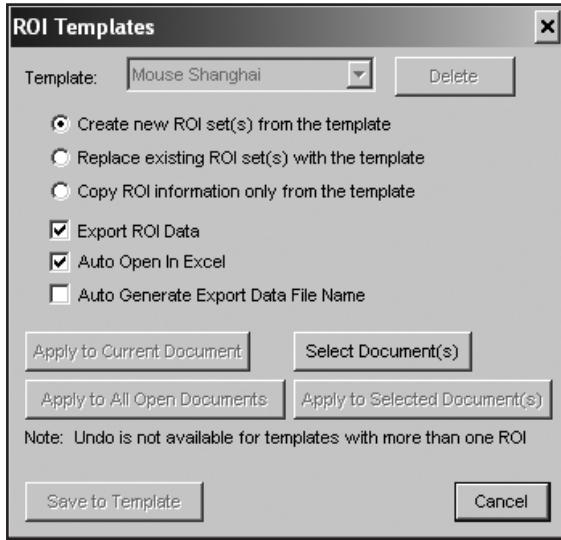
- 3** Click the Save Template button. The ROI Set Template dialog box appears.
- 4** Enter a name for the template in the New Template File Name text edit box.
- 5** Click OK
- 6** The ROIs in a template format is saved.

☞ NOTE: When naming a template, avoid using characters like a slash, colon, or semicolon.



Using a Saved ROI Template

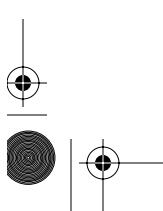
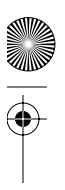
- 1** Acquire or open an image.
- 2** Select Templates from the Manual ROIs panel. The ROI Templates dialog box appears.



- 3** Choose the template you wish to apply using the Template pop-up menu and select any options that you want to apply.

NOTE: Some export options are not active until the Export ROI checkbox is selected.

- ✓ *Create new ROI sets(s) from the template* applies the template selected in the template pop-up list to the current project.
- ✓ *Replace existing ROI set(s) with the template* replaces the current ROIs on the current project with the saved template that is selected in the template pop-up list.
- ✓ *Copy ROI information only from the template* copies the names information from the selected template and applies it to the current set of ROIs
- ✓ *Export ROI Data* checkbox - exports the ROI data exported to a pre-named tab delimited text file once the selected ROI template has been applied to a file.
- ✓ *Auto open in Excel*—formats the data as an Excel file. This check box is only activated if Export ROI Data is checked.



✓ *Auto Generate Export Data File Name* checkbox—if checked, MI will use a date time stamp to auto generate a file name where ROI export data will be saved. This check box is only activated if Export ROI Data is checked.

4 Click the appropriate button to apply the template.

- ✓ *Apply to Current Document* button—applies selected ROI Template to currently active document in MI.
- ✓ *Apply to All Open Documents*—applies selected ROI template to all currently active documents in MI. In order to apply a template, the documents must be saved.
- ✓ *Select Document(s)* button—launches a default file selection dialog that allows you to select MI Project, *.bip, document(s) from a directory/folder of previously saved documents.
- ✓ *Apply to Selected Document(s)* button— applies selected the ROI Template to documents you have selected. This option is only available after you have selected at least one MI Project using the Select Documents button.
- ✓ *Save to Template* button—saves ROI set(s) of a currently opened document to a ROI template file. This option is disabled if there are currently no documents opened in MI.

5 Verify that the template was applied properly.

 NOTE: Click Center ROIs from the Manual ROIs panel to automatically center the ROI based on the centroid (center of mass or the 2nd moment of the intensity distribution).

Deleting a Saved ROI Template

- 1** Select Templates from the Manual ROIs panel. The Templates dialog box appears
- 2** Choose the ROI Template Set that you wish to delete using the Template pop-up menu.
- 3** Click Delete. A confirmation text box appears. Click Yes to delete the selected ROI Template Set.

Grid ROIs

You can perform measurements by creating grids of regions of interest (ROIs) on the image for analyzing slots, spots, arrays, or microplates that are regularly spaced in rows and columns. The Grid ROIs panel guides you through the making an ROI Grid. You can make a grid containing rectangles or ellipses. Once drawn, these ROIs are editable, movable, and can be duplicated.

Once you have drawn an ROI(s), you can choose to automatically center the ROI based on the centroid (center of mass or the 2nd moment of the intensity distribution).

You can designate ROIs as standards and use these standards to generate a mass curve to quantitate unknown ROIs. The ROI Analysis Data window shows all the ROI data in a spreadsheet format. You can select cells in the ROI Analysis Data window, copy them, and then paste them into other programs or export a tab-delimited text file.

You can choose to apply a pre-set grid or create your own grid. Once created, you can rotate, reposition or resize your grid and ROIs. If you are routinely performing similar experiments, you can save grid as a template. When you apply a template to an image, an exact copy of the previously defined ROIs is applied.

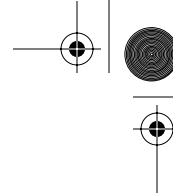
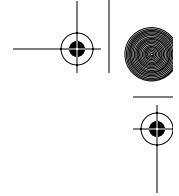
The Grid ROI Panel

The Grid Panel leads you through the process of making a grid of ROIs for analyzing slots, spots, arrays, or microplates that are regularly spaced in rows and columns.

The Grid ROIs Buttons



- ✓ *Set Grid Area*—defines the size of the grid. When this button is selected, a selection rectangle appears on-screen that can be edited to define the area you want to analyze. Use the Rectangle Selection tool to draw a new area. You can create an unlimited number of grids in an image.
- ✓ *Set Reference ROI*—defines the ROI shape (ROI Ellipse or ROI Rectangle). Use the ROI Ellipse or Rectangle to draw a boundary around the ROI within the grid.
- ✓ *Make New Grid*—uses the Reference ROI and the Set Grid Area to create a grid.
- ✓ *Set Standards*—opens the ROI Analysis Data window where you assign multiple ROIs as standards for the mass curve calculations. Select an ROI and click in the Standards (Std) column in the spreadsheet. The ROI Mass window opens so that you can assign a name and total mass to a standard in the ROI. Each ROI set must have its own set of standards.
- ✓ *Set Background*—lets you define how background for an ROI is calculated. Options include using the perimeter intensity values (median, mean, minimum, or maximum) around each ROI, using any constant value, or using the median, mean, minimum or maximum intensity of a selection.
- ✓ *Mass Curve*—once you have defined your standards you can select Mass Curve to open the ROI Mass Curve dialog box. Mass Curve displays an interactive plot of the standard data, which is used to calculate the experimental mass values (net intensity versus standard band mass). You can optimize the fit function to the data and to choose which ROIs to be included in the determination of mass.



- ✓ *Templates*—accesses the ROI Templates dialog box where you can create and apply a template from a defined ROI set to a new image. The ROI template recalls the number of ROIs and standard load amounts. This is especially useful if you routinely run similar experiments.
- ✓ *Center ROIs*—automatically centers ROIs based on the centroid (center of mass or the 2nd moment of the intensity distribution).
- ✓ *Delete ROI Set*—deletes the active ROI set.
- ✓ *View Options*—defines what ROI sets and what analysis data are displayed (e.g., ROI Boundaries, ROI IDs). In addition you can choose to show the ROI Analysis window and/or the Active ROI Info window.

The Grid ROIs Tools



Zoom Tools

Use the Zoom tools to adjust the size of the image through levels of magnification (0.25X, 0.33X, 0.50X, 1X, 1.5X, 2X, 4X, 6X, 8X, 16X, and 32X). To increase the magnification, click the image with the + Zoom tool. To reduce magnification, click the image with the - Zoom tool.

When you change the magnification with the Zoom tools, the software positions the point where you clicked in the center of the Image section. This differs from the Magnification slider which maintains the center of image in the window.



Pointer Tool

Use the arrow-shaped Pointer tool to select ROI Sets and ROIs. Shift-click to select multiple objects with the Pointer tool or click and drag the mouse over an area of the image containing more than one object.



White Point /Black Point Tools

Use the White Point/Black Point tools to select the white or black point values used to adjust the contrast in the image. To use, click the Black Point tool over the pixel in which you want to represent black point. Click the White Point tool over the pixel in which you want the to represent the white point. The image is then remapped using these points.

NOTE: The white point and black point can also be adjusted using the Image Display window.





Selection Rectangle Tool

Click and drag the Image Selection tool to define the grid size. The Selection Rectangle tool is also used to select a rectangle to make a selection for printing, exporting a selection as a TIFF or JPEG file, or copying to the clipboard.



ROI Ellipse Tool

The ROI Ellipse tool is used to draw a round or elliptically shaped ROI as a reference ROI for the grid. To access ROI Ellipse tool options, double-click on the tool. To draw a circle, select the Ellipse tool from the Grid ROI panel. Position the cursor in the center of the circle you want to draw, click and drag out to increase the size of the circle. Release the mouse and the drawn ROI is represented by moving red and white dashes (active ROI).

NOTE: To draw a perfect circle, press the Shift key while defining the ROI using the ROI Ellipse tool.

By double-clicking on the ROI Ellipse tool, you can choose your preference between drawing from the center or from the corner.



ROI Rectangle Tool

The ROI Rectangle tool is used to draw a box or rectangular shaped ROI as a reference ROI for the grid. To draw a circle, select the Ellipse tool from the Grid ROI panel. Position the cursor in the center of the circle you want to draw, click and drag out to increase the size of the circle. Release the mouse and the drawn ROI is represented by moving red and white dashes (active ROI). Do not record the ROI; the ROI must be active to be a reference. This tool is especially useful when the automated finding tools are not producing good results.

NOTE: To draw a perfect square, press the Shift key while defining the ROI using the ROI Ellipse tool.

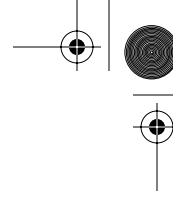
By double-clicking on the ROI Reference tool, you can choose your preference between drawing from the center or from the corner.



Move ROI Tool

The Move ROI tool is used to move or adjust the position of an ROI within the grid. You can select multiple objects clicking and dragging the mouse over an area of the image containing more than one object.





Resize All ROIs Tool

The Resize All ROIs tool is used to edit the shape of all ROIs within a grid. Click on the tool and then select an ROI to resize. Position the tool over a control point. Click and drag the control point in the direction that you want to resize the object. All ROIs within the grid will be resized.

NOTE: Magnify the image if you have trouble selecting the control point.



Rotate All ROIs Tool

The Rotate All ROIs tool is used to rotate all ROIs within a grid. Click on the tool and then select a control point on the ROI and rotate in any direction. All ROIs within the grid will be rotated.

NOTE: Magnify the image if you have trouble selecting the control point.



Resize Grid Tool

The Resize Grid tool resizes the spacing of the grid. Click on the tool and then select an ROI to reposition. Click and drag the ROI into position. The grid spacing will change, but the ROI size is not altered.



Rotate Selected ROI Tool

The Rotate Selected ROI tool is used to rotate a selected ROI within a grid. Click on the tool and then select a control point on the ROI and rotate in any direction. Only the selected ROI will be rotated.

NOTE: Magnify the image if you have trouble selecting the control point.

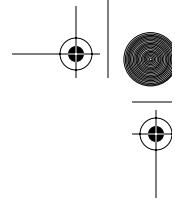


Resize Selected ROI Tool

The Resize Selected ROI tool is used to edit the shape of a selected ROI within a grid. Click on the tool and then select an ROI to resize. Position the tool over a control point. Click and drag the control point in the direction that you want to resize the object.

NOTE: Magnify the image if you have trouble selecting the control point.

You can select multiple ROIs by Shift-clicking objects or by dragging the Resize Selected ROI tool over the ROIs. When you resize on ROI, all selected ROIs will resize.



Magic Wand Tool

The Magic Wand tool automatically defines an ROI for you. To use, click and drag along a small portion of the edge of the ROI that you want to define. The gray level information from these pixels is used to define the boundary.

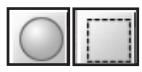
Alternately, you can click in the center of an ROI and the finder determines the edge using the threshold level value selected in the Magic Wand options. You can adjust how close the Magic Wand tool sets the threshold level with respect to its estimate of the background. To access the Magic Wand tool options, double-click on the tool.



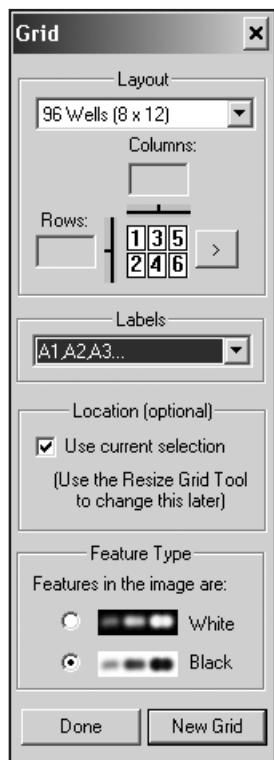
Making a Grid

You can create one or more grids on an image.

- 1 Select the Grid ROIs panel from the Navigation panel.
- 2 Choose the Set Search Area from the Grid ROIs panel. The Selection Rectangle tool is selected and a default selection rectangle is displayed.
- 3 Adjust the selection rectangle or redraw the rectangle by clicking and dragging to select the area of the image to be analyzed.



- 4 Choose Set Reference ROI, the ROI Ellipse or ROI Rectangle will become the active tool. Choose the tool that best represents the shape of the objects you want to measure within the grid.



NOTE: The software remembers the last ROI type drawn in Grid ROIs.

- 5 Draw an ROI to encompass the object you want to measure.

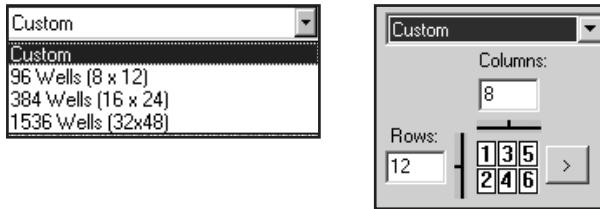


- 6 Click Make New Grid on the Grid ROIs panel. The Grid dialog box appears.

8

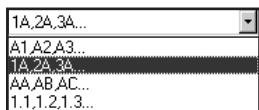
Grid Analysis

- 7** Choose the size grid you want drawn using the Grid pop-up menu. Several common grid sizes are available for selection or choose Custom to define your own.



NOTE: Once you have created a custom grid, you can save the grid as a template. Refer to *Using ROI Templates*, later in this chapter.

- 8** Use the Label pop-up menu to define how you want the analysis data to be labeled.



NOTE: The labels appear in the comments section of the ROI Analysis Data window.

- 9** Verify that the signal of the feature you are searching for is accurately defined as either white or black. This preference affects the Auto Find algorithms.

NOTE: If you are using a Carestream Imaging System, the selection you made when acquiring images is used by the software to determine signal orientation.

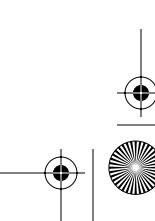
- 10** Click Make Grid. A grid is drawn.

NOTE: If you have made Set Grid Area with the Rectangle Selection tool, the Location checkbox is automatically checked. The grid will be drawn within the selection.

NOTE: Click Center ROIs from the Grid ROIs panel to automatically center the ROI based on the centroid (center of mass or the 2nd moment of the intensity distribution).

NOTE: You can add additional ROI using the Magic Wand tool or manual defining ROI shapes in the Manual ROI panel.

NOTE: You can create an unlimited number of ROI Sets by repeating the above steps.



Editing the Grid and Grid ROIs

You can move the grid's position and modify the spacing of the grid—as a group or individually. You can also modify the size and shape of the ROIs—as a group or individually.

Moving Selected ROIs within the Grid



The Move ROI tool is used to move or adjust the position of a selected ROI within the grid.

- ✓ To move a single ROI, click and drag the ROI to a new location. Only the selected ROI is repositioned.
- ✓ You can select multiple objects clicking and dragging the mouse over an area of the image containing more than one object.

Moving All ROIs within the Grid



The Resize Grid tool resizes the spacing of the grid. You can choose any ROI to reposition, all ROIs will move and be redistributed.

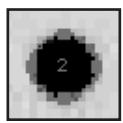
- ✓ Click on the tool and then select an ROI to reposition. Click and drag the ROI into position. The grid spacing will change, but the ROI size is not altered.

Editing the Shape of Selected ROIs within the Grid



Change the size or shape of a selected ROI within a grid using the Resize Selected ROI tool.

- ✓ Click on the Resize Selected ROI tool and then select an ROI to resize. Position the tool over a control point. Click and drag the control point in the direction that you want to resize the object.



- ✓ You can select multiple ROIs by Shift-clicking objects or by dragging the Pointer tool over the ROIs. Edit any of the selected ROIs, all selected ROIs will be edited.

NOTE: Magnify the image if you have trouble selecting the control point.



Editing the Shape of All ROIs within the Grid



The Resize All ROIs tool is used to resize the shape of all ROIs within a grid.

- ✓ Click on the tool and then select an ROI to resize. Position the tool over a control point. Click and drag the control point in the direction that you want to resize the object. All ROIs within the grid will be resized.

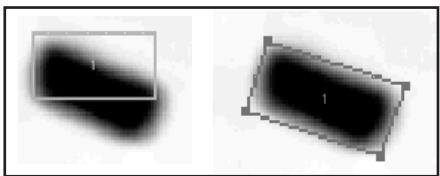
NOTE: Magnify the image if you have trouble selecting the control point.

Rotating an ROI within a Grid



The Rotate Selected ROI tool is used to rotate a selected ROI within a grid.

- ✓ Click on the tool and then select a control point on the ROI and rotate in the any direction. Only the selected ROI will be rotated.



Deleting Grid ROIs



- ✓ Delete a specific ROI by selecting the ROI with the Pointer tool and then pressing the Delete key.

 NOTE: You can select multiple objects by Shift-clicking the ROIs, by dragging the Pointer tool over the ROIs, or by selecting them in the ROI Analysis Data window.

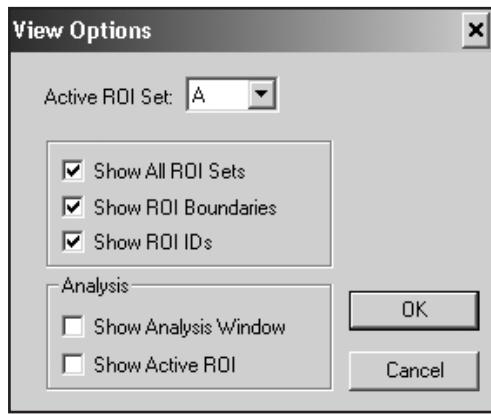


- ✓ Delete all the ROIs in a Set by choosing one of the ROIs in the set and clicking on Delete ROI Set from the Grid ROIs panel.

View Options

Define what ROI sets and what analysis data are displayed (e.g., ROI Boundaries, ROI IDs). In addition you can choose to show the ROI Analysis window and/or the Active ROI Info window.

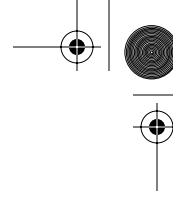
- 1 Click View Options from the Grid ROIs panel or choose ROI from the Show menu. The View Options dialog box appears.



- 2 Choose the features you want to be displayed on-screen.
 - ✓ Select the ROI set that you want active using the Active ROI Set pop-up menu.

NOTE: You can also make an ROI set active by selecting an ROI in the ROI set using the Pointer tool or by selecting the ROI set from the pop-up menu in the ROI Analysis window.

 - ✓ Choose Show All ROI Sets to display all ROI Sets.
 - ✓ Choose Show ROI Boundaries to display the ROI boundaries on-screen.
 - ✓ Choose ROI IDs to display the ROI ID numbers on-screen.
- 3 Choose the Analysis view options.
 - ✓ Select Show Analysis window to display all the data in the spreadsheet format.
 - ✓ Select ROI Info to display data for only a selected ROI.
- 4 Click OK to save your selections. These selections will be remembered when opening the project.



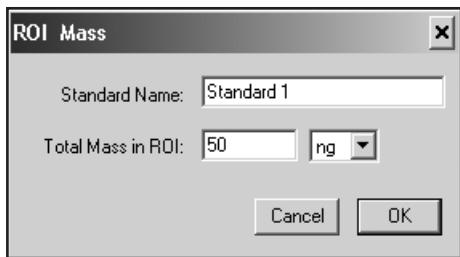
Setting Standards

You can designate ROIs as standards and use these standards to generate a mass curve to quantitate unknown ROIs.

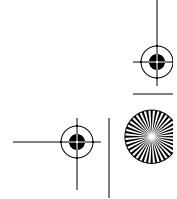
- 1** Select Set Standards from the Grid ROI panel. The ROI Analysis window opens.
- 2** Click in the Standard (STD) column to select an ROI as a standard.

R	S	Select All	Serial Number	Net Intensity	Mean Intensity	Name
E	S	<input checked="" type="checkbox"/>	32	710146	264.2203	Standard 1
E	S	<input type="checkbox"/>	33	814626	279.6112	
E	S	<input type="checkbox"/>	34	1410088	380.2306	
E	S	<input type="checkbox"/>	35	1068179	317.6816	
E	S	<input type="checkbox"/>	36	291932	217.6219	

- 3** The selected ROI is added to the standards list and ROI Mass dialog box appears.



- 4** Enter the standard name and the total mass in the Standard Name and the Total Mass in ROI text edit boxes, respectively.
 - ✓ The maximum number of characters in a standard name is 32.
 - ✓ The units in the Total Mass in ROI pop-up menu corresponds to the options in the Preferences menu.
 - ✓ Each ROI set, must have its own mass curve and set of standards.
- 5** Click OK. The ROI is assigned a mass value and is used in the mass calculation.

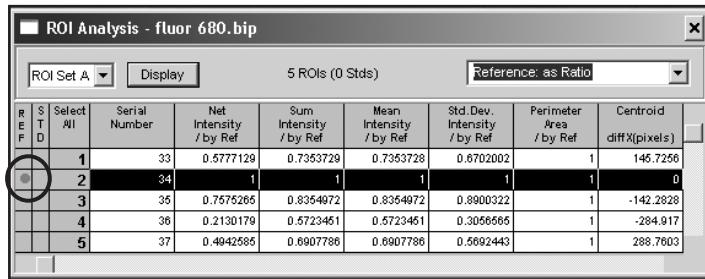




Using References

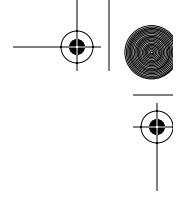
The Reference Selector in the ROI Analysis Data window designates a single ROI as a reference to measure against all other ROIs.

- Select a row in the ROI Analysis Data window as a reference by clicking in the adjacent Reference (REF) column.



R	S	Select	Serial Number	Net Intensity / by Ref	Sum Intensity / by Ref	Mean Intensity / by Ref	Std.Dev. Intensity / by Ref	Perimeter Area / by Ref	Centroid diffX(pixels)
E	T	All							
F	D								
		<input checked="" type="checkbox"/>	1	33	0.5777129	0.7353729	0.7353728	0.6702002	1 146.7256
		<input type="checkbox"/>	2	34	1	1	1	1	0
		<input type="checkbox"/>	3	35	0.7575265	0.8354972	0.8354972	0.8900322	1 -142.2828
		<input type="checkbox"/>	4	36	0.2130179	0.5723451	0.5723451	0.3056655	1 -284.917
		<input type="checkbox"/>	5	37	0.4942685	0.6907786	0.6907786	0.5692443	1 288.7603

- Choose how you want your data to be displayed using the Reference pop-up menu.
 - Don't Use*—is the default and will not compare the data against the reference set.
 - as% Difference*—displays the ROI data as a percent difference between the reference and the experimental ROIs.
 - as Ratio*—displays the ROI data as a ratio between the reference and the experimental ROIs.

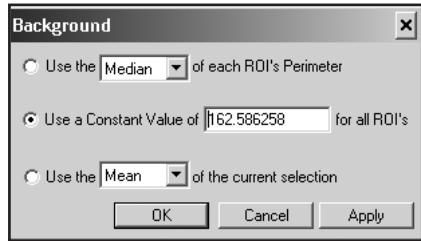


Setting Background

You can define how background for an ROI is calculated. Options include using the perimeter intensity values (median, mean, minimum, or maximum) around each ROI, using any constant value, or using the median, mean, minimum or maximum intensity of a selection. The default is median of each ROI of the perimeter since it is less susceptible to single pixel noise.

Set Background 1 Select Set Background from the Grid ROIs panel. The Background dialog box appears.

2 Select from one of the three options:



- ✓ *Use the perimeter of each ROI*—each ROI uses perimeter intensity information to calculate a local background. Use the pop-up menu to choose Median, Mean, Minimum or Maximum of the each ROI's perimeter.
- ✓ *Use a constant value for all ROIs*—you can enter any value in the text edit box. The number (gray levels) that you enter will be subtracted from each pixel within the ROI as background.
- ✓ *Use the current selection to generate a background for all the ROIs*—allows you to define a selection using the Selection Rectangle tool. The intensity value from this selection are used to calculate the background. Use the pop-up menu to choose Median, Mean, Minimum or Maximum of the selection as the background.

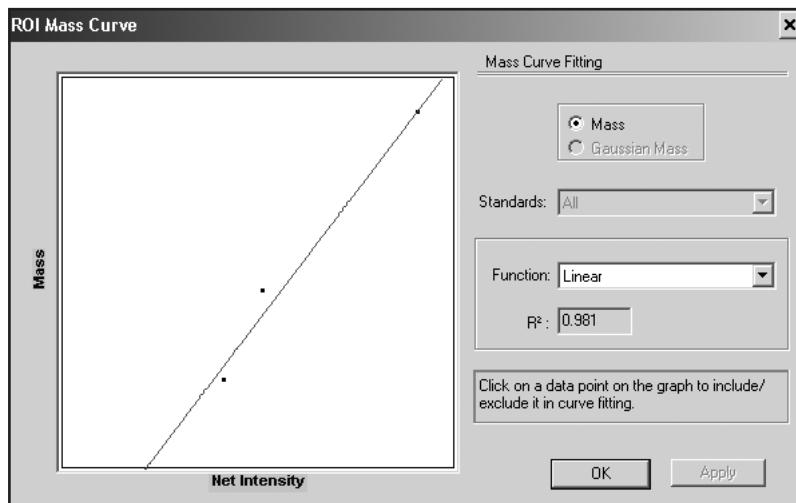
3 Click Apply to update the background.



Reviewing the ROI Mass Standard Curve

Once you have defined standards. The standard data is plotted on a graph which is used to calculate the experimental mass values (net intensity versus standard mass). You can optimize the fit function to the data and choose which ROIs to be included in the determination of mass.

- 1 Access the Mass Curve dialog box by selecting Mass Curve from Grid ROIs panel. The Mass Curve dialog box appears.



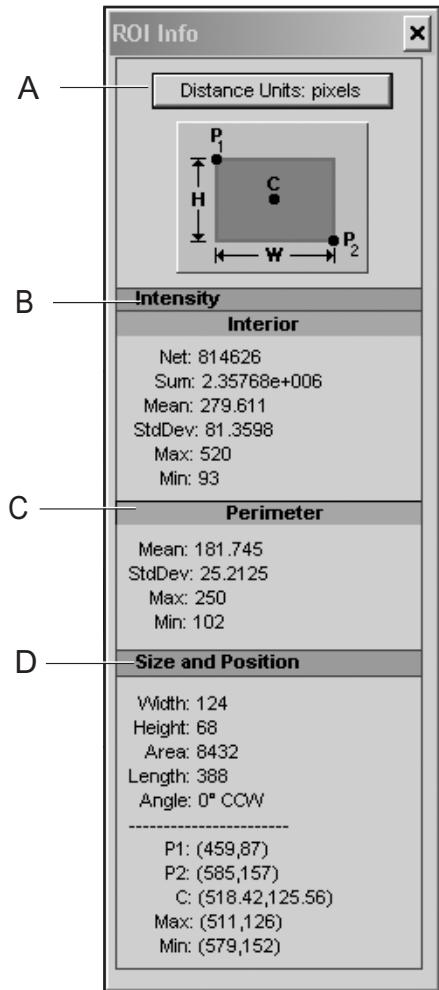
- 2 To maximize the accuracy of your mass determination, review the curves to make sure that the appropriate points are included.
- 3 Choose the fitting function that best represents the data using the Function pop-up menu. The R^2 value aids you in determining the best fit.

ROI Info Window

Once you have defined a region of interest, view the analysis data for the current selection using the ROI Info window.

To display the window:

- ✓ Choose to display the Active ROI Info window by selecting Show Active ROI in the ROI Options dialog box or click Active ROI Info from the Show menu. The Active ROI Info window appears.



A *Distance Units* pop-up menu allows you to define the units for position and area measurements. Use the pop-up to choose pixels, centimeters, or inches.

B *Interior* provides net, sum, mean, standard deviation, maximum, and minimum intensity values of the interior of the object.

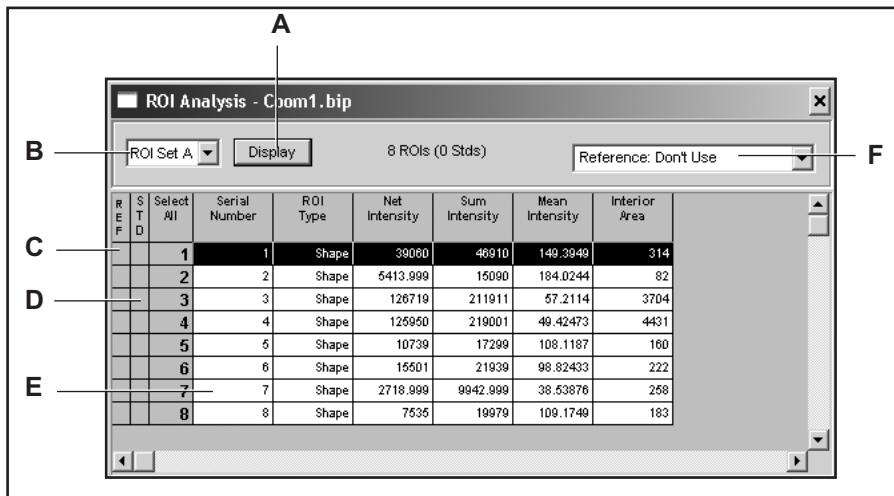
C *Perimeter* provides mean, standard deviation, maximum, and minimum intensity values of the perimeter of the object.

D *Size and Position* provides width, height, area, length, and rotation angle of the object. The data provides information on positioning of the centroid and minimum and maximum intensities.

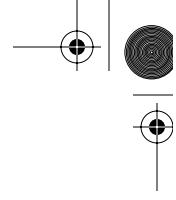
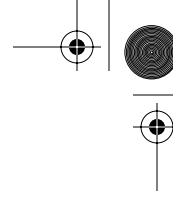
ROI Analysis Data Window

The ROI Analysis Data window shows all the ROI data in a spreadsheet format. You can select cells in the ROI Analysis Data window, copy them, and then paste them into other programs or export a tab-delimited text file.

Although all of the statistics should be familiar, a few statistics need to be defined in more detail in the context of digital imaging. Not all of the statistics apply to all ROIs. For example, the area of an ROI can only be calculated if the ROI has interior pixels. Other statistics like width and height have different meanings for different ROI types.



- A The *Display button* accesses the Analysis Display dialog box where you can choose the type of data you want displayed in the ROI Analysis Data window. You can also choose how the background is calculated.
- B The *ROI Set pop-up menu* allows you to choose the ROI set you want to display. The active ROI set is the default.
- C The *Reference Selector* designates a single ROI as a reference to measure against all other ROI's. Select a row as a reference by clicking in the adjacent Reference (REF) column.
- D The *Standard Selector* designates an ROI as a standard point. Select by clicking in the adjacent Standard (STD) column. A ROI Mass window opens allowing you to assign an ROI name and assign a mass load. At least two standard points are required to generate a mass curve.
- E *Data Fields* contain the measurement values and calculated data for each ROI.
- F The *Reference pop-up menu* allows you to display the referenced values (if a reference has been designated) as either a ratio or a % difference. The option default is *Don't Use*.



Viewing the ROI Analysis Data

The ROI Analysis Data can display the following information for each ROI.

ROI General Data

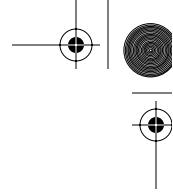
- ✓ *Serial Number*—is a unique identifier for ROIs. If the ROI is deleted, the serial number is never reused. To reset the serial numbers, all ROIs need to be deleted.
- ✓ *ROI Type*—specifies the type of ROI (Rectangle, Ellipse, Shape, or Line).
- ✓ *Comments*—annotate ROI with information about the ROI (up to 255 characters).
- ✓ *Name*—assign a name to the ROI (up to 255 characters).
- ✓ *Mass*—for an experimental ROI, is determined by comparing the sum of the background subtracted intensities (Net Intensity) of all the pixels in the ROI with the background subtracted intensities of all pixels in the standard ROIs.

ROI Intensity Data

- ✓ *Net*—is the sum of the background-subtracted pixel values within the ROI.
- ✓ *Sum*—adds together all the pixel intensities within the ROI (includes background).
- ✓ *Mean*—is the average intensity of the pixels within the ROI.
- ✓ *Background*—provides the background value. This is defined within the ROI Analysis Display dialog box.
- ✓ *Maximum*—provides the maximum pixel intensity within the ROI.
- ✓ *Minimum*—provides the minimum pixel intensity within the ROI.
- ✓ *Standard Deviation*—is the square root of the sum of the squared deviation of each pixel value from the mean pixel value. The standard deviation is a useful measure of the statistical error or noise in your data. To find the noise level of your image, create an ROI that includes only background pixels typical of the image. The standard deviation for this ROI is a good indicator of the random variations you can expect for other pixels in the image.

ROI Geometry Data

- ✓ *Width/Height*—for all ROIs except the rectangle and oval, are the horizontal and vertical distances between the centers of the *top left* and *bottom right* pixels described above. For rectangular ROIs, width and height are the number of interior pixels in the horizontal and vertical direction when the ROI is in its unrotated position. Carestream MI Software makes this special case so that area equals width x height for unrotated rectangles, as expected. For ellipses the width and height are always the width and height of the major and minor axes of the interior pixels in the unrotated ellipse. The width and height values of rectangles and ovals do not



change when they are rotated. Width and height may change for other ROIs when rotated, due to changes in the bounding rectangle.

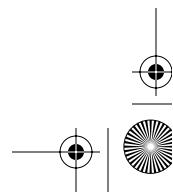
- ✓ *Area*—for each ROI is calculated by counting the number of interior pixels. This is done so you know exactly how many pixels used to calculate other statistics, such as the mean and r.m.s. or the interior pixels. If an ROI has no interior pixels (e.g., a line or an open polygon) the area is zero. Note that the area is not calculated from geometry (e.g., W x H for a rectangle) because when ROIs are rotated the number of interior pixels may change due to the nature of a digital imaging.
- ✓ *Angle*—(for all ROIs except the line ROI) is the current counter clockwise rotation angle of the ROI. For the line ROI the angle is reported as the angle from the +x axis to the line. Note that it makes a difference which end of the line is the starting point and which is the end point of the line. If you click and draw a line ROI up the angle is +90°. If you click and draw the line down the angle is -90° although the two line ROIs appear the same on the image.
- ✓ *Perimeter Length*—for all ROIs, except the Polygon and ellipse ROI, the perimeter length is calculated by summing the Pythagorean distances between the centers of the vertices (nodes) of the ROI and/or the centers of the end points. For the free form ROI which has no control points, the perimeter length is the length of the line that connects the centers of the perimeter pixels in the original order that the perimeter points were drawn. When ROIs are rotated their perimeter lengths may change as a result of the new locations of the perimeter pixels, end points or vertices. For oval ROIs, a formula is used to calculate the perimeter of the ellipse that passes through the centers of the perimeter pixels. This is more accurate than using the techniques for arbitrary shapes and polygons because continuous curves and like ovals are not as well represented in digital imaging.

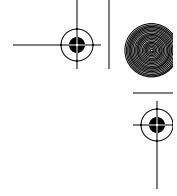
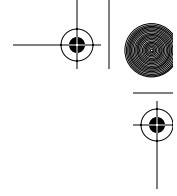
NOTE: The centers of the perimeter pixels is used to calculate the perimeter lengths because this is less ambiguous for very complicated shapes or unclosed figures like open polygons and lines that have no “inside” or “outside.”

- ✓ *Perimeter Area*—is the area that is designated as the perimeter.

ROI Position Data

- ✓ *Centroid*—for all ROIs, the (x,y) location of the “center of mass” or the 2nd moment of the intensity distribution. You can calculate the geometrical center of the ROI from the *top left* and *bottom right* values. The centroid is calculated because it is a better indicator of the position of the ROI; the 2nd moment finds the position of the feature inside the boundary of the ROI even if the boundary is not well centered on the object.

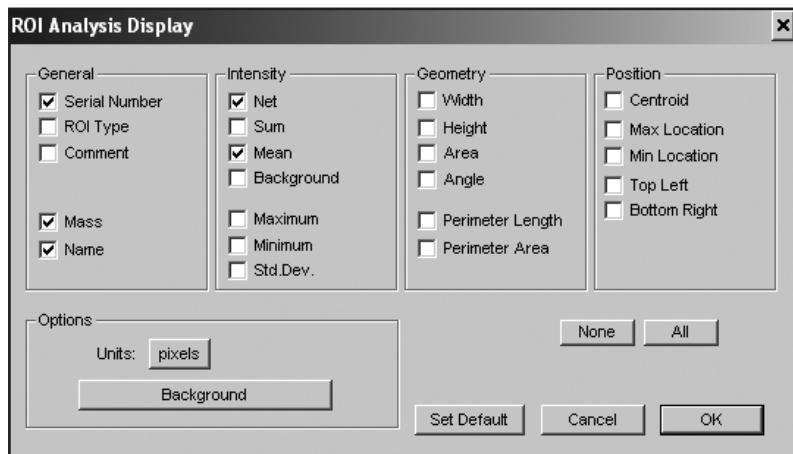




- ✓ *Max Location*—defines the pixel that has the highest intensity value.
- ✓ *Min Location*—defines the pixel that has the lowest intensity value.
- ✓ *Top Left/Bottom Right*—are determined by finding the smallest rectangle that completely encloses the perimeter pixels of the ROI. The (x,y) coordinates of the pixels in the *top left* and *bottom right* corners of the bounding rectangle are what is reported for *top left* and *bottom right*. When you rotate an ROI, its bounding rectangle may change, therefore, the *top left* and *bottom right* values for the ROI may change after it is rotated.

You can choose the ROI set and the analysis data type to be displayed.

- 1 Click Analysis from the Quick Access bar or choose ROI Analysis Data from the Show menu. The ROI Analysis window appears.
- 2 Choose the ROI Set you want to display using the ROI Set pop-up menu.
- 3 Select Display button. The ROI Analysis Display box appears.



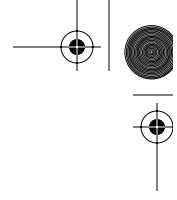
- 4 Select the variables that you want to display for all ROIs.
 - 5 Click OK. The data appears in the ROI Analysis Data window.
- NOTE:** As you click on an ROI analysis data field, that ROI is selected in the Image section. As you select an ROI in the Image section, the data is field is highlighted.

Sorting the ROI Analysis Data

As ROIs are created, ROIs are added to the bottom of the list. You can, however, sort the data by any column.

- ✓ Click any column heading, this sorts the data in descending order.
- ✓ Double-click any column heading to sort in ascending order.
- ✓ To sort in original order, click on the Serial Number heading.

You can save ROIs and define them as a template. When you apply a template to an image, an exact copy of the previously defined ROIs is applied.

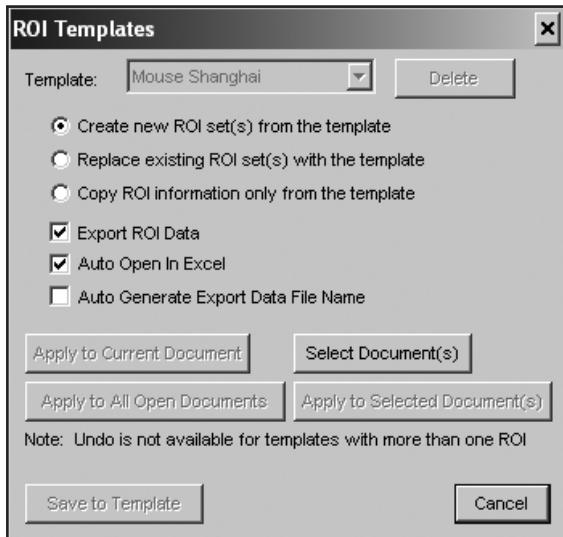


Using ROI Templates

You can save ROIs and define them as a template. When you apply a template to an image, an exact copy of the previously defined ROIs is applied.

Saving an ROI Template

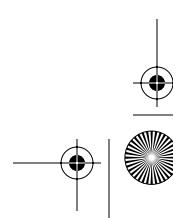
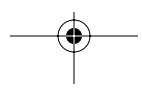
- 1** Define manual ROIs.
- 2** Select Templates from the Grid ROIs panel. The Templates dialog box appears.



NOTE: If you have not previously saved an ROI template, a warning may appear stating that there are no saved templates files. Click OK to access the ROI Template dialog box.

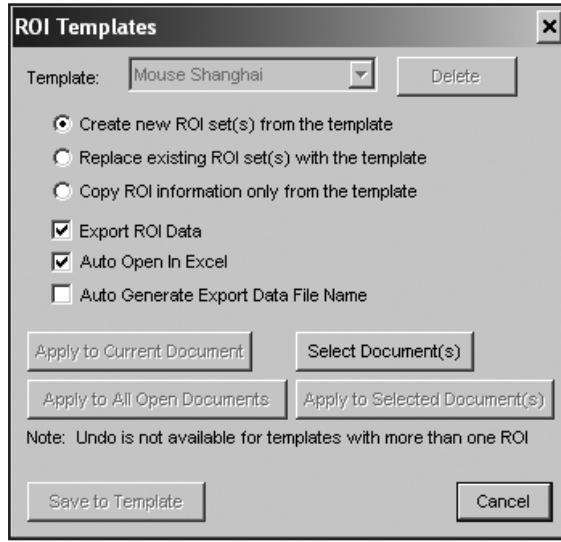
- 3** Click the Save Template button. The ROI Set Template dialog box appears.
- 4** Enter a name for the template in the New Template File Name text edit box.
- 5** Click OK
- 6** The ROIs in a template format is saved.

NOTE: When naming a template, avoid using characters like a slash, colon, or semicolon.



Using a Saved ROI Template

- 1** Acquire or open an image.
- 2** Select Templates from the Manual ROIs panel. The ROI Templates dialog box appears.



- 3** Choose the template you wish to apply using the Template pop-up menu and select any options that you want to apply.
 - NOTE:** Some export options are not active until the Export ROI checkbox is selected.
 - Create new ROI sets(s) from the template* applies the template selected in the template pop-up list to the current project.
 - Replace existing ROI set(s) with the template* replaces the current ROIs on the current project with the saved template that is selected in the template pop-up list.
 - Copy ROI information only from the template* copies the names information from the selected template and applies it to the current set of ROIs
 - Export ROI Data* checkbox - exports the ROI data exported to a pre-named tab delimited text file once the selected ROI template has been applied to a file.
 - Auto open in Excel*—formats the data as an Excel file. This check box is only activated if Export ROI Data is checked.
 - Auto Generate Export Data File Name* checkbox —if checked, MI will use a date time stamp to auto generate a file name where ROI export data will be saved. This check box is only activated if Export ROI Data is checked.

- 4** Click the appropriate button to apply the template.
 - ✓ *Apply to Current Document* button—applies selected ROI Template to currently active document in MI.
 - ✓ *Apply to All Open Documents*—applies selected ROI template to all currently active documents in MI. In order to apply a template, the documents must be saved.
 - ✓ *Select Document(s)* button—launches a default file selection dialog that allows you to select MI Project, *.bip, document(s) from a directory/folder of previously saved documents.
 - ✓ *Apply to Selected Document(s)* button— applies selected the ROI Template to documents you have selected. This option is only available after you have selected at least one MI Project using the Select Documents button.
 - ✓ *Save to Template* button—saves ROI set(s) of a currently opened document to a ROI template file. This option is disabled if there are currently no documents opened in MI.
- 5** Verify that the template was applied properly.

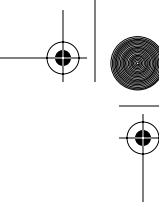
 NOTE: Click Center ROIs from the Manual ROIs panel to automatically center the ROI based on the centroid (center of mass or the 2nd moment of the intensity distribution).

Deleting a Saved ROI Template

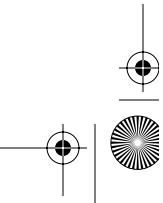
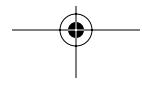
- 1** Select Templates from the Manual ROIs panel. The Templates dialog box appears
- 2** Choose the ROI Template Set that you wish to delete using the Template pop-up menu.
- 3** Click Delete. A confirmation text box appears. Click Yes to delete the selected ROI Template Set.



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9*Annotations*

Prepare images for presentation or publication from within Carestream Molecular Imaging Software. The Annotation window is a separate mode, where you can annotate, resize an image, create custom views, and label your data. Any annotations or modifications you make to the image is restricted to this mode and will not affect your analysis results. You may open the Annotation window any time during the process of analyzing an image.

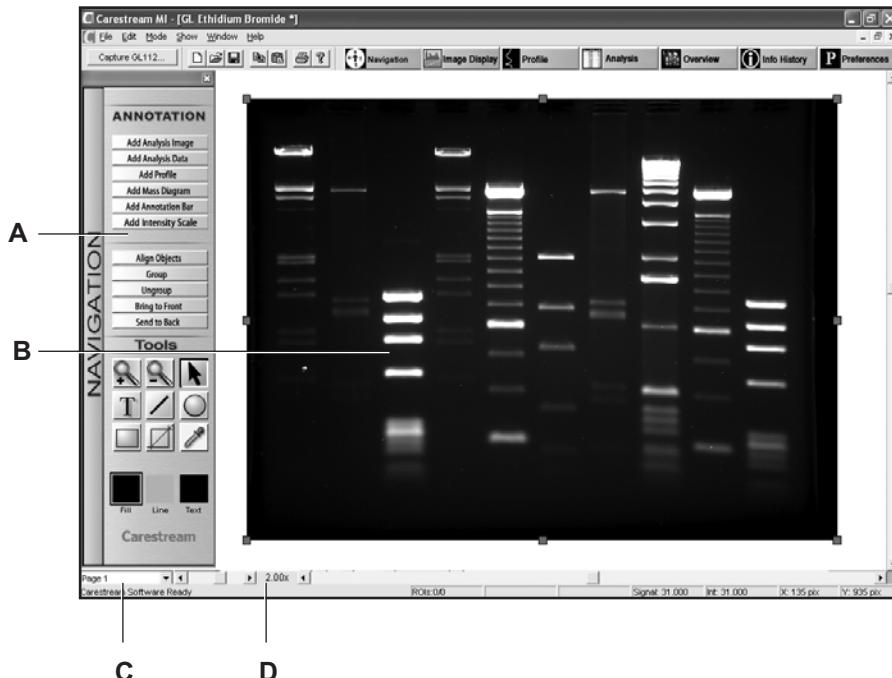
You can create up to ten pages of annotations. The size of the page is defined by your printer selection. You can use the area outside the printable area to store notes or make comments on your experiment.

The tools available in the Annotation panel are complete with a Text tool, Line tool, and a Shape tool. In addition, you can assign any color to your drawn annotations or text using the Color Selector. Annotation pages can also be exported for publication.

You can drag and drop analysis data onto the annotation pages as either individual values or as columns of data. Once on the Annotation page, the font and size can be changed and the text can be edited. If you are doing lane analysis, you can also place individual lane profiles and mass standard curves data onto an Annotation page.

Opening the Annotation Window

- 1 Choose Annotation from the Navigation panel. The Annotation window appears.



- A *Annotation panel* provides you with the features and tools preparing your images and data for presentation and publication.
- B The *Annotation window* displays a printable area that corresponds to the default printer and Page Setup selections.
- C *Page pop-up menu*—create up to 10 pages of annotations per project allowing you to store detailed information about your experiment in your project.
- D The *Magnification slider* provides digital magnification from 0.25X to 8X. The Magnification slider maintains the center of the image. This differs from the Zoom tool which shifts the center of the image to wherever the tool is clicked.

The Annotation Panel

The Annotation window provides you with a copy of the image for adding comments, labeling, or preparing the image for publication.

The Annotation Panel Buttons



- ✓ **Add Analysis Image**—adds an image to the Annotation window. When selecting Add Analysis Image, the Annotation Image Layout window opens. You can customize the size, zoom in on features of interest, and choose the portion of the image you want to display.
- ☞ NOTE: You can cut and paste images from other projects or sources, i.e., Powerpoint, Photoshop, JPG or TIFF.
- ✓ **Add Analysis Data**—opens the Analysis window and allows you to copy the data from the Analysis worksheet as individual values or as columns of data.
- ✓ **Add Profile**—appends a lane profile to the image. The lane profile is only available if the image has been analyzed for lanes and bands. The profile that is appended to the image is the lane(s) that is displayed in the Profile window prior to entering the Annotation mode.
- ✓ **Add Mass Diagram**—displays the mass standard curve in your annotations, if you analyzed your image and assigned mass standards.
- ✓ **Add Annotation Bar**—When acquiring images using Carestream Imaging Systems, you have the option to append an Annotation bar to your image. Once you have made the selection to append an Annotation bar, the information can be edited in the Annotation window.
- ✓ **Add Intensity Scale**—provides a visual index of intensities. The color index is defined in the Advanced Image Display window.
- ✓ **Align Objects**—lines up selected objects precisely along a horizontal or vertical axis, as well as distributes them evenly across a horizontal or vertical axis. If you are aligning to the right, all selected objects align to the right edge of the selected object furthest to the right.



- ✓ *Group*—takes individual objects and fuses them into a single unit that can be moved as one object.
- ✓ *Ungroup*—separates “fused” objects that had been grouped. Each individual object can be edited independently.
- ✓ *Bring to Front*—you can change the stacking order of objects that are overlapped. The Bring to Front button rearranges the stacking order, placing the selected object on top of the stack.
- ✓ *Send to Back*—you can change the stacking order of objects that are overlapped. The Send to Back button rearranges the stacking order, placing the selected object behind all other objects in the stack.

The Annotation Panel Tools

Tools in the Annotation panel are specific to this mode and are designed to help you annotate your image.



Zoom Tools

Use the Zoom tools to adjust the size of the image through levels of magnification (0.25X, 0.33X, 0.5X, 0.75X, 1X, 1.25X, 1.5X, 1.75X, 2X, 2.5X, 3X, 4X, 6X, and 8X). To increase the magnification, click the image with the + Zoom tool. To reduce magnification, click the image with the - Zoom tool.

When you change the magnification with the Zoom tools, the software positions the point where you clicked in the center of the Image section. This differs from the Magnification slider, which maintains the center of image in the window.



Pointer Tool

Use the arrow-shaped Pointer tool to select objects to move or resize. Shift-click on multiple objects with the Pointer tool or drag the mouse over an area of the image containing more than one object.



Text Tool

Use the Text tool to annotate with image using text. You can select custom fonts, sizes, styles, and rotation of the text. To use, select the Text tool, define the area in which you want the label to appear by clicking and dragging on the image.



9**Annotations**
 **Line Tool**

The Line tool is designed to draw lines, arrows, or brackets on the image.

 **Ellipse Tool**

The Ellipse tool is designed to draw circles or ovals which can either be filled, framed, or filled, and framed. Double-click on the Ellipse tool to access drawing options.

 **Rectangle Tool**

The Rectangle tool is designed to draw boxes which can either be filled, framed, or filled and framed. Double-click on the Rectangle tool to access drawing options.

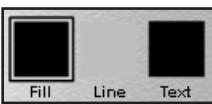
 **Crop Tool**

The Crop tool masks part of the object using a rectangle to trim the edges of the image so they are not displayed.

 **Dropper Tool**

Use the Dropper tool to select a new foreground or background color by clicking the Dropper tool on the object.

 NOTE: The currently selected color (as shown by the frame) corresponds to whether or not the Dropper tool selects a foreground or background color.


Fill, Line, and Text Color Options

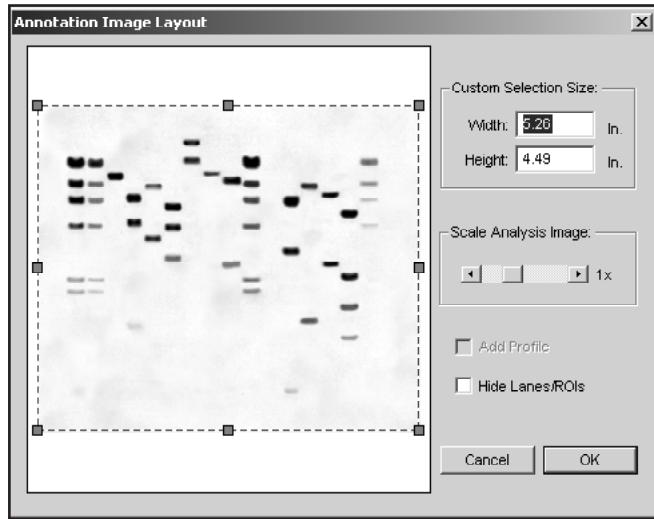
Use the Fill, Line or Text Color option buttons to define color. Click the Color Selector to adjust the color. For color reproducibility, use the More Choices button to access the color wheel.

Using Images in Annotations

Adding an Analysis Image

If you are preparing a presentation, you can add multiple copies of your experimental image to highlight important features. This enables you to show specific bands at different magnifications or eliminate lanes that are not of interest.

- 1 Choose Add Analysis Image from the Annotation panel. The Annotation Image Layout window appears.



- 2 Use the Custom Selection Size text edit boxes to define the size (in inches) of the image you want the image to appear on the Annotation page.
- 3 Adjust the Scale Analysis Image slider to magnify or reduce the image size between 0.25X and 8X. The image will not resize in the Annotation Image Layout window, however, the portion of the image that will be displayed is bounded in a red Selection Rectangle. The magnification factor appears to the right of the Scale Analysis Size slider.
- 4 Click and drag the Selection Rectangle to the region of the image you want to display. The region inside the selection is displayed in the main Annotation window.
- 5 To show the Lane/ROI or Profile, select the Hide Lanes/ROI or Profile checkboxes.

NOTE: You can use the View Options on the Lanes and ROI panels to show/hide the Lane Markers, Lane Lines, Band Labels, ROI boundaries, or ROI IDs.

9*Annotations*

 NOTE: You can also manually adjust the image view, in the Annotation window, by pressing down the Shift key (Options key for Macintosh) while clicking and dragging.

- 6** Click OK (Set for Macintosh).

 NOTE: You can add multiple copies of the image to highlight important features. This enables you to show specific features at different magnifications or eliminate parts of the image that are not of interest.

Cropping the Image Using the Grab Handles

-  **1** Using the Crop tool, click once on the image to select the object. Nine grab handles appear.
- 2** When you drag the cursor, over a grab handle, the cursor changes into one of three different icons.

-  Change size vertically.
-  Change size horizontally.
-  Change both vertical and horizontal size of the image.

 NOTE: This operation is not a true cropping of the image. It merely moves a mask over the image so as to display only the region of interest defined by the selection window. The underlying image remains intact and can be viewed, whole or in part, by readjusting the grab handles or by moving the entire selection window.

- 3** Click OK when finished.

Repositioning the Image

You can move the image anywhere within the Annotation window. If you drag the cursor over the image, the cursor changes to an open hand.

- ✓ To change the image placement on the page, click and drag. As you move the cursor, the image can be repositioned.
- ✓ Click and drag to move the entire image. Click and drag with the Shift key (Options key in Macintosh) depressed to reposition the image within the region outlined by grab handles.

Importing Image Objects into Annotations

If you are preparing a presentation, you can import objects via the clipboard using the copy and paste functions. The imported object is a separate item within the Annotation window.

Images of any color type can be imported—full color, 256 index color, grayscale, and black and white.

 NOTE: If you want to copy images between projects, make a selection, copy from within the Image window, and paste into the Annotation window. The image is pasted as an object.

Editing Graphic Objects

These objects can be edited in Annotations.

- 1 Double-click the object. The Annotation Image Layout window appears.
- 2 Use the Custom Selection Size text edit boxes to define the width and height of an image in the Annotation window.
- 3 Adjust the Scale Analysis Image slider to magnify or reduce the image size between 0.25X and 8X. The image will not resize in the Annotation Image Layout window, however, the portion of the image that will be displayed is bounded in a red Selection Rectangle. The magnification factor appears to the right of the Scale Analysis Size slider.
- 4 Select the region of interest. If you have selected an image size which is too large, you may not be able to view the entire image in the Annotation window. Reposition the selection region by clicking and dragging the red selection rectangle in the window. The region inside the selection is displayed in the main Annotation window.

 NOTE: Adjust the image view in the Annotation window, by pressing down the Shift key (Option key for Macintosh) while clicking and dragging.

Adding Analysis Data

Lanes, bands, and ROI data can be labeled by dragging and dropping text from an Analysis Data window.

- 1** Open Analysis data set by selecting Analysis from the Quick Access bar.
- 2** Display all columns and values needed for annotation.
- 3** Choose Annotation from the Navigation panel.
- 4**  Select a cell using the Pointer tool. Click and press until a light blue line or gray background appears around the selected cell.
 NOTE: Any cell from the Analysis Data windows can be moved to the Annotation window. When you drop a text object, the text object maintains the attributes of the previous text object, i.e., font, size, style, and rotation.
- 5** Adjust the label position using the Pointer tool. Click and drag to reposition the label.
- 6** Adjust label size with the Pointer tool. Double-click on a label to adjust the format using the Text Options dialog box.

Dragging and Dropping a Column from an Analysis Data Window

A column of data can be dragged and dropped from an Analysis window.

- 1** Open Analysis data set by selecting Analysis from the Quick Access bar.
- 2** Display all columns and values needed for annotation.
- 3** Choose Annotation from the Navigation panel.
- 4** Move an entire column by clicking and dragging the column label to the Annotation window.
 -  The Lane Number Label moves all data points (Lane Analysis only).
 -  The column heading moves a column of data without a table. NOTE: The graphic retains its shading and gridlines. You cannot edit these text fonts.
- 5**  Use the Pointer tool to adjust the label position. Click and drag to reposition the label.

6 To adjust label size, select any of the grab handles to resize the text block or double-click to access the Annotation Image Layout window.

7 Use the Custom Selection Size text edit boxes to define the width and height of an image.

8 Adjust the Scale Analysis Image slider to magnify or reduce the image size between 0.25X and 8X.

 **NOTE:** The image will not resize in the Annotation Image Layout window, however, the portion of the image that is displayed is bounded in a red Selection Rectangle. The magnification factor appears to the right of the Scale Analysis Size slider.

9 Select the region of interest. If you have selected an image size which is too large, you may not be able to view the entire image in the Annotation window. Reposition the selection region by clicking and dragging the red selection rectangle in the window. The region inside the selection is displayed in the main Annotation window.

 **NOTE:** Manually adjust the image view, in the Annotation window, by pressing down the Shift key (Option key for Macintosh) while clicking and dragging.

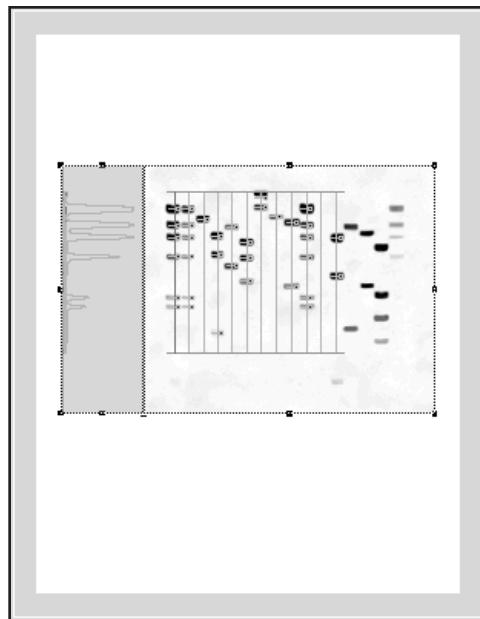
Adding Lane Profiles

You can append a lane profile to an image or add multiple lane profiles to the Annotation window.

When you display an image in the Annotation window, you can also choose to append a profile to the image. The lane profile is only available if the image has been analyzed for lanes and bands. The profile that was appended to the image is the lane(s) that is displayed in the Profile window prior to entering the Annotation mode.

Appending a Lane Profile to an Image

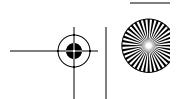
- 1 In the Lanes panel, choose the lane profile you want to append in the Profile window.



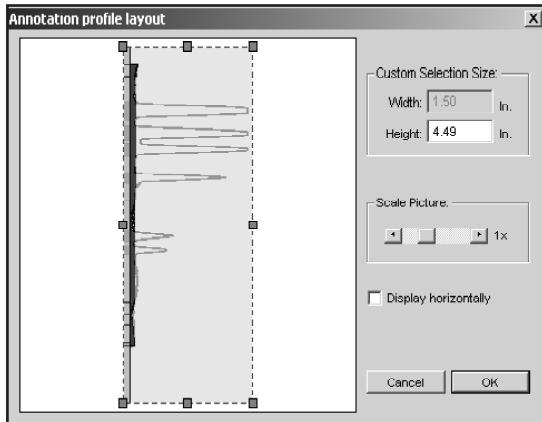
- 2 Click Annotation on the Navigation panel or choose Annotation from the Mode menu.

- 3 Select Add Profile from the Annotation panel. The profile appears next to the image.

 NOTE: Notice that the profile is scaled to fit your image. The profile is a separate object and can be moved, reoriented, and resized using the grab handles or by double-clicking the profile to view the Profile Layout window.



- 4** Double-click on the profile with the Pointer tool. The Profile Layout window appears.



- 5** Select the height, scale the profile, or change the orientation of the profile to horizontal (90° CCW).

 NOTE: The Scale Profile options are 0.25X, 0.33X, 0.5X, 0.75X, 1X, 1.25X, 1.5X, 1.75X, 2X, 2.5X, 3X, 4X, 6X, and 8X.

- 6** Click OK.

Adding Multiple Profiles

You can add multiple profiles to compare lanes as follows:

- 1** In the Lanes panel, choose the lane profile you want to append in the Profile window.

- 2** Choose Annotation from the Navigation panel.



- 3** Double-click the image with the Pointer tool. The Annotation Image Layout window appears.

- 4** Click the Add Profile checkbox and click OK or choose Add Profile from the Annotation panel. The profile is placed next to the image.

- 5** Double-click on the profile with the Pointer tool. The Profile Layout window appears.

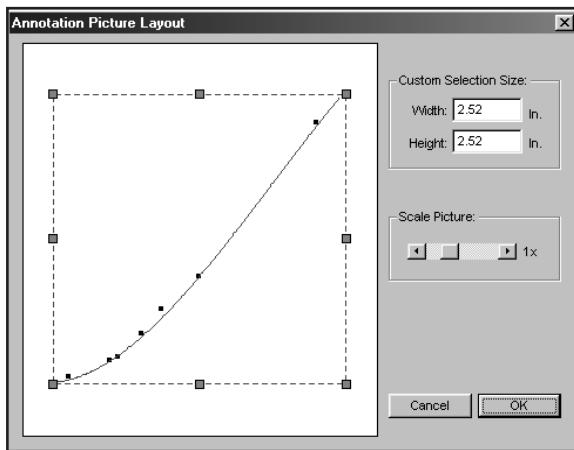
- 6** Select the height, scale the profile, or change the orientation of the profile to horizontal (90° CCW).

- 7** Click OK. Repeat the process to add additional profiles.

Adding the Mass Curve

If you performed Mass analysis and assigned standards for mass, you can display the mass standard curve in your annotations.

- 1 Choose Add Mass Diagram from the Annotation panel. The Add Mass Diagram dialog box appears.
- 2 Choose the Analysis Type and Curve Type.
- 3 Click OK. The graphic is placed on the Annotation page.
- 4 With the Pointer tool, double-click the object. The Annotation Image Layout window appears.



- 5 Use the Custom Selection Size text edit boxes to define the width and height of the mass curve.
- 6 Adjust the Scale Analysis Image slider to magnify or reduce the image size between 0.25X and 8X.

 NOTE: The image will not resize in the Annotation Image Layout window, however, the portion of the image that is displayed is bounded in a red Selection Rectangle. The magnification factor appears to the right of the Scale Analysis Size slider.

- 7 Select the region of interest. If you have selected an image size which is too large, you may not be able to view the entire image in the Annotation window. Reposition the selection region by clicking and dragging the red selection rectangle in the window. The region inside the selection is displayed in the Annotation window.

Adding the Annotation Bar

When acquiring images using Carestream Imaging Systems, you have the option to append an Annotation bar to your image. Once you have made the selection to append an Annotation bar, the information can be edited in the Annotation window.

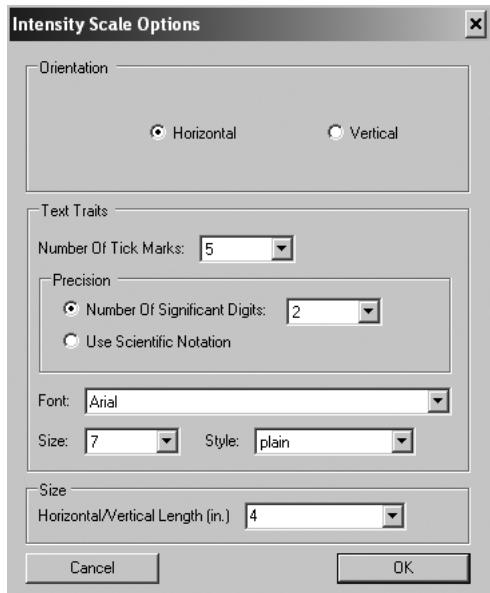
- 1 Choose Add Annotation bar from the Annotation panel. The Annotation bar is placed on the Annotation page. The Annotation bar information is grouped. To edit, use the Pointer tool to select the Annotation bar and then click Ungroup from the Annotation panel.

 NOTE: The Annotation bar text can be edited and reformatted as any annotation described in this chapter.

Adding the Intensity Scale

You can add an intensity scale on your images. The intensity scale is dictated by the selections you have made in the Image Display window.

- 1 Choose Add Intensity Scale from the Annotation panel. The intensity Scale Options opens.



2 Select options

- ✓ Orientation—rotational or vertical.
- ✓ Text Traits—including number of tick marks, significant digits, scientific notation and text attributes
- ✓ Size—horizontal and vertical length.

- 3 The intensity scale is placed on the Annotation page.

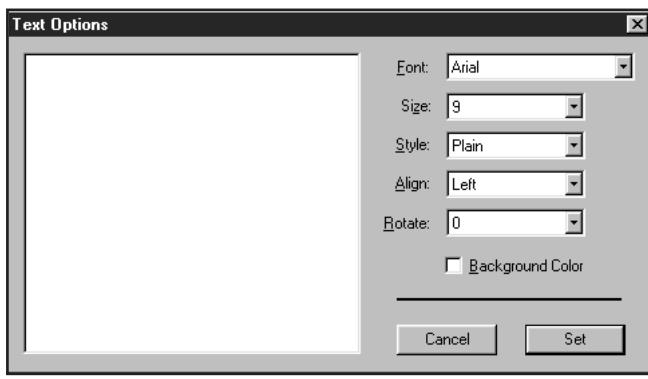
 NOTE: If you are overlaying images, you can choose to add one or both of the intensity scales used in the overlay.

Text Formatting Options

Add titles or other labels to the image using the Text tool.

T 1 Select the Text tool from the Annotation panel.

2 Using the Text tool, click and drag on the image where the label is desired. The Text Options dialog box appears.



3 Type text and then set the format with the pop-up menus.

- ✓ *Font*—use any available font on your computer.
- ✓ *Size*—use default sizes or use the other option to define size.
- ✓ *Style*—choose from Plain, Bold, and Italics.
- ✓ *Align*—align text to the right, center, or left.
- ✓ *Rotate*—orient text at 0°, 45°, and 90°.
- ✓ *Background color*—define a background color using the Color Selector.

4 Click Set. The text appears in the Annotation window.

5 Using the Pointer tool, click and drag to position the text box.

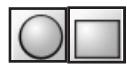
6 To adjust the format, double-click on the Text Tool or the Text. The Text Options dialog box appears.

Drawing Objects

Advanced presentations can be created by adding lines and shapes to the image. Using Carestream MI it is simple to do.

Drawing Shapes

The Shape tool can draw either rectangles or ovals and display them as filled or framed.



- 1 Choose the Ellipse tool or Rectangle from the Annotation panel. The cursor changes to a crosshair.



NOTE: To draw a perfect circle or square, press the Shift key down while drawing.

- 2 Click and drag on the image to create a shape. The shape appears with 9 grab handles.



- 3 Using the Pointer tool adjust the size and shape of objects using the grab handles. The cursor changes when you move it over a grab handle.



Change size vertically



Change size horizontally



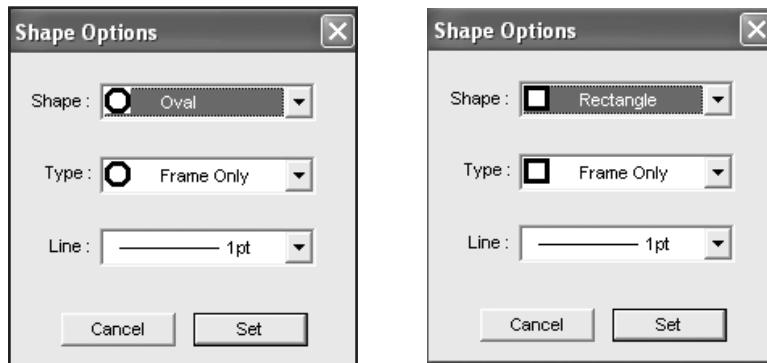
Change both vertical and horizontal size of the image

- 4 Click and drag with the Pointer tool to position the shape correctly.

9*Annotations****Ellipse and Rectangle Options Dialog Box***

The Ellipse and Rectangle tools can draw shapes that can either be filled, framed, or filled and framed.

- 1 Double-click on the tool or the drawn shape to change the format. The Options dialog box appears.



- ✓ The *Shape pop-up menu*—selects a shape to draw rectangles or circles.
- ✓ The *Type pop-up menu*—selects the fill and frame of the object.
- ✓ The *Line pop-up menu*—selects the thickness of the line.

- 2 Click Set when finished.

Drawing Lines

The Line tool draws lines, arrows, or brackets.

-  1 Select the Line tool from the Annotation panel. The cursor changes to a crosshair.

 NOTE: To easily draw a straight line, press the Shift key while drawing. This constrains lines to 0°, 45°, and 90° angles.

- 2 Click and drag on the image to create a line. The line appears on the image with two grab handles.

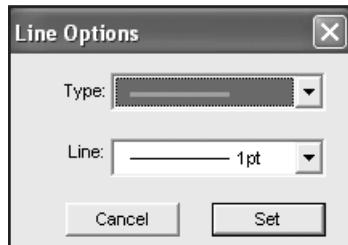
-  3 Adjust the size with the grab handles using the Pointer tool.

- 4 Click and drag the line with the Pointer tool to position it on the page.

Line Options Dialog Box

The Line tool is designed to draw lines, arrows, or brackets on the image.

- 1 Double-click on the Line tool or on a drawn line to change the format. The Line Options dialog box appears.



✓ *Type pop-up menu*—choose from lines, arrows, or brackets.

✓ *Line pop-up menu*—choose line weights from 0.5 pt to 12 pt.

- 2 Click Set when finished.

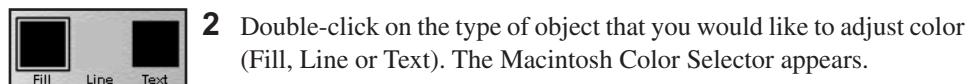
Using the Color Selector

The Color Selector sets the foreground and background color for text and objects.

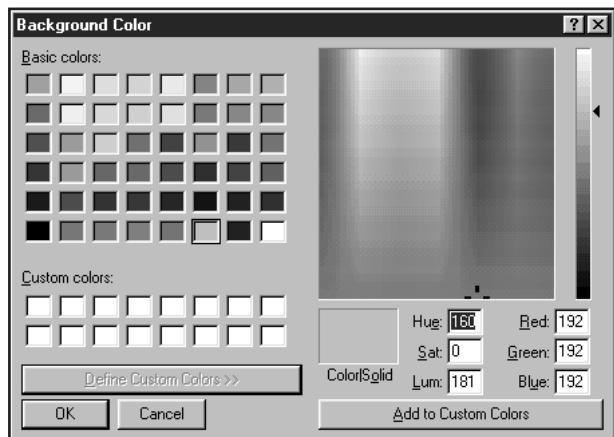
Using the Color Selector—Windows

The Color Selector sets the foreground and background color for text and objects. You can set the color for an object either before or after the object is created or placed.

-  1 If the text or object is already placed, click and drag to highlight the text or click to select the object using the Pointer tool.



- 3 Select a color from the color palette or click on the Color Selector to apply a custom color. Click on either the foreground or the background to select the current color setting with the Dropper tool.

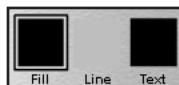


- 4 Click OK when done.

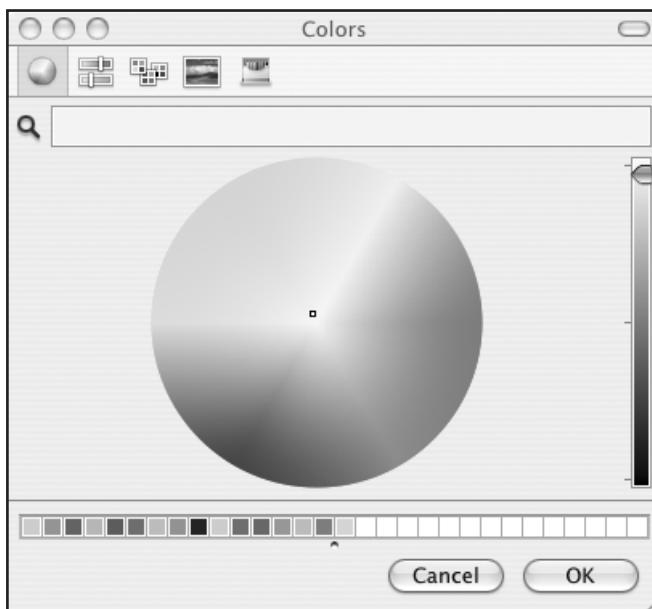
Using the Color Selector—Macintosh

The Color Selector sets the foreground and background color for text and objects. You can set the color for and object either before or after the object is created or placed.

 1 If the text or object is already placed, click and drag to highlight the text or click to select the object using the Pointer tool.

 2 Double-click on the type of object that you would like to adjust color (Fill, Line or Text). The Macintosh Color Selector appears.

3 Select a color from the color palate or click on the Color Selector to apply a custom color. Click on either the foreground or the background to select the current color setting with the Dropper tool.



4 Click OK when done.

Aligning Objects

Labels, lines, and shapes can be automatically aligned. For example, to center your labels.

-  1 Select the Pointer tool from the Annotation panel.
- 2 Shift-click for Macintosh to highlight two or more objects. The objects are outlined by grab handles.
- 3 Choose Align Object from the Annotation menu and select your alignment preference. The objects are realigned.

Grouping/UnGrouping Annotation Objects

When annotating an image, it is important to be able to group objects together. This allows you to move a series of objects together.

Grouping Objects

-  1 Select the Pointer tool from the Annotation panel.
- 2 Shift-click or click and drag to highlight one or more labels, lines, or shapes. The objects are outlined by grab handles.
- 3 Choose Group from the Annotation panel. The objects are grouped together—individual grab handles are replaced by one set of grab handles for all items in grouped object and can be moved together.

 NOTE: Objects that are grouped together cannot be edited. You must ungroup the objects to edit.

Ungrouping Objects

- 1 Use the Pointer tool from the Annotation panel to select the grouped object.
- 2 Choose Ungroup from the Annotation panel. The objects now ungroup. The individual objects are outlined by grab handles.

Layering Objects

When annotating an image, it is important to be able to layer objects. This process involves sending objects *Back* to another layer or bringing objects *Forward*. You can move any object in the Annotation window *Forward* and *Back* and place text in shapes.

- 1 Select the Pointer tool in the Annotation Toolbar (Tool Palette for Macintosh).
- 2 Shift-click on multiple objects or click and drag over multiple labels, lines, or shapes. The objects are outlined by grab handles.
- 3 Choose Send to Back from the Annotation menu. The highlighted object(s) is/are put behind the current object or image.
- 4 Choose Bring to Front from the Annotation menu. The highlighted object(s) is/are put in front of the current object or image.
- 5 For adding additional colors, click Define Custom Colors. Select the color from the palette and click Add to Custom Colors.
- 6 Choose a color and click OK.

Multiple Pages

You can create up to 10 pages of annotations per project allowing you to store detailed information about your experiment in your project.

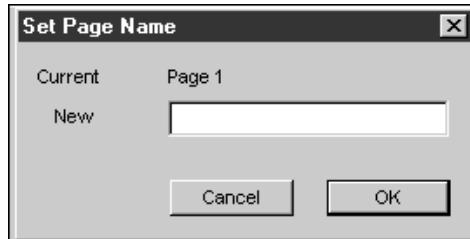
Selecting a Page



- 1** Use the Page pop-up menu to select a page.
- 2** The new page appears in the window.

Renaming a Page

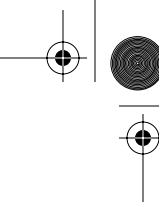
- 1** Choose Change Name from the Page pop-up menu. The Set Page Name dialog box appears.



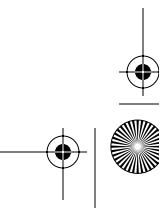
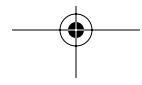
- 2** Type a new name for the page in the text edit box—up to 21 characters.
- 3** Click OK. The new name is updated in the Page pop-up menu.



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9-24



Managing Projects

In this chapter, you will learn the essentials of managing your images.

When you acquire an image or open a TIFF or JPEG file, a new project is automatically opened. Once saved, the project contains the original images as acquired, in addition to file information gathered during capture, analysis results, standards information, annotations, and preference settings. Carestream MI Software, like most programs, lets you to save the same image with different names and also revert to the last saved version. The software offers you the option of exporting the original image in a variety of formats.

The file information records the original capture settings and tracks any changes to the image that were destructive, i.e., cropping or rotating. In addition, custom fields are provided for you to enter any user information pertaining to the image. The image, file information, analysis data, and annotations can be printed and exported.

Carestream MI Software includes an image databasing feature that uses data stored in the File Information to employ an advanced search strategy to locate and open a specific stored project.

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Managing Projects

Saving Files

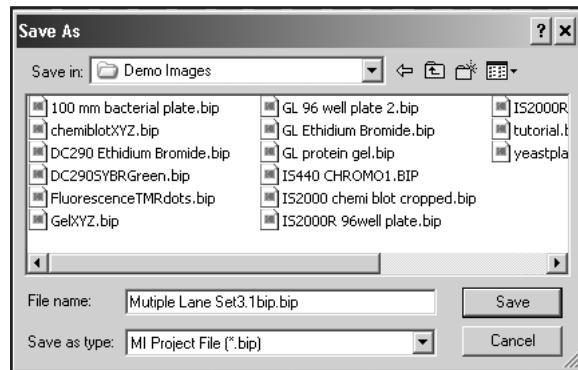
Let's review the save options:

- ✓ *Save as a MI Project File*—saves the image analysis results, standards information, annotations and file information containing historical capture information and user information as a project.
- ✓ *Save a Selection*—saves a selection of your image.
- ✓ *Revert to Saved*—allows you to revert to the last saved version of your project.

Saving a New Project

All information created in a project is saved when you issue the Save command. The first time you save the file, the File Information dialog box is opened for you to store archival information concerning the project. This information is indexed by the database and can be used to search and sort images

- 1 Choose Save from the File menu. The Save to Database dialog opens.
- 2 Select one of the following options—*Add to Image Database*, *Add Later*, or *Don't Add*. Refer to *Using the Database*, later in this chapter for more details.
- 3 The Save As dialog box appears.

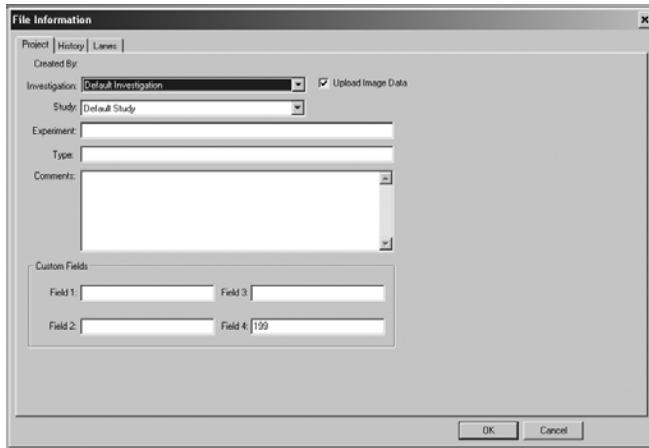


- 4 Select a destination drive, folder, and a filename for the project.
- 5 Click Save. The File Information dialog box appears.

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Managing Projects

- 6** Enter the project information in the various text edit boxes in the Projects tab.



 NOTE: If you database the image, the information can be used to retrieve or sort saved projects.

- 7** Click History tab to review the history of the image.

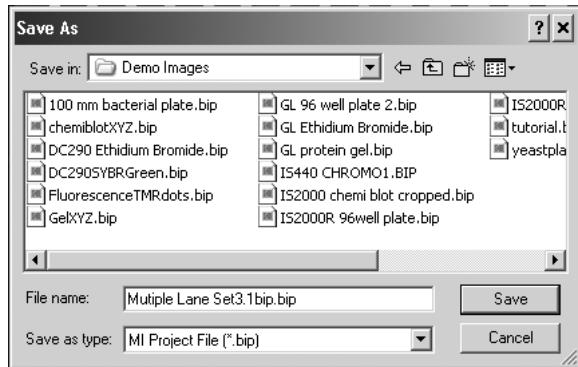
The history records any capture information from your imaging system. Once acquired, it tracks any changes that were destructive to the image, i.e., cropping, rotating. This dialog box cannot be edited.

- 8** Click Lanes tab to review lane information of lanes, or use the Edit button in this tab to designate or rename lanes.
- 9** Click OK. The file information is saved.

Using the Save As Command

You can use the Save As command whenever a project is open and you would like to save a new version of the file. The new version becomes the active project and the original file is stored as the previously saved version. The original project is not affected by any changes made to the new file.

- 1 Choose Save As from the File menu. The Save As dialog box appears.

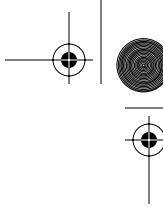


- 2 Select a destination drive, folder, and a new filename for the project.
- 3 Click Save. The project is saved with the new name and becomes the active project. All projects are saved as MI Project File (*.bip) which saves the image, file information, analysis results, standards information, annotations, and preference settings.

Saving a File

It's a good idea to periodically save your work during a session.

- 1 Choose Save from the File menu. The file is saved on disk.
 - ✓ If you haven't made any changes since the last Save, the Save option is dimmed.
 - ✓ If you choose Close from the File menu after making changes, a dialog box warns you to save your work.
 - ✓ MI Project File(*.bip)—saves the image, file information, analysis results, standards information, annotations, and preference settings



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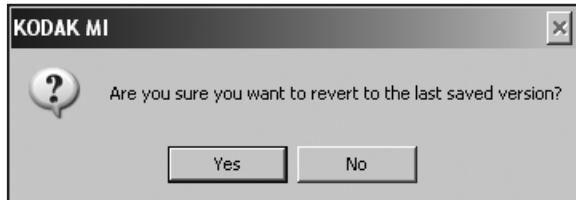
Managing Projects

Reverting to a Previous Version

It may be occasionally necessary to revert to a previously saved version of a file. Once you save your project, you can revert to the last saved version at any time. But keep in mind that you will lose the changes you've made since the last save.

NOTE: This option is only available if you have previously saved the image.

- 1 Choose Revert To Saved from the File menu. A dialog box asks for confirmation to continue.



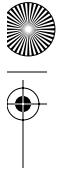
- 2 Click Yes (Revert for Macintosh). The current project reverts to its last saved version.

Closing an Existing File

When you close an existing file, the File Information dialog box does not appear.

- 1 Choose Close from the File menu. If you have made changes to the file, you are prompted with an alert that asks if you want to save the changes.
- 2 Click Save. The file is saved with the changes you made, or click Don't Save to revert to the version as previously saved or Cancel to exit the Save dialog box.

NOTE: If you have opened an existing file but have not made any changes to it, the file automatically closes and no confirmation dialog box appears.

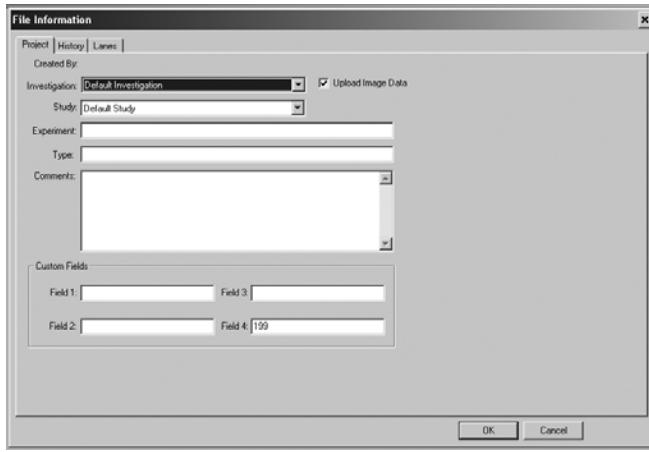




Editing and Viewing the File Information

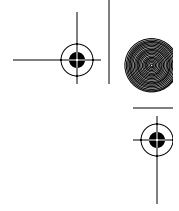
The File Information dialog box appears only when a project is saved for the first time. To access the File Information dialog box:

- 1 Click Info History from the Quick Access bar or choose File Info from the File menu. The File Information dialog box for the current project appears.



- 2 Enter your changes to the in the Projects or Lanes tabs.
- 3 Review the History tab. The History maintains details provided by the capture device, and any subsequent changes that were destructive to the image, i.e., cropping, rotating. This dialog box cannot be edited.
- 4 Click the Lanes tab. The Lanes tab appears. To label lanes, click Edit to access the Lane Information dialog box. Navigate through lanes using dialog arrows or select the lane you want to label using the pop-up menu. Click OK, if you've edited any text fields, to return to the File Information dialog box. Click Cancel, if you did not make any changes.
- 5 Click OK.

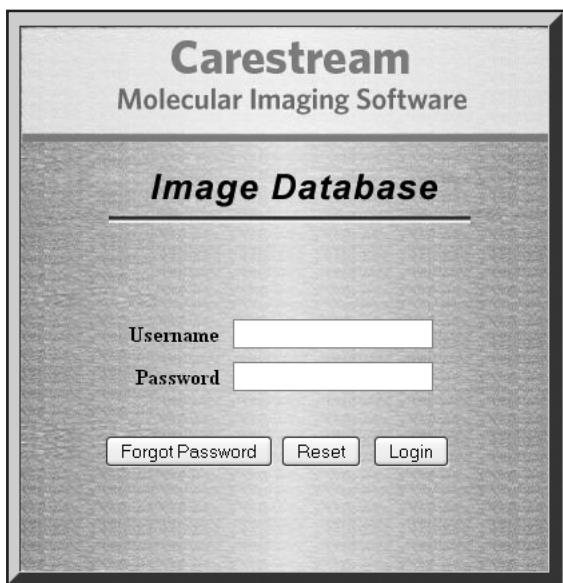




Using the Image Database

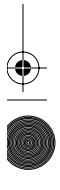
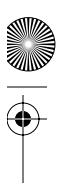
Carestream MI provides a databasing feature where you can archive important retrieval information to manage your projects. The database is made up of individual records and pointers to the source file. Information that is populated into the database record is parsed from the KODAK MI project File Information. Once populated, you can sort and manage projects, and also do advanced data comparisons with images containing Lane Analysis Data.

- 1 Click on the Database button on the Navigation panel. The Carestream MI Security Login window may appear, the first time you log into the Carestream MI Security Manager, enter Admin as the Username and enter password as the Password. Click the Login button.

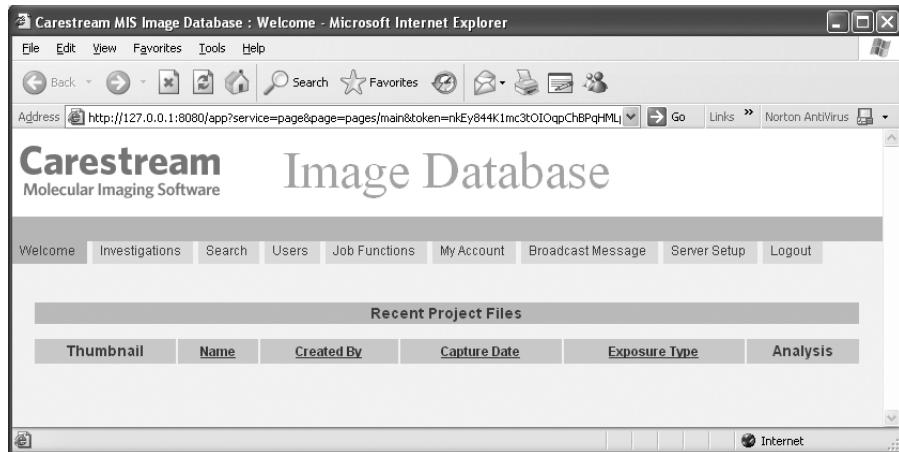


10

Managing Projects



2 The Welcome window appears.



 NOTE: Access to the various functions within the Security Manager is assigned by Job Function. The Admin login or an Administrator Job Function can access and make changes in all tabs.

- 3** Click on the My Account tab. The My Account window appears.

The screenshot shows the 'User Information' section with the following fields:

- First Name:** Admin **
- Last Name:** Administrator **
- Username:** Admin **
- Password:** [redacted] **
- Confirm Password:** [redacted] **
- Email:** yourname@yourcompany.com **
- Office Location:** none
- Lab Location:** none
- Office Phone:** [redacted]
- Lab Phone:** [redacted]

The 'Account Information' section includes:

- Active:** Yes
- Last Modification Date:** [redacted]
- System Administrator:**
- Job Function:** Administrator
- Attempt Count:** 0
- Notes:** no notes at this time

- 4** Enter your first and last name in the First Name and Last Name text edit boxes, respectively.
- 5** Enter a new password in the Password text edit box and re-enter your new password in the Confirm Password text edit box.
- Note:** We strongly recommend that you change your password immediately to ensure security and integrity of the database.
- 6** Enter your E-mail address in the E-mail text edit box.
- 7** Optional: Enter your office and laboratory locations in the Office Location and Lab Location text edit boxes, respectively.
- 8** Optional: Enter your office and lab phone numbers in the Office Phone and Lab Phone text edit boxes, respectively.
- 9** Click Update.

Managing Job Functions

Job Functions, each associated with specific permission levels should be designated. Once these Job Functions are set up, Users can be associated with each Job Function.

Adding Job Functions

We provide default job descriptions to get you started. You can use these defaults or delete them to create your own. You cannot delete the Administrator Job Function. Once a Job Function is assigned to a User, that Job Function can no longer be deleted.

To add a new Job Function:

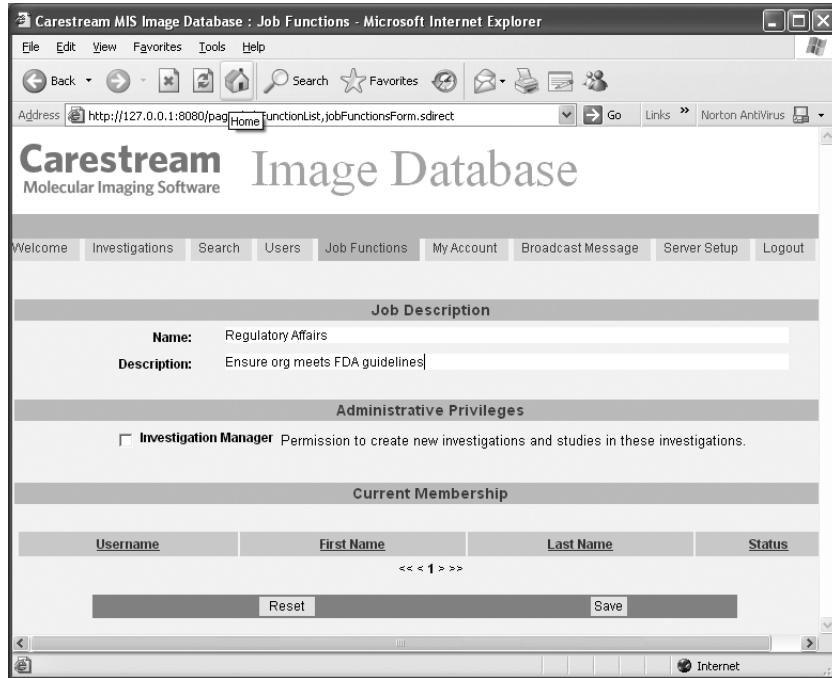
- 1 Click on the Job Functions tab. The Job Functions tab appears.

Delete	Name	Description
<input type="checkbox"/>	Administrator	Administers the Image Database
<input type="checkbox"/>	Principal Investigator	Manages investigations
<input type="checkbox"/>	Research Scientist	Performs experiments to generate project files

<< < 1 > >>

Add Job Function

- 2** Click the Add Job Function button. The Add Job Function window appears.



- 3** Enter a new Job Function in the Name text edit box.
- 4** Provide a description of the Job Function in the Description text edit box.
- 5** Select the administrative privileges that are associated with the new Job Function (if applicable).
 - Approver* has the authority to approve or reject projects.
 - Investigation Manager* has the authority to create new investigations.
- 6** Click Save.

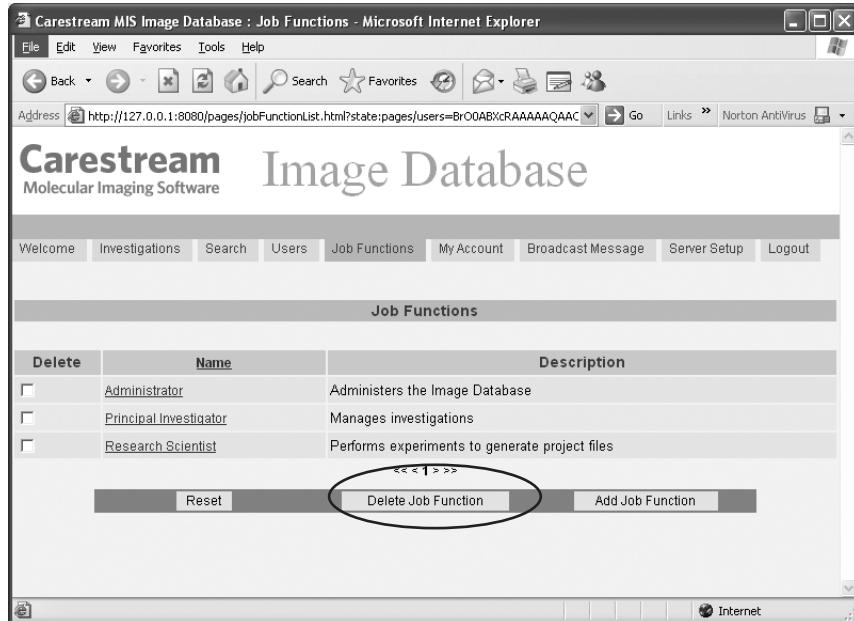
NOTE: The Reset button clears the fields and reverts to the defaults.

Deleting Job Functions

We provide default Job Functions to get you started, including an Administrator Job Function. Once a Job Function is assigned to a User, that Job Function can no longer be deleted.

To delete a job function:

- 1 Click on the Job Functions tab. The Job Functions window appears.



- 2 Select the Job Function that you want to delete.

NOTE: You can not delete the Administrator Job function.

NOTE: You cannot delete a Job Function if any User has ever been assigned that Job Function.

- 3 Click the Delete Job Function button. The Job Function is deleted.

10

Managing Projects

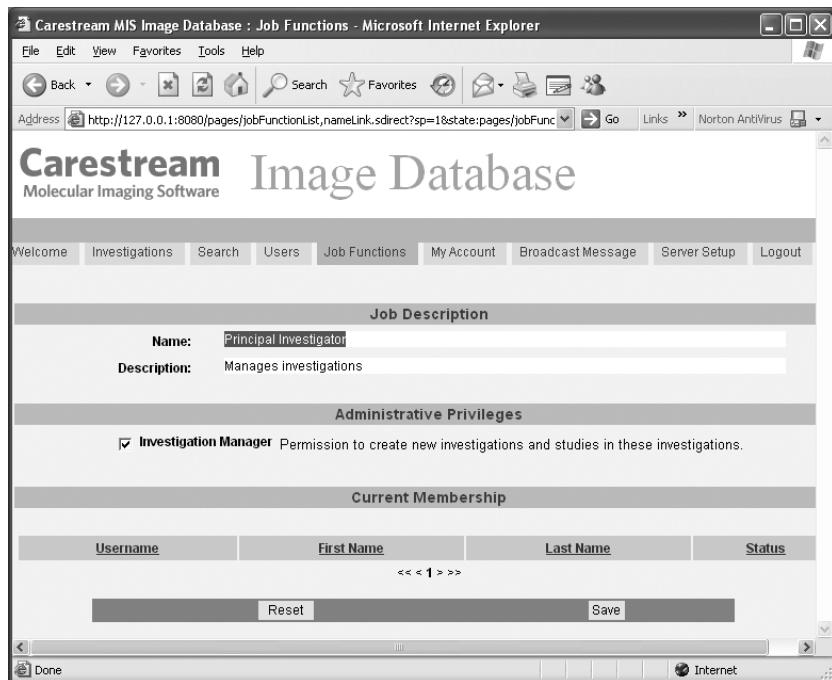
Reviewing Users Assigned to a Job Function

Once a User is assigned to a Job Function, you can review all Users associated with that Job Function.

- 1 Click on the Job Functions tab. The Job Functions window appears.
- 2 Click the Job Function you want to review.

Delete	Name	Description
<input type="checkbox"/>	Administrator	Administers the Image Database
<input type="checkbox"/>	Principal Investigator	Manages investigations
<input type="checkbox"/>	Research Scientist	Performs experiments to generate project files

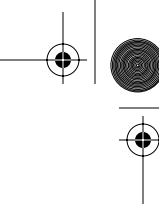
3 The Job Functions Description window appears.



- ✓ You can update the Name/Description.
- ✓ You can change Administrative Privileges for this job function.
- ✓ You can access and update a specific Users Information by double-clicking on their name.

4 To exit, click Save.

 NOTE: The Reset button clears the fields and reverts to the defaults.



Managing Users

Once Job Functions are designated, you can set up Users that are associated with each Job Function.

Adding Users

We provide default Job Functions, including the Administrator to help you get started. Once a User is assigned a Job Function, that Job Function can no longer be deleted. Once a User is added, that User cannot be deleted. However, the User can be deactivated.

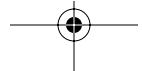
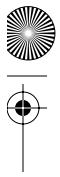
To add a new user:

- 1 Click on the Users tab. The User Administration window appears.
- 2 Click Add User.

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Managing Projects

- 3 The New User Information window appears.



- 4 Enter your first and last name in the First Name and Last Name text edit boxes, respectively.

The screenshot shows a Microsoft Internet Explorer window titled "Carestream MIS Image Database : User Information - Microsoft Internet Explorer". The address bar shows the URL <http://127.0.0.1:8080/pages/users/form.sdirect>. The main content area is titled "User Information". It contains a note: "Fields marked with ** are required." and "Password must be between 5 to 12 characters." Below this, there are several input fields:

User Information	
First Name:	Hannah **
Last Name:	White **
Username:	Hwhite **
Password:	***** **
Confirm Password:	***** **
Email:	*
Office Location:	
Lab Location:	
Office Phone:	
Lab Phone:	

Below the "User Information" section is another section titled "Account Information". At the bottom left of the form is a "Done" button, and at the bottom right is an "Internet" icon.

NOTE: You cannot delete the Admin User.

NOTE: All fields marked with ** are required.

- 5 Enter a password in the Password text edit box and re-enter the new password in the Confirm Password text edit box.

NOTE: Passwords need to be between 5-12 alphanumeric characters long.

NOTE: You will need to notify the new Users of their temporary passwords that you have assigned. On their first login, they will be prompted to reset their password. When resetting passwords, a new password must be entered.

- 6 Enter the User's E-mail address in the E-mail text edit box.
- 7 Optional: Enter your office and laboratory locations in the Office Location and Lab Location text edit boxes, respectively.

- 8 Optional: Enter your office and lab phone numbers in the Office Phone and Lab Phone text edit boxes, respectively.
- 9 Select the User's Job Function using the Job Functions pop-up window.
- 10 Enter any notes you want to add pertaining to this user in the Notes text edit box.
- 11 Click the System Administrator checkbox if these privileges will be assigned to the User.
- 12 Click Create.

Unlocking a User

If a User's account becomes locked because of greater than three consecutive failed logins, an administrator must unlock the account. To unlock an account:

- 1 Click the Users tab. The User Administration window appears.
- 2 Select the User(s) that you want to unlock by clicking the checkbox(es). Click Unlock.

Select	Username	First Name	Last Name	Email	Job Function	Status
<input type="checkbox"/>	Admin	Admin	Administrator	youname@yourcompany.com	Administrator	Active
<input checked="" type="checkbox"/>	Gwood	Graham	Wood	gwood@csh.com	Research Scientist	Locked
<input type="checkbox"/>	Hwhite	Hannah	White	hannah.white@csh.com	Research Scientist	Active

NOTE: The Status column should indicate any Users that are locked out.

- 3 The Users' account(s) are unlocked.

Deactivating a User

Once a User account is created, that User cannot be deleted. However, it can be deactivated by the Administrator. Once deactivated, a User can reactivate in the event that they return to the study.

- 1 Click on the Users tab. The User Administration window appears.
- 2 Select the user(s) that you want to deactivate by clicking the checkbox(es). Click the Deactivate button.

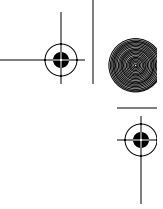
Select	Username	First Name	Last Name	Email	Job Function	Status
<input type="checkbox"/>	Admin	Admin	Administrator	yourusername@yourcompany.com	Administrator	Active
<input type="checkbox"/>	Gwood	Graham	Wood	gwood@csh.com	Research Scientist	Active
<input checked="" type="checkbox"/>	Hwhite	Hannah	White	hannah.white@csh.com	Research Scientist	Active

<< < 1 >>

[Reset](#) [Add User](#) [Activate](#) [Deactivate](#) [Unlock](#)

NOTE: The Admin account cannot be deactivated.

- 3 The user(s) are deactivated.



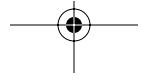
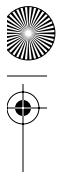
Activating a User

- 1 Click on the Users tab. The User Administration window appears.
- 2 Select the user(s) that you want to activate by clicking the checkbox(es). Click the Activate button.

The screenshot shows the 'User Administration' page of the Carestream MIS Image Database. At the top, there's a message: '1 user(s) deactivated.' Below that is a table with columns: Select, Username, First Name, Last Name, Email, Job Function, and Status. Three users are listed: Admin (Active), Gwood (Active), and Hwhite (Deactivated). At the bottom of the table is a navigation bar with buttons: Reset, Add User, Activate (circled in red), Deactivate, and Unlock.

Select	Username	First Name	Last Name	Email	Job Function	Status
<input type="checkbox"/>	Admin	Admin	Administrator	youname@yourcompany.com	Administrator	Active
<input type="checkbox"/>	Gwood	Graham	Wood	gwood@csh.com	Research Scientist	Active
<input checked="" type="checkbox"/>	Hwhite	Hannah	White	hannah.white@csh.com	Research Scientist	Deactivated

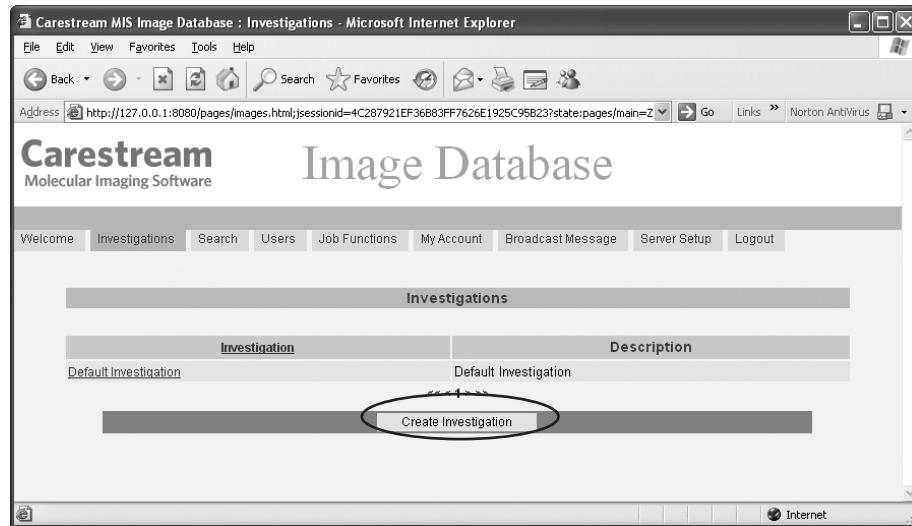
- 3 The user(s) are activated.



Creating an Investigation

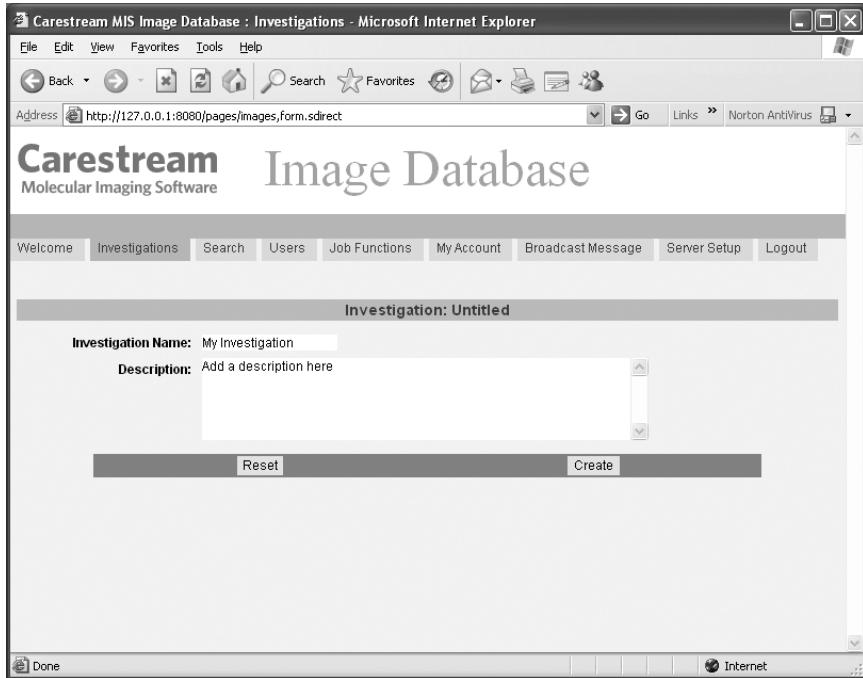
Carestream MI organizes your Carestream MI projects into Investigations and Studies. Each Investigation may contain multiple Studies. Each Study has a assigned group of Users.

- 1 Click the Investigations tab. The Investigations window appears.



- 2 Click the Create Investigation button. An Untitled Investigation window appears.

- 3 Enter a name in the Investigation Name text edit box.
- 4 Enter a description in the Description text edit box.



- 5 Click Create to save. Click Reset to clear the fields.

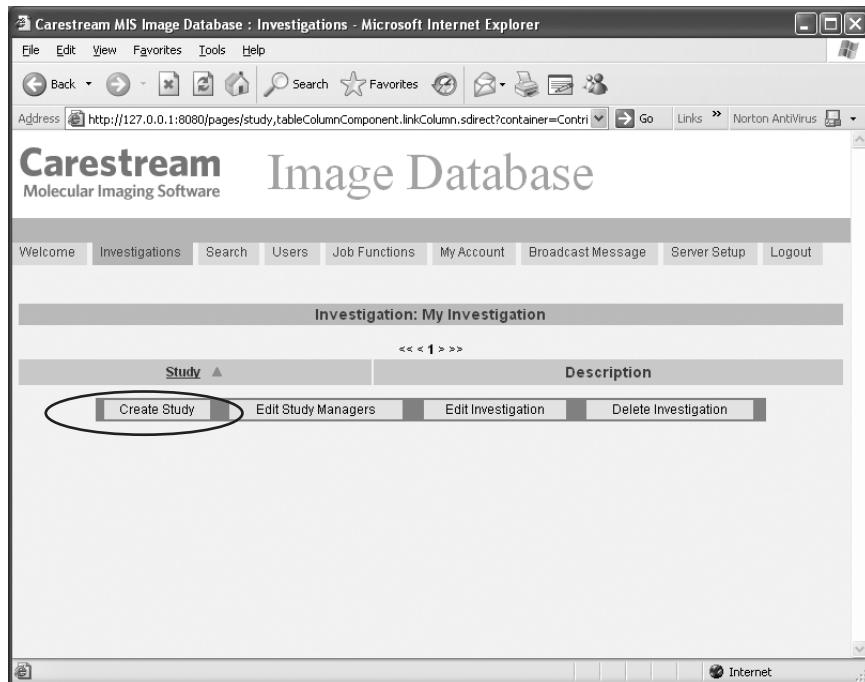
 NOTE: The Reset button clears all the fields and reverts to the defaults.

Setting Up Studies Under Investigations

Once an Investigation has been set up, you can set up Studies.

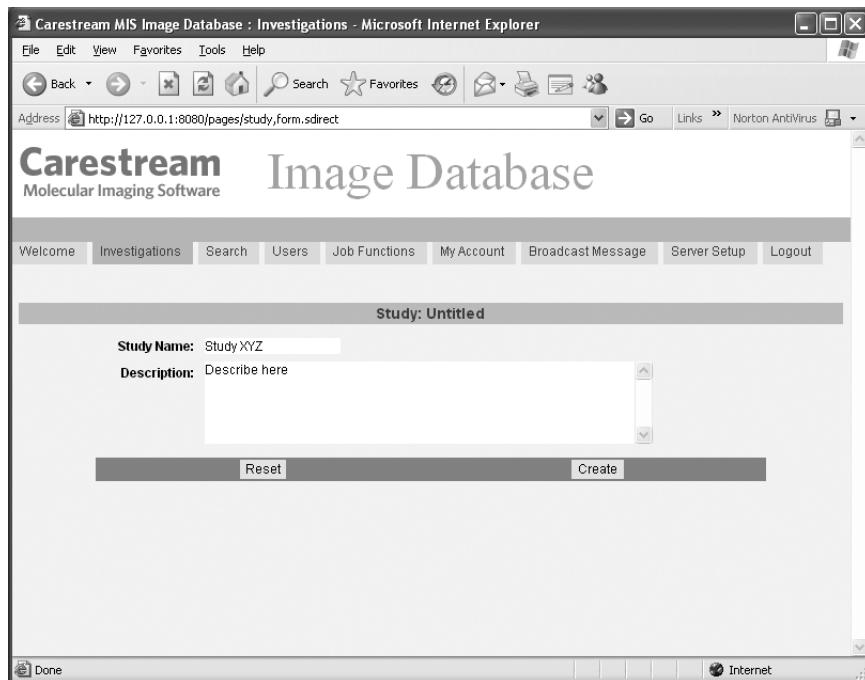
Creating Studies

- 1 Click the Investigations tab. The Investigations window appears.
- 2 Click the Investigation to which you want to associate a new Study.
- 3 The Study window appears. Click the Create Study button.



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- 6** Click Create. The Study is created. Projects can now be assigned to the Study.

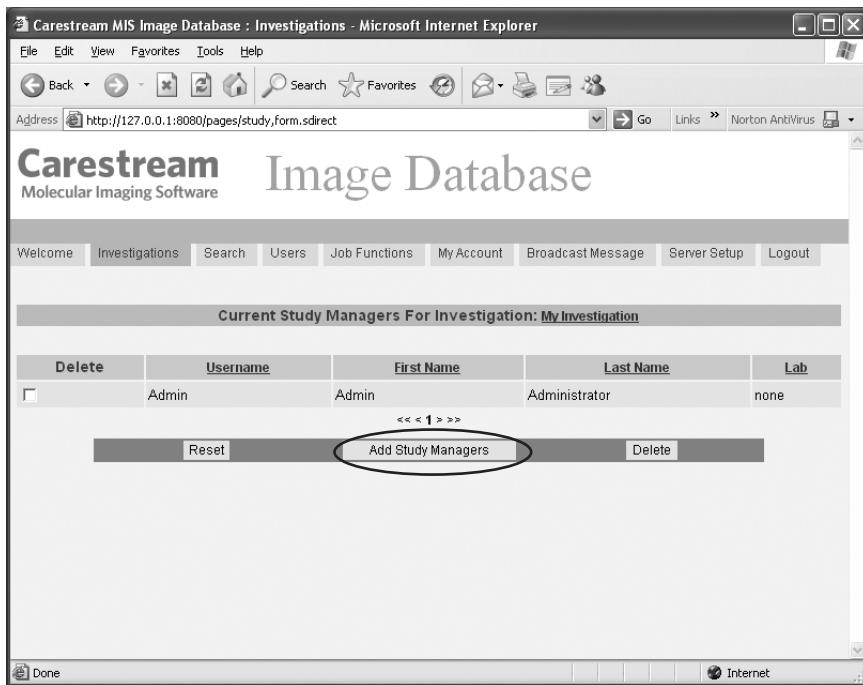
NOTE: An Investigation can contain an unlimited number of Studies.

NOTE: The Reset button clears all the fields and reverts to the defaults.

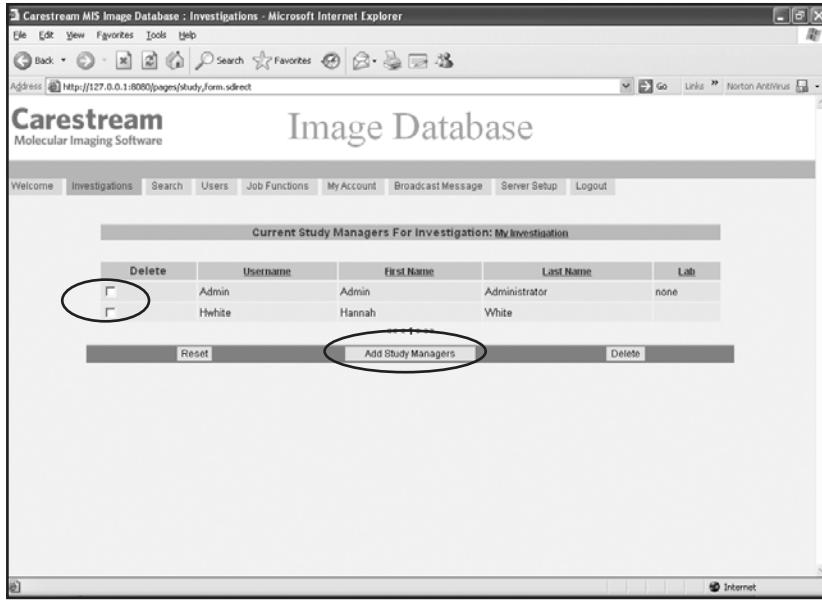
Assigning Study Managers

Once you have set up Investigations and Studies, you can assign a Study Manager(s) to the Investigation. A Study Manager has privileges to add or modify the studies within an Investigation.

- 1** Click the Investigations tab. The Investigations window appears.
- 2** Click the Investigation to which you want to designate a Study Manager. The Study window appears.
- 3** Click Edit Study Manager. The Study Manager window appears.
- 4** Click Add Study Manager.



- 5** The Select Users window appears. Choose any User(s) to create a Study Manager by clicking the checkbox(es).



- 6** Click Add. The User(s) is now assigned as a Study Manager.

NOTE: The Reset button clears the fields and reverts to the defaults.

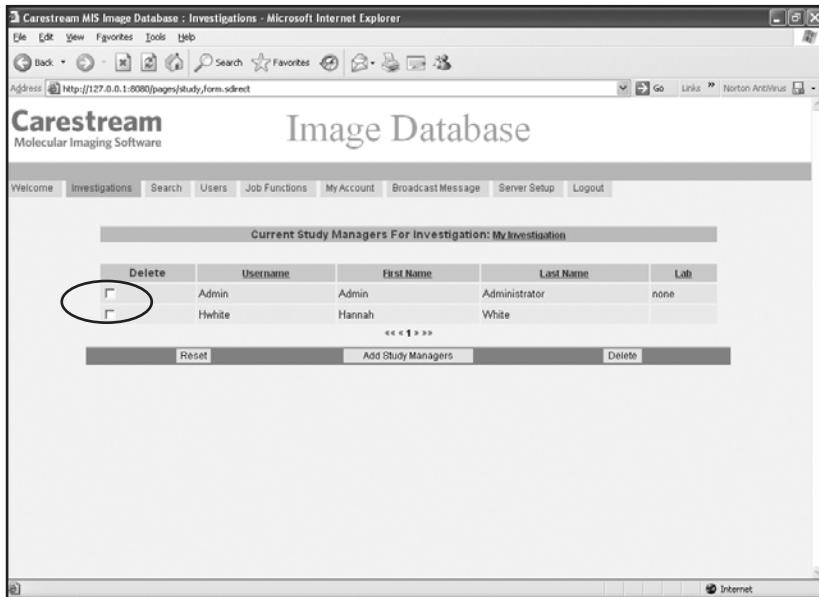
NOTE: To assign a User as a Study Manager, that User must already be entered as a User to that Study.

10

Managing Projects

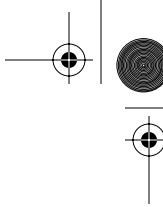
Deleting Study Managers

- 1 Click the Investigations tab. The Investigations window appears.
- 2 Click the Investigation that contains the Users that you want to delete. The Study window appears.
- 3 Click Edit Study Manager. The Study Manager window appears.
- 4 Select the Study Manager(s) you want to delete by clicking the checkbox(es).



- 5 Click Delete.

NOTE: The Reset button clears the fields and reverts to the defaults.



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Assigning Study Users

- 1** Click the Investigations tab. The Investigations window appears.
- 2** Click the Investigation containing the Study to which you want to add Users. The Study window appears.
- 3** Click the Study to which you want to add Users. The specific Study window appears.
- 4** Click Manage Users. The Assigned Users window appears.

Username	First Name	Last Name	Lab	Read/Write	Read Only
Hwhite	Hannah	White		<input checked="" type="checkbox"/>	<input type="checkbox"/>
Admin	Admin	Administrator	none	<input checked="" type="checkbox"/>	<input type="checkbox"/>

<< < > >>

Add User

NOTE: The Reset button clears the fields and reverts to the defaults.



- 5** Click Add User. The Add Users window appears. Add new User(s) from the list of Users to receive Read/Write or Read Only privileges using the checkbox(es). Click Add.

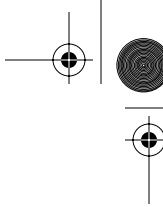
Username	First Name	Last Name	Lab	Read/Write	Read Only
Hwhite	Hannah	White		<input checked="" type="checkbox"/>	<input type="checkbox"/>
Gwood	Graham	Wood		<input type="checkbox"/>	<input checked="" type="checkbox"/>

<< < 1 > >>

[Reset](#) Add

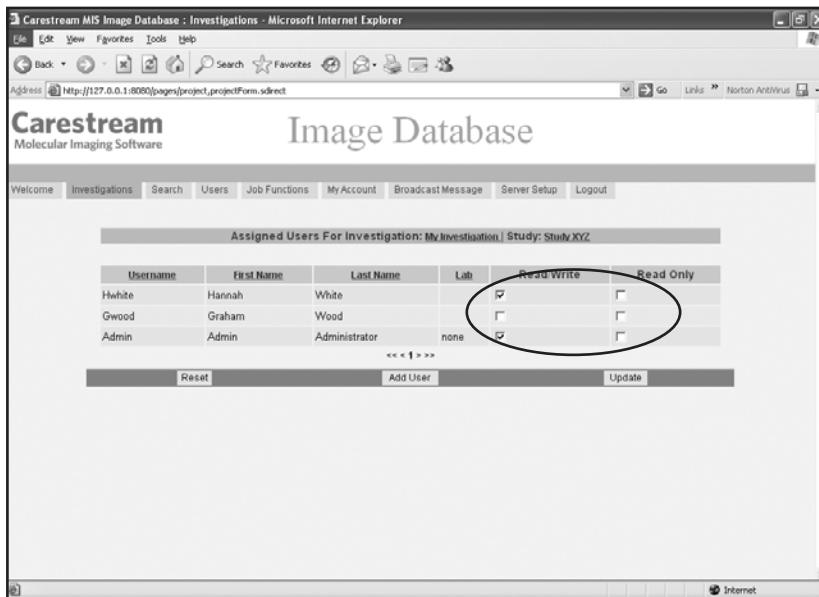
- 6** The User is now assigned those privileges to the study.

NOTE: The Reset button clears the fields and reverts to the defaults.



Removing or Editing User Access to a Study

- 1 Click the Investigations tab. The Investigations window appears.
 - 2 Click the Investigation containing the Study from which you want to remove user access. The Study window appears.
 - 3 Click Manage Users. The Assigned Users window appears.
 - 4 Deselect Read/Write and Read Only checkbox(es) to remove access to a User.



10

Managing Projects

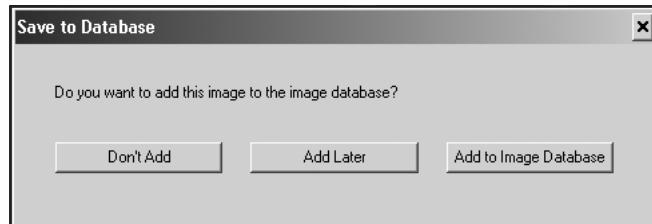
- 5** Click Update. The User(s) privileges to the Study are removed or edited.

 NOTE: The Reset button clears the fields and reverts to the defaults.

Saving an Image to the Database

When you save an image, you will be presented with the options related to saving your image to the database.

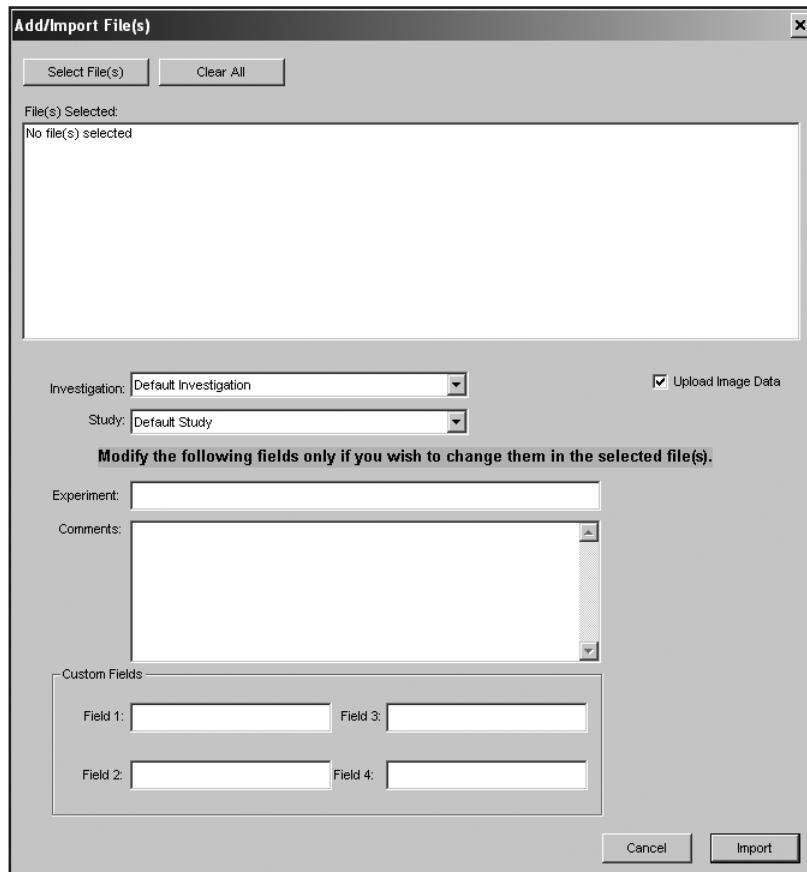
- 1 Initiate adding an image by choosing Save (the first time you save a project). The Save to Database dialog box opens.



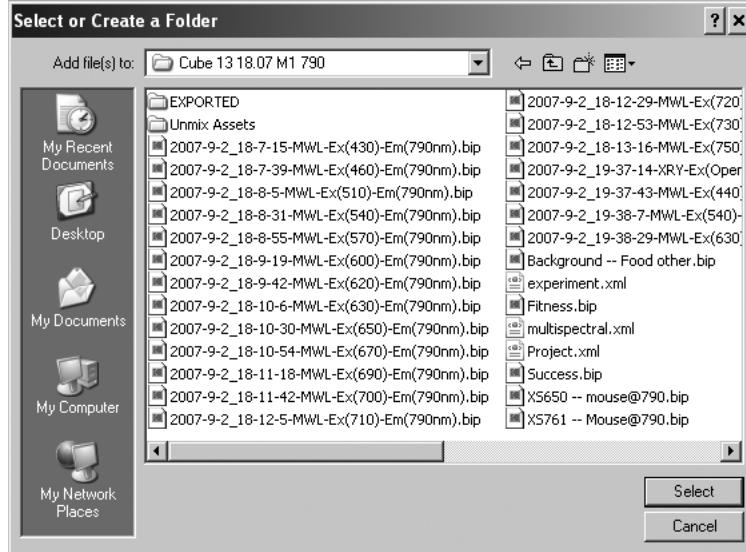
- 2 Choose *Add to Image Database*. Other options include *Add Later* or *Don't Add*.

 NOTE: You can choose an option to automatically add images upon saving. See *Image Database Preferences* later in this chapter.

- 3 The information is added to the database.



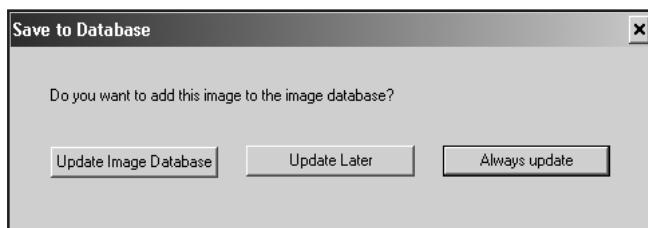
- 2** Choose the files you want to import by clicking on the Select File(s) button. The Select File(s) dialog opens.



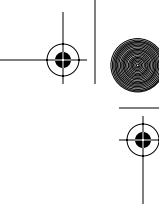
- 3** Select the files you want to import. For multiple images, use the control key (shift for Macintosh) to select the files.
- 4** Click OK. The files will appear in the Add/Import Files(s) window.
- 5** Click Export. The file(s) are exported.

Updating Images in the Database

If you make changes to your project that result in changes in the data used by the database as search criteria, upon saving you will be presented with the option to update the database.



- ✓ *Update Image Database*—updates the database with the changes.



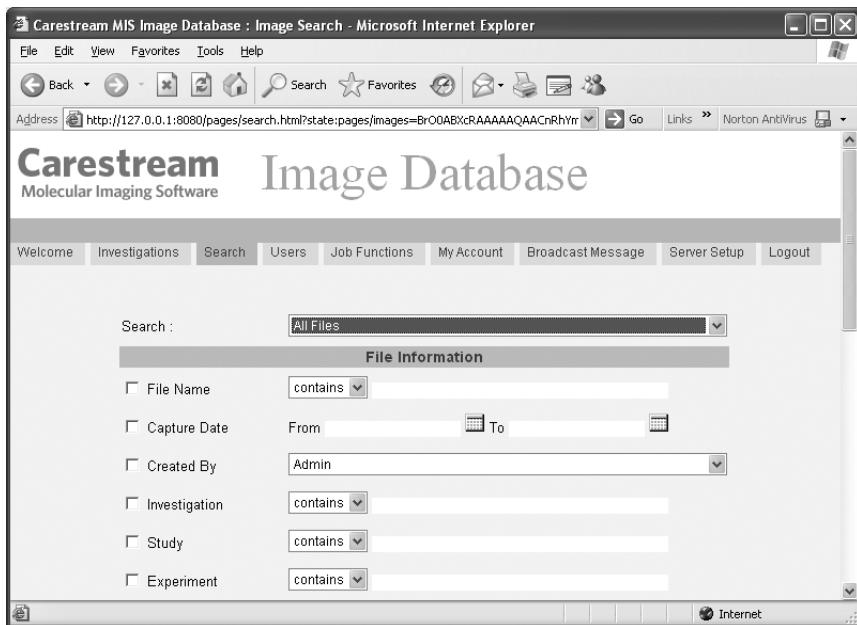
10

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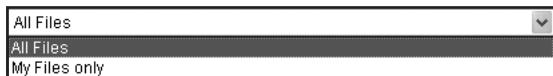
- ✓ *Update Later*—will not update the Image Database with the changes.
- ✓ *Always Update*—automatically saves any changes to the database whenever changes occur and open the Save to Database dialog box.

Searching the Database

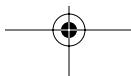
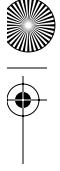
- 1 Click Search tab or choose Search from the File Menu. The Search window appears.



- 2 Use the *Search pop-up menu* to select to search the all files in the database (*All Files*) or only your own files (*My Files Only*).



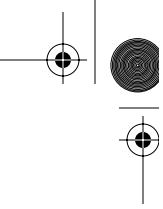
- 3 Define the search criteria you want to use to find the images of interest by clicking the checkbox and defining the qualifiers. The fields include:
 - ✓ *File Name*—searches for a file based on the name you assigned when saving the file. Qualifiers are *contains* and *is* and provides a text edit box to enter text. The search field is not case sensitive.
 - ✓ *Capture Date*—searches for a file based on the date the image was captured.



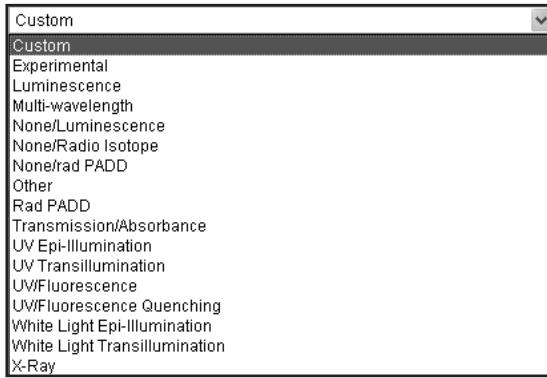
Highlight the month, date or year using the arrows to scroll up and down to define the from and to date.



- ✓ *Created By*—searches by user name. All users assigned to that have captured images are listed in the pop-up window.
- ✓ *Investigation*—searches for all projects related to a specific Investigation. The investigation is assigned in the Project tab in the File Information window when the file is saved. Qualifiers are *contains* and *is* and provides a text edit box to enter text. The search field is not case sensitive.
- ✓ *Study*—searches for all projects related to a specific study. The study is assigned in the Project tab in the File Information window when the file is saved. Qualifiers are *contains* and *is* and provides a text edit box to enter text. The search field is not case sensitive.
- ✓ *Experiment*—searches for a specific experiment. The Experiment name is assigned in the Project tab in the File Information window when the file is saved. Qualifiers are *contains* and *is* and provides a text edit box to enter text. The search field is not case sensitive.
- ✓ *Type*—searches for specific project type. The field is located in the Project tab in the File Information window. Qualifiers are *contains* and *is* and provides a text edit box to enter text. The search field is not case sensitive.
- ✓ *Comment*—searches for specific comment in the comment text. The Comment field is located in the Project tab in the File Information window that opens the first time you save the file. Qualifiers are *contains* and *is* and provides a text edit box to enter text. The search field is not case sensitive.
- ✓ *Field 1, 2, 3 and 4*—are custom fields that you can use to code specific files for fast retrieval. The field is located in the Project tab in the File Information window that opens the first time you save the file. Qualifiers are *contains* and *is* and provides a text edit box to enter text. The search field is not case sensitive.
- ✓ *History*—searches for specific information that is recorded in the History file. The History file maintains a record of changes to the file. The History is located in the History tab in the File Information window.
- ✓ *Illumination Source*—searches for files based on the illumination type that is



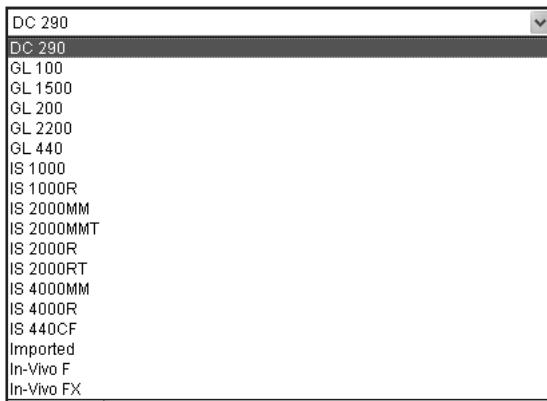
recorded from Carestream Imaging Systems. This information is contained in the History tab of the File Information window.



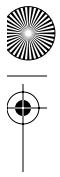
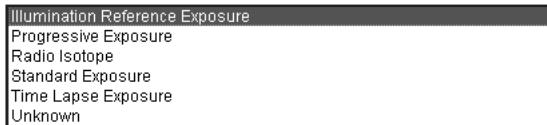
10

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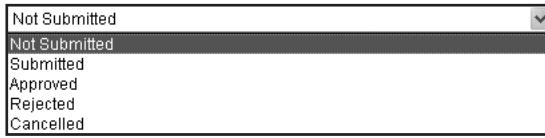
- ✓ *Capture Source*—searches for files associated to a Carestream capture device that was used to capture the image. The pop-up window lists the different Carestream Imaging Systems, files that are captured from non-Carestream systems are marked as Imported. This information is contained in the History tab of the File Information window.



- ✓ *Exposure Type*—searches for files based on the type of exposure sequence used when capturing the image. The pop-up window lists the different types that are supported by the various Carestream capture devices.



- ✓ *Approval Status*—searches for files associated to the approval status. Select the status using the pop-up window.



- ✓ *Band MW*—searches the analysis data for files containing bands with a specific molecular weight range. Use the min and max text edit fields to define the molecular weight range (bp).

Min	Max	(bp)
-----	-----	------

- ✓ *Band Mass*—searches the analysis data for files containing bands with a specific mass range. Use the min and max text edit fields to define the mass range (ng).

Min	Max	(ng)
-----	-----	------

- ✓ *Band Model Mass*—searches the analysis data for files containing bands with a specific modeled mass range. Use the min and max text edit fields to define the modeled mass range (ng).

Min	Max	(ng)
-----	-----	------

- ✓ *Standard Type*—searches files for specific lists Standards you have used in Lane Analysis. This information is contained in the Lanes tab of the File Information window.

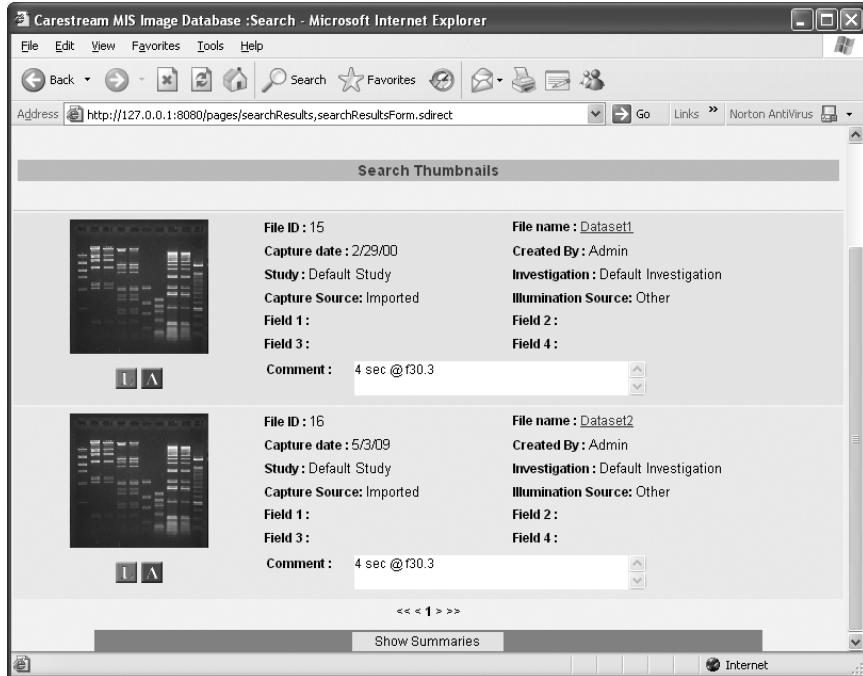
- ✓ *Lane Name*—searches for specific lane names used in Lane Analysis. Qualifiers are *contains* and *is* used with your specific text. This information is contained in the Lanes tab of the File Information window. The search field is not case sensitive.

- 4** Click Search. The Search Results Summary window opens.

Delete	File ID	File Name	Created By	Capture Date	Capture Source	Illumination Source
<input type="checkbox"/>	1	chemblotXYZ.bip	Admin	3/16/99	Imported	Other
<input type="checkbox"/>	5	GelXYZ.bip	Admin	3/16/99	Imported	Other
<input type="checkbox"/> Delete All						
<< < > >>						
Show Thumbnails				Delete		

- ✓ *File ID*—is a unique identifier that every image is automatically assigned.
- ✓ *File Name*—identifies the image that you have selected in the Search Results window. Clicking on the File Name opens the File data window.
- ✓ *Created By*—identifies the user that originally saved the file.
- ✓ *Capture Date*—identifies the date that the file was originally saved.
- ✓ *Capture Source*—lists the Carestream camera type that was used to capture the image.
- ✓ *Illumination Source*—lists the illumination method used when capturing the image.
- ✓ *Status*—lists the status of the approval process.
- ✓ *Delete*—marks the project for deletion from the database. Use the Delete All checkbox to mark all the files found for deletion. This action does not eliminate the file from the server but only eliminates the data from the database and relinquishes the control from the database.

✓ *Thumbnails (Hide or Show)* is a visual display of your file.



5 Click Delete. All projects marked for deletion are removed from the database.

Opening a MI Project from Your Database

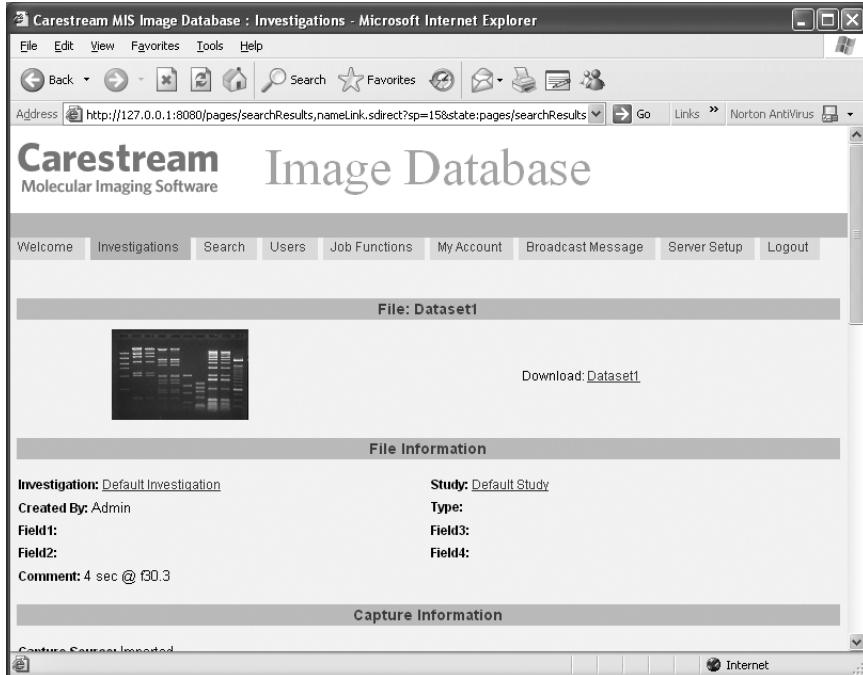
Once you have defined and executed a database search you can open files in Carestream MI from the database.

- 1** Click Image Database from the Navigation panel, log into the Security Manager.
- 2** Click the Search tab. The Search window appears.

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- 3** Use the search criteria to sort the records that you want to open. Click Search, the Search Results window opens.
- 4** Click on the File Name. The File Data window appears.



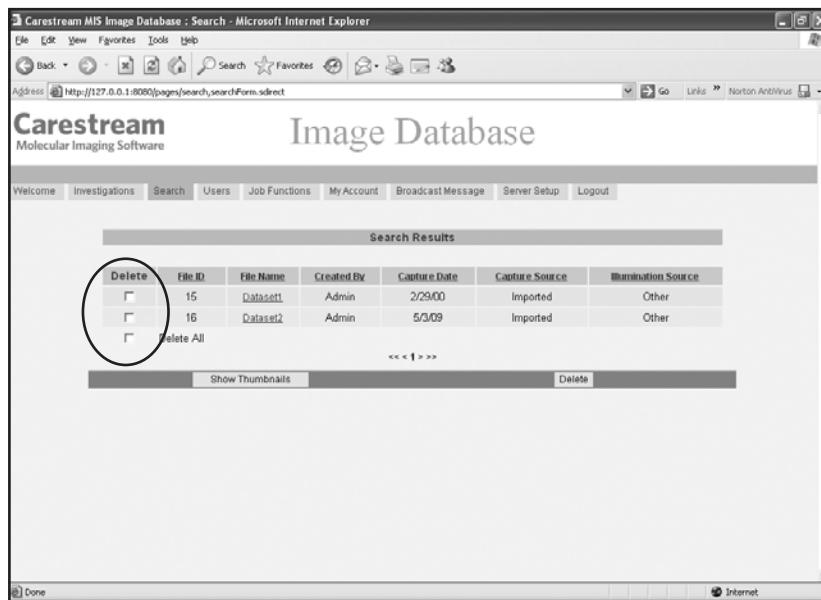
- 5** Click on the download link. The project opens on your desktop.

NOTE: The project is downloaded to your local machine. When you save the image it will automatically be saved back to the server location.

Removing Images from Your Database

In the course of databasing your images, you may want to remove records from your database. Remember, removing the image from the database does not delete the image from your computer/server permanently, it is only removing the record that indexes information about your project.

- 1** Click Image Database from the Navigation panel, log into the Security Manager.
- 2** Click the Search tab. The Search window appears.
- 3** Use the search criteria to sort the records that you want to remove. Click Search, the Search Results window opens.
- 4** Mark individual files for deletion by selecting in the Delete column checkboxes next to the file or click on the Delete All checkbox.



- 5** Click Delete.

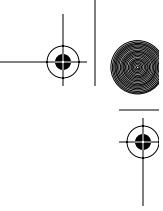


Image Database Preferences

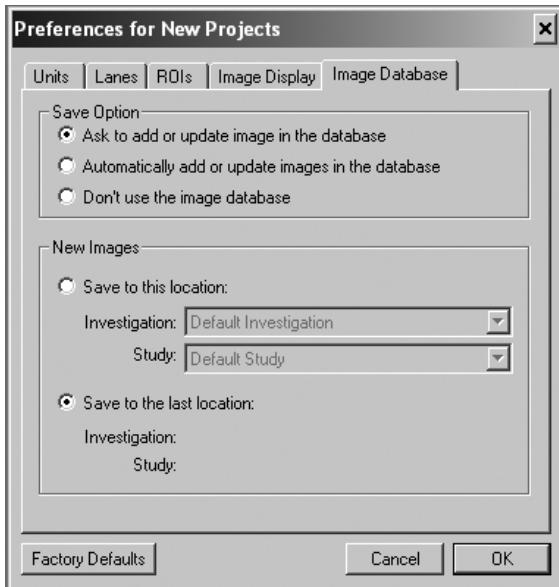
For all projects, you can set Preferences related to saving images to a database. You can set either a project or a new project preferences using the menu items from the Edit menu or by clicking the Preferences on the Quick Access bar.

New Project Preferences

If you want to change the Preferences for all new images related to the Image Database, use the New Project Preferences.

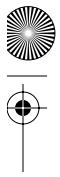
1 Open the Preferences window.

- ✓ To set the Preferences for all new projects—choose New Project Preferences from the Edit menu or click on the Preferences button on the Quick Access bar when no projects are open.
- ✓ To change the Preferences for the open project—choose Project Preferences from the Edit menu or click on the Preferences button on the Quick Access bar with the project open and in the active window.



2 Choose one of the three options:

- ✓ *Ask to add or update image in the database*—selection prompts you every time you save an image if you want to save the image to the database.



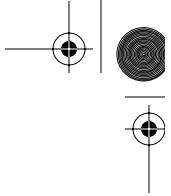
- ✓ *Automatically add or update images in the database*—does not prompt you but saves and updates the database whenever an image is saved.
- ✓ *Don't use the database*—does not save any images or info in the database and cannot be searched.

3 Choose new image options

- ✓ *Save to this location* enables you to select the destination folder to save all new images. The images are saved to the selected investigation and study.
- ✓ *Save to the last location* saves the files to the last saved destination folder. The images are saved to the selected investigation and study.

4 Click OK.

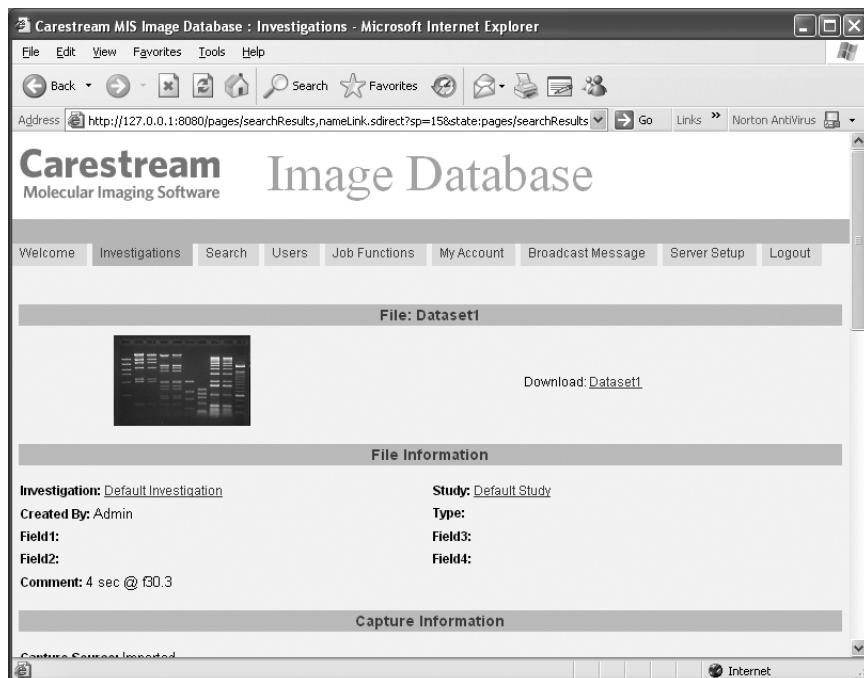
 NOTE: The default selection is to *Ask to add or update the database*.



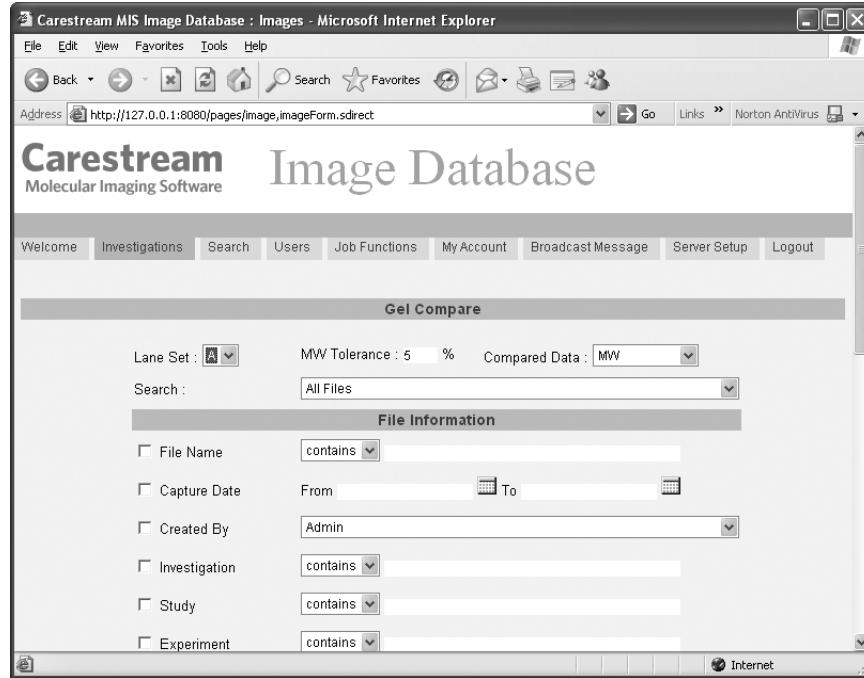
Advanced Database—Gel Comparisons

Gel Comparisons compare the presence or absence of bands across multiple gels, and displays the results of the analysis in a sorted order (closest match at top of list). This is an advance search feature of your database. To perform this search, the images must be in the image database.

- 1 Click Image Database from the Navigation panel, log into the Security Manager.
 - 2 Click the Search tab. The Search window appears.
 - 3 Use the search criteria to find the records that you want to compare. Click Search, the Search Results window opens.
 - 4 Clicking on the File Name to access the File data window. The File Data window appears.



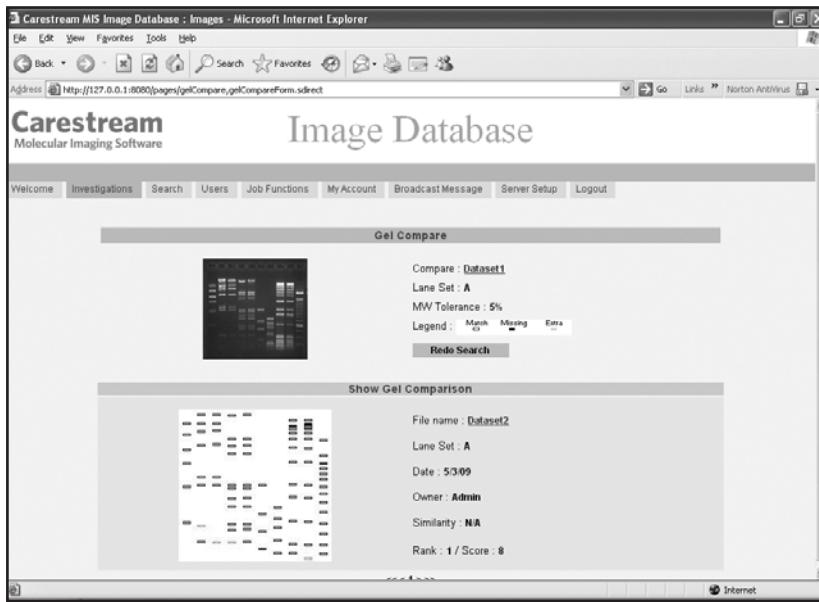
- 5 Click Gel Compare button from the bottom of the window. The Gel Comparison window appears.



- 6 Use the *Search pop-up menu* to select to search the all files in the database (*All Files*) or only your own files (*My Files Only*).
- 7 Define the search criteria to find the images that you want to search against using the File Information section.
- 8 Use the Lane Set pop-up menu to select the Lane Set(s) want to compare as the reference.
- 9 Enter a % tolerance in the MW Tolerance test box. This sets the molecular weight tolerance that the gel comparison algorithms uses to determine if individual bands are matches. The default tolerance is set to 5%.
- 10 Choose whether you want to use the Molecular Weight or Synthetic Molecular weight using the Compared Data pop-up menu.

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11 Click Display. The Results window opens.



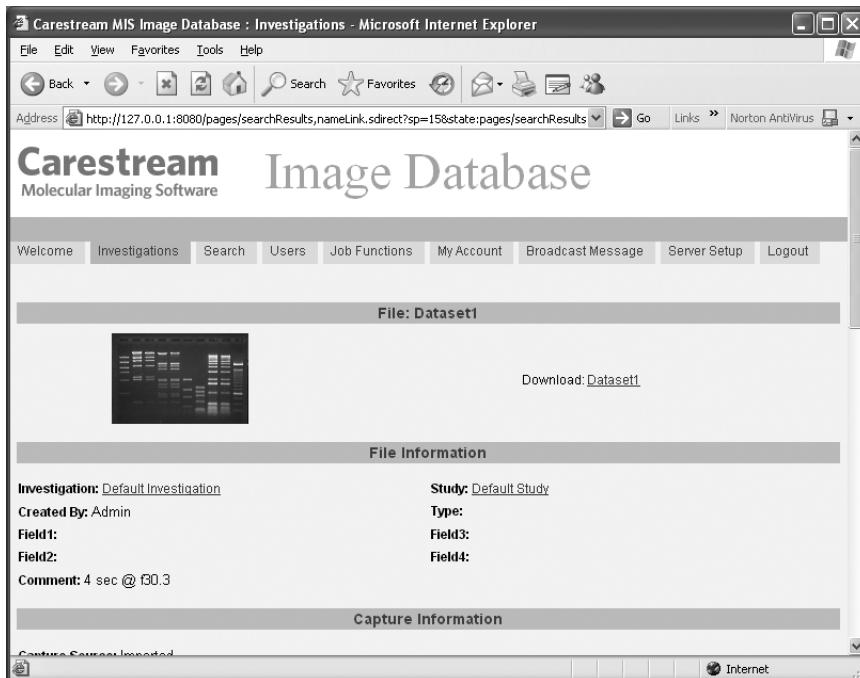
- ✓ The *Reference Image*—to which you want to compare all the other images, is placed at the top of the window.
- ✓ The *Results*—provides tools to scroll through the set of comparison gels and visually be able to see the differences. Each gel is identified with key information including the name of the image, lane set, date the image was created and the% similarity. The color of the bands correspond to whether or not they appear in both gels or are unique to one of the comparison gels.
 - open (filled with white) corresponding to bands in both the reference gel and comparison gel.
 - black bands corresponding to bands only found in the reference gel.
 - green bands corresponding to bands that are only found in the comparison gel.

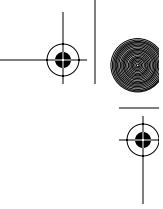
12 Click Print to print your results (optional).

Advanced Database—Differential Lane Display

Differential Display uses one lane as a reference and compares the reference lane to all the other lanes in the image. Lanes are compared on a band by band basis to determine if the band masses (or if no mass calibration is available, the band net intensities) are increasing or decreasing. If there is a selected lane, then the selected lane are designated the reference lane, otherwise Lane 1 of the active lane set is the default reference lane.

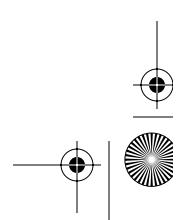
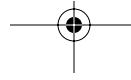
- 1** Click Image Database from the Navigation panel, log into the Security Manager.
- 2** Click the Search tab. The Search window appears.
- 3** Use the search criteria to find the records that you want to compare. Click Search, the Search Results window opens.
- 4** Clicking on the File Name to access the File Data window. The File Data window appears.



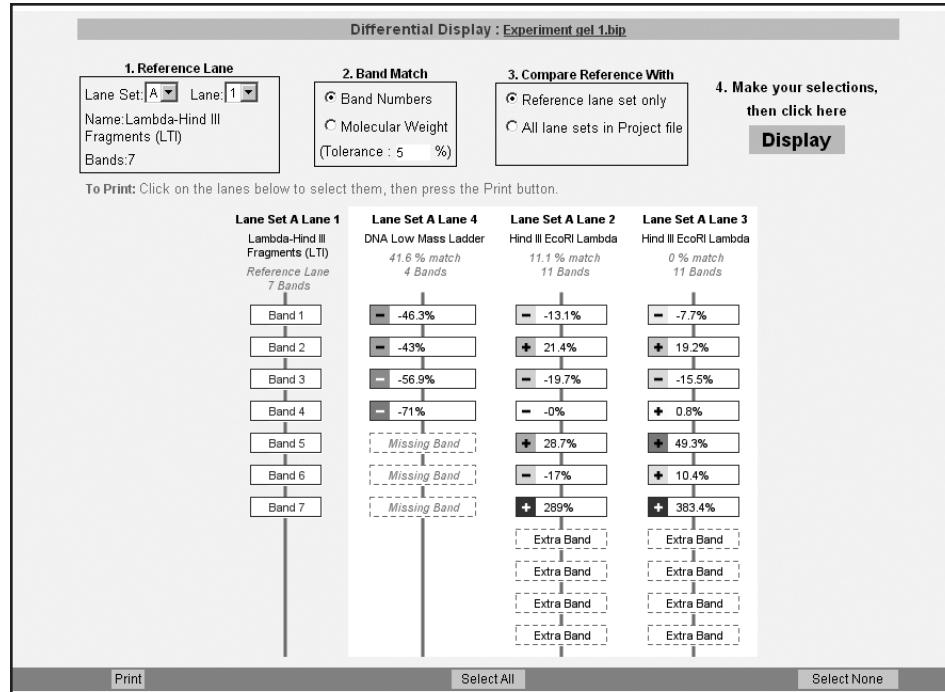


- 5** Click Differential Display button from the bottom of the window. The Differential Display window appears.

- 6** Use the Reference Lane box to select the Reference Lane and the Reference Lane Set.
- 7** Use the Band Match box to select how band matching is accomplished. Select Band Numbers or Molecular Weight.
- ✓ *Band Numbers*—bands with the same number in the reference set are compared with bands of the same number in the comparison set.
 - ✓ *Molecular Weight*—the molecular weights of each band are compared to within the given tolerance level.
- 8** Choose whether or not you want to generate a plot using all the lanes in the lane set or just the lanes of the reference lane set using the Compare Reference with buttons.



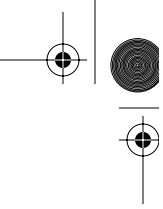
9 Review the data generated.



The Band boxes along the lane line show each match and the% difference in net intensity (or band mass) between the reference bands and the comparison bands. The boxes are scored and colored according to the following rules:

- ✓ If a band in the comparison lane matches a band in the reference lane—a box is drawn at position of reference lane band. When a standard is used the band masses are used to find the % difference, otherwise the net intensity is used. The box is shaded red if percent is negative, with pure red for 100%, 50% red for a 50% difference, etc. The box is shaded blue if percent is positive, with pure blue for 100% or greater, 50% blue for a 50% difference, etc.
- ✓ If a band is in the comparison lane but not in the band reference lane—a box is drawn at position of comparison lane band. The box is green and labeled as Extra band.
- ✓ If a band is in the reference lane but no band is in comparison lane—a box is drawn at the position of reference lane band. The box is black and is labeled Missing.
- ✓ The overall score for each lane is the sum of the absolute values of the % differences for Case 1 bands, plus 100 for every missing or extra band. Therefore, if the max score is zero, the match is 100%—all the lanes are identical.

10 Click Print to print your results (optional).

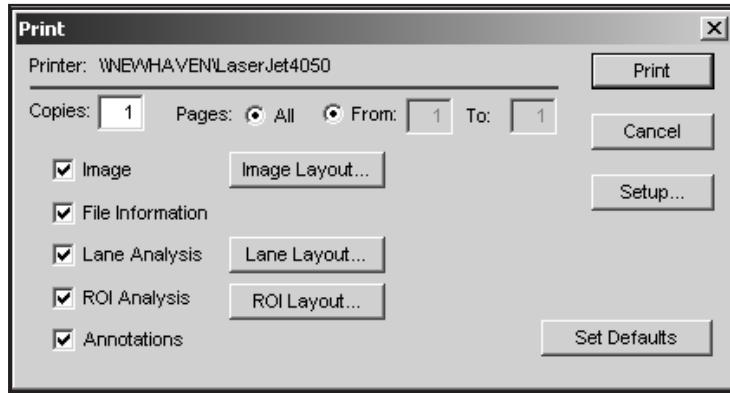


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Printing

Carestream MI allows great flexibility in printing your project. You can print the image, file information, analysis results, annotations, and preference settings.



- ✓ *Image* checkbox—prints the full image or a specified region. The Image Layout dialog box allows you to select magnification or specify the area of interest.
- ✓ *File Information* checkbox—prints the file information for the current project.
- ✓ *Lane Analysis*—prints lane analysis results. The Lane Layout dialog box provides you with all the options for printing analysis results. The summary option is a selection within the dialog box.
- ✓ *ROI Analysis*—prints ROI analysis results. The ROI Layout dialog box provides you with all the options for printing ROI results.
- ✓ *Annotations*—prints all annotations pages that have information on them.

NOTE: Gel Comparison and Differential Display results are printed through the results window and are not available in the Print dialog box.

The checkboxes in the Print dialog box determine what information is printed. You can select or deselect each checkbox independently. For example, by only selecting the Print File Information checkbox you only print the file information for the project.

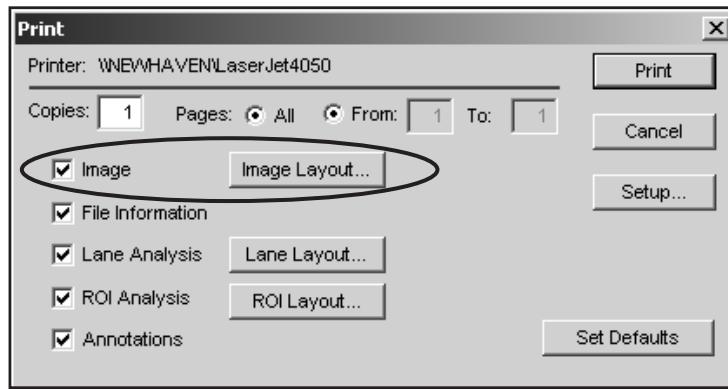
The *Set Defaults* button applies the checked printing selections to the current project, when printing. These new settings are saved with the project for future sessions and become default settings for subsequent image captures or new image files opened that have not yet been saved as projects. Printing default settings for a given project may be changed at any time.



Printing the Full Image

To print the entire image at 100% magnification:

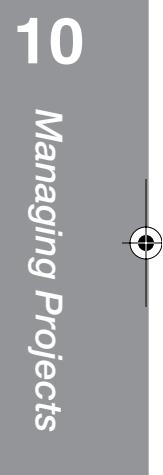
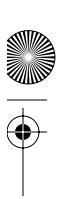
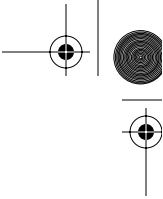
- 1** Choose Print from the File menu. The Print dialog box appears.
- 2** Select the Image checkbox.



- 3** Click the Image Layout button to preview the image placement.

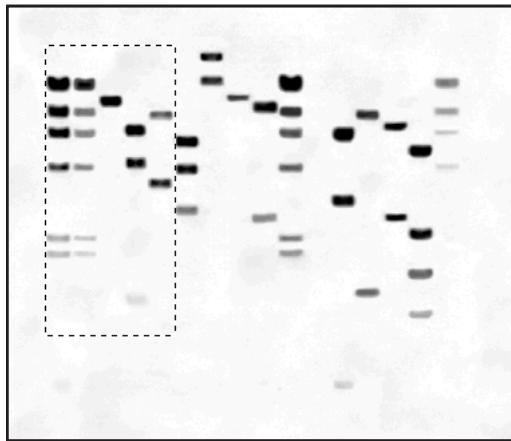
☞ NOTE: The Image Layout dialog box allows you to set the magnification. This option can also be used to print an area of interest. The same result can be obtained by making a selection in the image and choosing Print Selection.

- 4** Click Print.



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- 3** Choose Print Selection from the File menu. A Print dialog box appears.
- 4** Select the Image checkbox. Make changes to image sizing and placement, as desired by choosing the Image Layout button.
- 5** Click Print.

The software automatically centers the selected area when it prints your image. If the selected area is larger than the size of a page, the image is cropped to fit on the page.

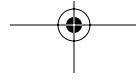
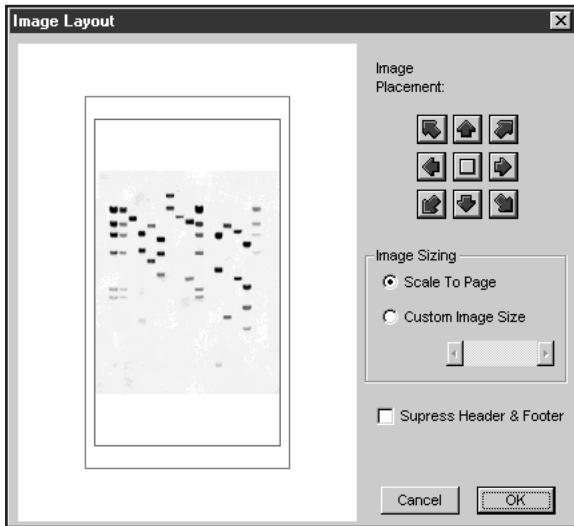


Image Layout

The Print dialog box contains an option for previewing the image layout before printing.

- 1** Choose Print from the File menu.
- 2** Select the Image checkbox and click the Image Layout button. The Image Layout dialog box appears.

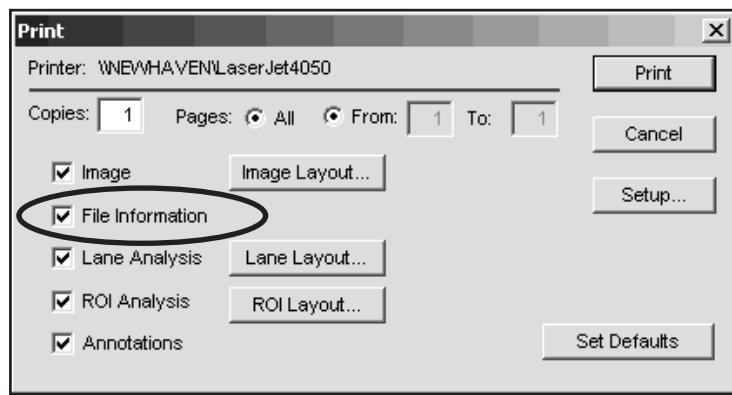


- ✓ If the selection is larger than the page size, a frame appears around the part of the image that fits on the page. This is the print area.
- 3** Use the Image Placement arrows or the cursor to position the print selection on the page.
 - ✓ Click an Image Placement arrow. The frame around the selected image moves in the direction of the arrow.
 - ✓ Place the cursor over any area of the miniature page. The cursor changes to a hand. Click and drag to reposition the frame on the page.
- 4** Size the image using Scale to Page buttons or select the Custom Image Size button and adjust the slider.
- 5** Check Suppress Header & Footer if you do not want the project border to appear on the printed image.
- 6** Click OK, to return to the Print dialog box.
- 7** Click Print.

Printing the File Information

When you select the File Information checkbox, the file information prints for the current project.

- 1** Choose Print from the File menu. The Print dialog box appears.
- 2** Select the File Information checkbox.

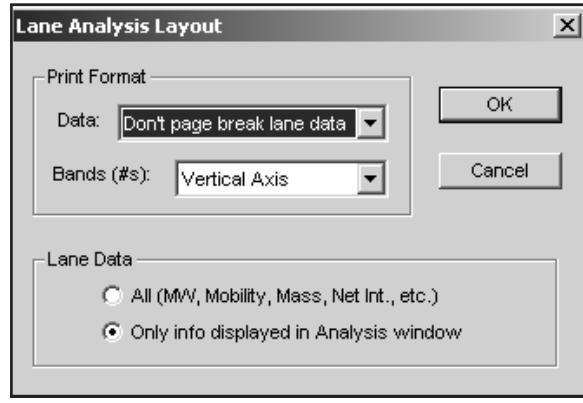


- 3** Click Print.

Printing the Lane Analysis Data Results

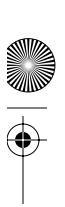
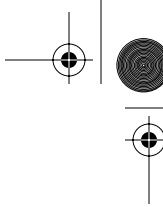
You may choose to print all or a selection of the data currently displayed in the Lane Analysis Data window.

- 1 Choose Print from the File menu. The Print dialog box appears.
- 2 Select Lane Analysis and click the Lane Layout button to access the custom options. The Lane Analysis Layout dialog box appears.



- 3 Select the layout option that best suits your needs by using the Data pop-up menu.
 - ✓ *One page summary (w/image)*—option provides a scaled copy of the image, the lane names as defined in the Lane Information dialog box and a single analysis variable. Once selected, a pop-up menu appears that allows you to select the variable.
 - ✓ *Page break lane data*—prints analysis results with the minimum number of pages possible. Lane data for images with large numbers of bands are printed on multiple pages.
 - ✓ *Don't page break lane data*—keeps all lane data together. If you are printing lane data with large numbers of bands, it may not fit on the existing page. In this case, the program moves the entire lane data to a new page, printing the lane data continuously.
 - ✓ *Single lane per page*—prints data from each lane on separate pages.

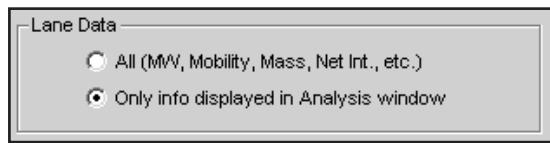
NOTE: Depending upon the layout option selected above, the Analysis Layout dialog box may appear differently.



4 Select the orientation of the printed analysis data using the Bands (#s) pop-up menu.

- ✓ *Bands Horizontal Axis* prints the band numbers across the horizontal axis and the analysis data along the vertical axis.
- ✓ *Bands Vertical Axis* prints the band numbers across the vertical axis and the analysis data along the horizontal axis.

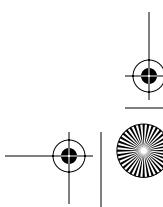
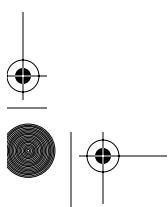
5 If appropriate, select the data you want to print.



- ✓ Choose *All (MW, Mobility, Mass, Net Int. etc.)*—to print all lanes of information and all analysis variables.
- ✓ Choose *Only info displayed in Analysis window*—to only print the lanes and analysis variables displayed in the current project's Lane Analysis Data window.

6 Click OK, to return to the Print window.

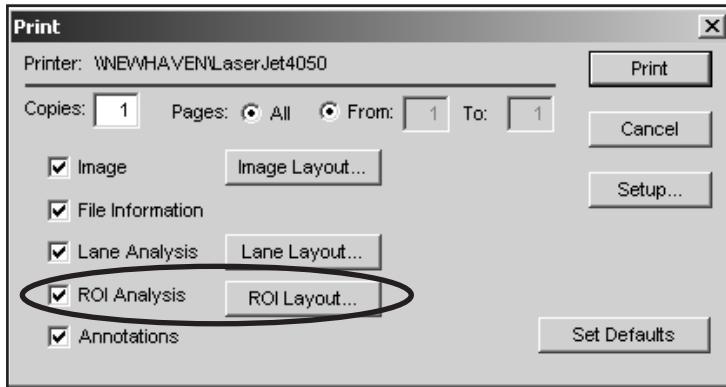
7 Click Print.



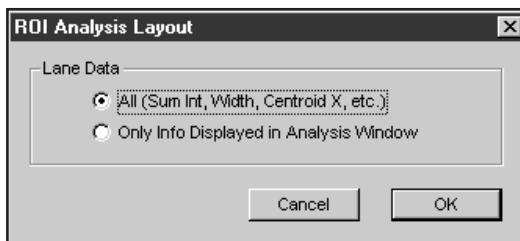
Printing ROI Analysis Data Results

When you select ROI Analysis, the ROI data prints from your image. You may choose to print all of the data or only a selection of the data currently displayed in the ROI Analysis Data window.

- 1** Choose Print from the File menu. The Print dialog box appears.
- 2** Select ROI Analysis and click the ROI Layout button to access the custom options. The ROI Analysis Layout dialog box appears.



- 3** Use the ROI Analysis buttons to select one of the options:

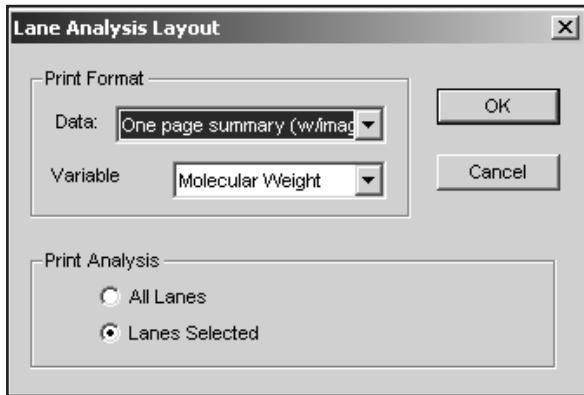


- ✓ Choose *All (sum, Int, Width, Centroid X, etc.)*—to Print all ROI data.
 - ✓ Choose *Only Info Displayed in Analysis Window*—to print only the information displayed in the ROI Analysis Data window.
- 4** Click OK, to return to the Print window.
 - 5** Click Print.

Printing a Summary

The Print Summary option offers a one page summary of your analysis results. The Image with Summary option displays a copy of the image, lane names as defined in the Lane Information dialog box and results for a single analysis variable.

- 1 Choose Print from the File menu. The Print dialog box appears.
- 2 Click the Lane Analysis checkbox.
- 3 Select the Lane Layout button to access the custom options.
- 4 Select *One page summary (w/image)* from the Data pop-up menu.

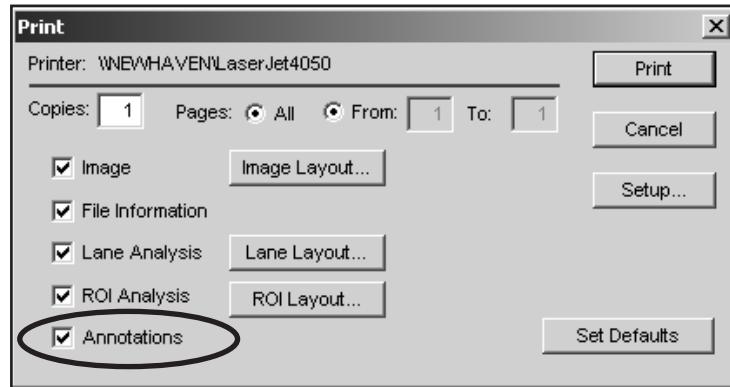


- 5 Select an analysis variable from the Variable pop-up menu.
- 6 Select the *All Lanes* button to print the analysis data for all lanes or *Lanes Selected* for only the lanes displayed in the Lane Analysis Data window.
- 7 Click OK, to return to the Print window.
- 8 Click Print.

Printing Annotations

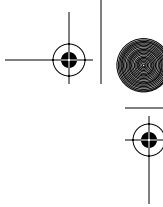
When you select Annotations, the Annotations window prints for the current project.

- 1 Choose Print from the File menu. The Print dialog box appears.
- 2 Select the Annotations checkbox.



- 3 Click Print. All page containing annotations are printed.

NOTE: If you want to print a lane profile, display a profile in Annotations.



Exporting Images and Data

You can export your image(s) and all your analysis data for use in other programs or for publication. The image export options include a variety of TIFF, BMP, JPEG, and PICT (Macintosh only) formats.

You can export the lane analysis data, profile analysis data, ROI analysis data, and histogram information. The data is exported as a tab-delimited text file, which can be read by most spreadsheet programs.

A variety of formats are available in which you can export your image. Let's review the options:

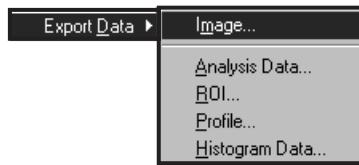
- ✓ *8-bit TIFF File—Scaled to Display Min/Max (*.tif)* saves a 8-bit indexed color image which uses the current image display color table (grayscale, rainbow, etc.), inversion state, and current max and min settings. This is the best format to use when saving 8-bit TIFF files for use in graphics programs.
- ✓ *8-bit TIFF File—Scaled to Image Min/Max (*.tif)* saves a 8-bit grayscale image which maps the current image minimum to 0 and the current image maximum to 255
- ✓ *8-bit TIFF—No Scaling (*.tif)* saves a 8-bit image which simply converts the floating point data to a 1 byte value. Data values above 255 are set to 255, values below 0 are set to 0.
- ✓ *16-bit TIFF File—Scaled to Display Min/Max (*.tif)* saves a 16-bit intensity image using the inversion state and current max and min settings
- ✓ *16-bit TIFF File—Scaled to Image Min/Max (*.tif)* saves a 16-bit intensity image which maps the current image minimum to 0 and the current image maximum to 65,535. This format contains the maximum image information and should be the preferred file type when saving 16-bit TIFF files.
- ✓ *16-bit TIFF File—No Scaling (*.tif)* saves a 16-bit image which simply converts the floating point data to 16-bit intensity values. Data values above 65,535 are set to 65,535, values below 0 are set to 0.
- ✓ *PICT File*—a common Macintosh file format that is compatible with many presentation packages.
- ✓ *JPEG File (*.jpg)*—a common file format that uses a excellent compression scheme for images. This option is only available for exporting image.
- ✓ *Windows Enhanced Metafile Format (*.emf)*—Saves the image and annotations pages.
- ✓ *BMP File (256 Color or True Color)(*.bmp)*—a common cross-platform file format used in the graphics industry. This option is available for use with the Carestream DC290 Zoom Digital camera for exporting unprocessed color images.

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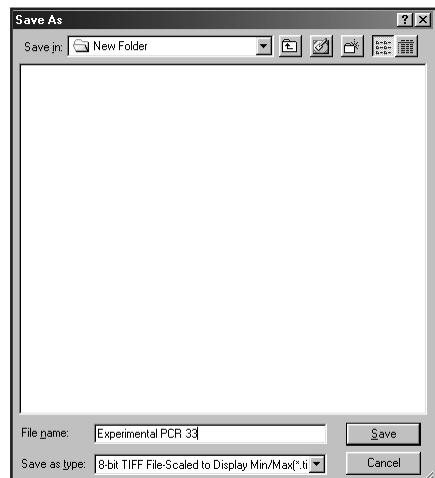
Managing Projects

Exporting an Image

- 1 Choose Image from the File menu and the Export Data submenu.



- 2 The Save As dialog box appears.

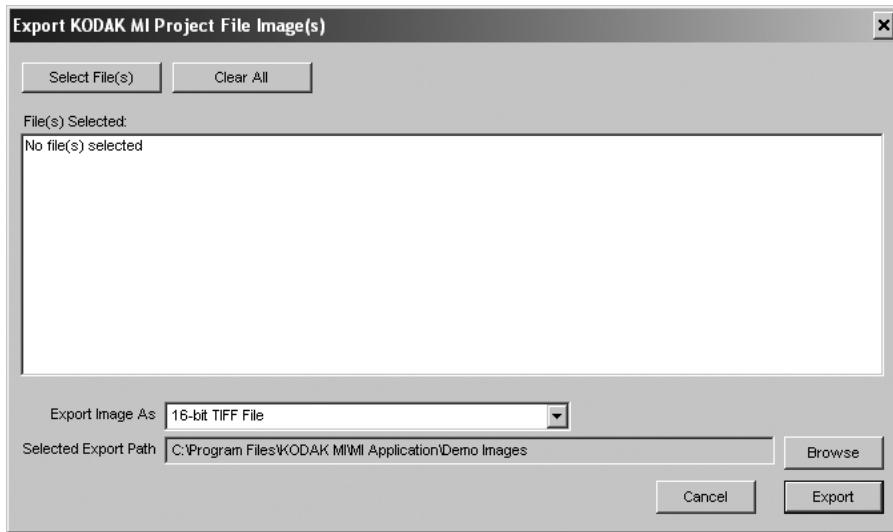


- 3 Choose the file format in which you would like your image saved.
- 4 Select the destination drive, folder, and choose a filename for the image.
- 5 Click Save. The file is saved in the new file format.

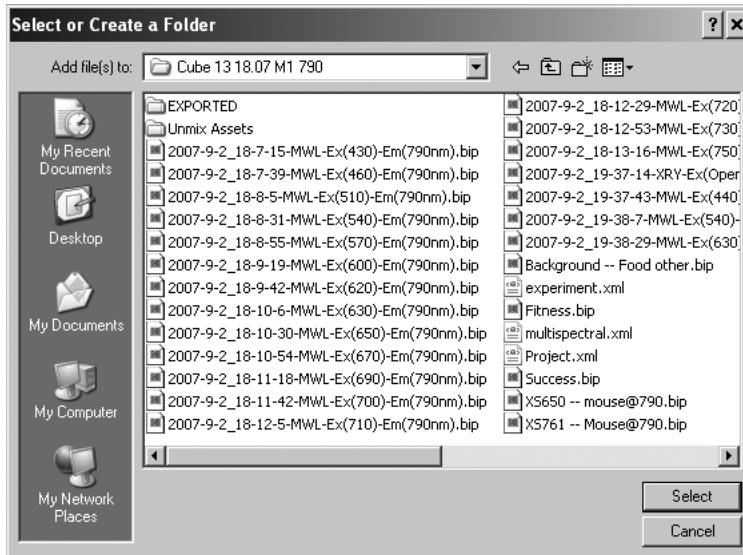
 NOTE: When saving 8-bit or 16-bit TIFF files, the contents of the selection is saved.

Exporting Multiple Images

- 1 Choose Export Multiple File Image(s) from the File menu. The Export Carestream MI Project File Image(s) appear.



- 2 Choose the files you want to export by clicking on the Select File(s) button. The Select File(s) dialog opens.



- 3** Select the files you want to export. For multiple images, use the control key (shift for Macintosh) to select the files.
- 4** Choose the file format in which you would like your image exported.
- 5** Select the destination drive and folder.
- 6** Click OK. The file(s) are exported.

Exporting Lanes/Bands Analysis or Profile Data

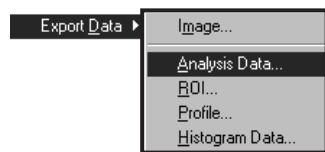
You can export the data in the Lane Analysis Data window and the data used to generate the profile.

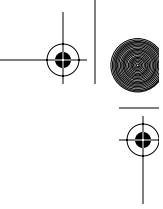
To prepare a selection of data for export:

- 1** Click Analysis from the Quick Access bar or select Lane Analysis Data from the Show menu. The Lane Analysis Data window appears.
- 2** Use the Lanes pop-up menu to select which lanes to display. Remember to hide the Profile window so you can display the analysis data for all of the lanes of interest.
- 3** Select the Display button. Use the Lane Analysis Display dialog box to select the data for export. For example, choose Molecular Weight (MW) and Band Mass (Mass). The data is displayed below the Lane Label.

To export your data:

- 1** Choose Analysis Data or Profile from the File menu and the Export Data submenu.

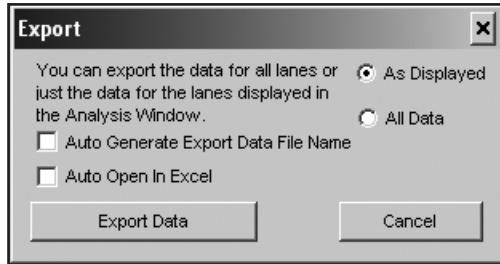




10

Managing Projects

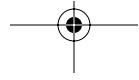
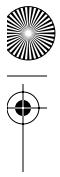
- 2** A dialog box appears asking you what data to export. Select the data to export.



- ✓ *As Displayed*—exports only the data shown in the Lane Analysis Data window.
 - ✓ *All Data*—exports all the lane data.
 - ✓ *Auto Generate Export Data File*—if checked, MI will use a date time stamp to auto generate a file name where Lane export data will be saved.
 - ✓ *Auto open in Excel*—formats the data as an Excel file.
- 3** Click Export Data.
- 4** Once selected, the Save As dialog box appears. Select a destination drive, folder, and a filename.
- 5** Click Save.

 NOTE: The default*.txt extension is required by Windows-based computers. To open in a spreadsheet program, launch the spreadsheet application and use the Open command from the File Menu to open the document.

- 6** The lane analysis data appears as it is displayed in the Lane Analysis Data window. The profile data exported includes the following data fields:
- ✓ Normalized Mobility Information (scaled 1% to 100%) of the distance down the profile
 - ✓ Mobility in pixels
 - ✓ Intensity values of the profile (non-scaled)
 - ✓ Background intensity Fit intensity (if available)



Exporting ROI Analysis Data

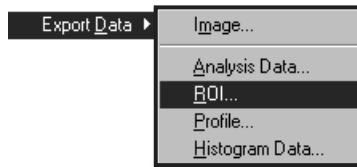
You can export the data in the ROI Analysis Data window.

Preparing a Selection of Data for Export

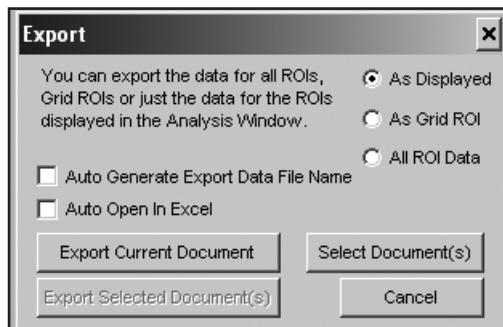
- 1 Choose ROI Analysis Data from the Show menu and the ROI submenu. The ROI Analysis Data window appears.
- 2 Select the Display button. Use the ROI Analysis Display dialog box to select the variables to display.

Exporting the ROI Analysis Data

- 1 Choose ROI from the File menu and the Export Data submenu.



- 2 A dialog box appears asking you what data to export. Select the data to export.



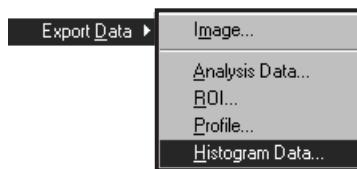
- ✓ *As Displayed*—exports only the data shown in the ROI Analysis Data window.
- ✓ *As Grid ROI*—exports all the ROI data in the Grid format.
- ✓ *All ROI Data*—exports all the ROI data.
- ✓ *Auto Generate Export Data File*—if checked, MI will use a date time stamp to auto generate a file name where ROI export data will be saved.
- ✓ *Auto open in Excel*—formats the data as an Excel file.

- 3** Click Export Current Document or for multiple images the click Select Documents. Use the control key (shift for Macintosh) to select the files.
- 4** Once selected, the Save As dialog box appears. Select a destination drive, folder, and a filename.
- 5** Click Save.
 - NOTE:** The default*.txt extension is required by Windows-based computers. To open in a spreadsheet program, launch the spreadsheet application and use the Open command from the File Menu to open the document.
 - NOTE:** The *.txt extension is required by Windows-based computers. When opening in a spreadsheet program, launch the spreadsheet application and use the Open command from the File Menu to open the document.
- 6** Click Select Document(s) to export the ROI Data from project files that have been saved previously that contain ROI data. Use the dialog box to select the project files that contain ROI data you wish to export. Use the Control key (Shift for Macintosh) to select the files.
- 7** Click OK.
- 8** Click Export Selected Document(s) to export.
 - NOTE:** The Export Select Document(s) is only active when multiple files have been selected.

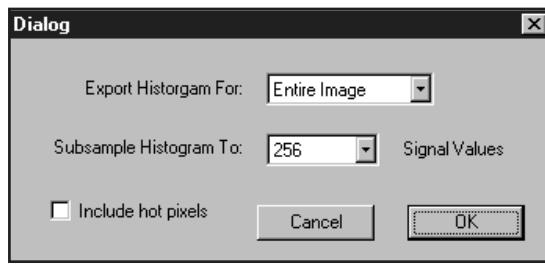
Exporting the Image Histogram Data

You can export the data in the Image Histogram.

- 1 Choose Histogram Data from the File menu and the Export Data submenu.



- 2 The Options dialog box appears.



- ✓ Use the *Export Histogram For* pop-up menu—to choose the data to export, either a selection or the entire image.
- ✓ Use the *Subsample Histogram To* pop-up menu—to subsample the data down to 8-bit, 12-bit, or 16-bit.
- ✓ The *Include hot pixels* checkbox controls how data is scaled/subsampled. When checked, the software uses image min/max values, while unchecked the software uses the Image Display min/max value.

- 3 Click OK.
- 4 Once selected, the Save As dialog box appears.
- 5 Select a destination drive, folder, and a filename.
- 6 Click Save. The file is saved as a tab-delimited text file with a *.txt extension. This type of text file can be read by spreadsheet programs.

 NOTE: The *.txt extension is required by Windows-based computers. When opening in a spreadsheet program, launch the spreadsheet application and use the Open command from the File Menu to open the document.

Guided Tour

This Guided Tour shows you how to analyze images with Carestream Molecular Imaging Software (MI) by walking you through sample projects. The Guided Tour includes five stops:

- ✓ Tour Stop 1: *Software Overview* gives you an overview of MI's Image window and analysis tools.
- ✓ Tour Stop 2: *Lane and Band Analysis* shows you how to analyze a gel for mass and molecular weight using MI's automatic lane and band finder.
- ✓ Tour Stop 3: *Analyzing Spots Using Manual ROI Tools* teaches you how to analyze spots using the ROI tools and view the ROI Analysis Data.
- ✓ Tour Stop 4: *Counting Colonies Using the Automated ROI Finder* teaches you to use the automatic ROI finder to count colonies.
- ✓ Tour Stop 5: *Analyzing Microtitter Plate Assays Using the Grid ROI Tools* shows you how to analyze ROI that are in a grid.
- ✓ Tour Stop 6: *Overlaying Two In Vivo Images*—Using the Advanced Image Display window, you can display one image on top of another image. You will use these tools to overlay an X-ray image with a fluorescent image.
- ✓ Tour Stop 7: *Annotating the Image* prepares an image for presentation using MI's powerful annotation tools.

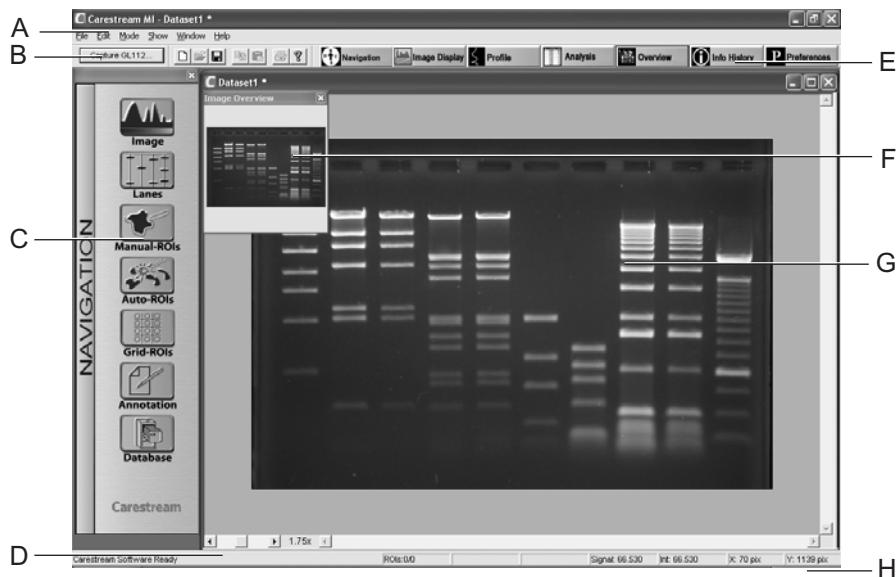
Opening an Image

Let's open the sample file, Tutorial. MI can open any image generated with Carestream capture systems including Gel Logic and Image Station systems, JPEG, TIFF or images acquired via a TWAIN compliant scanner or digital camera.

- 1 Launch MI. The MI Project window appears.
- 2 Choose Open from the File menu. Navigate to the Demo Image subfolder in the Carestream MI folder.
- 3 Select Tutorial.bip from the Demo Images folder.
- 4 Click Open. MI opens the project and displays the image in the Image window.

Tour Stop 1: Software Overview

After acquiring a new image or selecting a saved image, Carestream MI Software opens the Project window. From this window, you can control how the image is displayed on-screen and what Quick Access features and tools appear. Lets review the Project window.



- A The *Menu bar* contains the Carestream MI Software commands. The Menu bar is organized under 6 items—File, Edit, Mode, Show, Window, and Help (Windows) or Carestream MI, File, Edit, Mode, Show, and Window (Macintosh).
- B The *Capture button* accesses your Carestream Imaging System or TWAIN Image Acquire window. If installed, the Capture button displays the currently selected device.
- C Use the *Navigation panel* to optimize the image display, quantify lanes, regions or grids, and annotate and database your image.
- D The *Magnification slider* provides digital magnification from 0.25X to 32X. The Magnification slider maintains the center of the image. This differs from the Zoom tool which shifts the center of the image to wherever the tool is clicked.
- E The *Quick Access bar* accesses frequently used windows—Navigation, Image

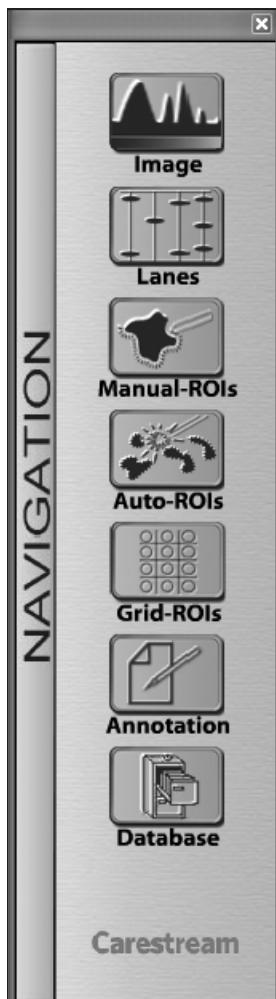
- Display, Profile, Analysis, Overview, Info History, and Preferences.
- F The *Overview Image* contains the scaled version of the entire image. A red box indicates the portion of the image appearing in the Image Section. You can navigate what is displayed in the Image Section by repositioning the rectangle. This feature is especially useful when you are zoomed in on the image.
 - G The *Image Section* represents the image as acquired. This is where you work with the image.
 - H The *Status bar* provide important information as you work through your project. The Status bar changes as you perform various functions.

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Guided Tour

The Navigation Panel

The Navigation panel guides you through optimizing the image display, quantification of lanes, regions or grids, annotation and databasing of your image. Click the buttons on the Navigation panel to access the desired mode. To return to the Navigation panel, click your cursor along the Navigation bar on the left side of the panel.



You can choose to display or hide the Navigation panel by selecting Navigation from the Quick Access bar or choosing Navigation from the Show menu. Hide the Navigation panel to maximize your viewing area when you want to display images on-screen.

- ✓ *Image*—opens the Image panel where you can crop, rotate, or flip your image. In addition, you can access the Image Display window where you can adjust the appearance of the on-screen and printed image.
- ✓ *Lanes*—opens the Lanes panel where you can analyze gels or blots as lane sets.
- ✓ *Manual ROIs*—opens the Manual ROIs panel where you can draw regions of interest and generate intensity, geometric and location data.
- ✓ *Auto ROIs*—opens the Auto ROIs panel where you are guided through the process of automatically defining ROIs using different methods.
- ✓ *Grid ROIs*—opens the Grid ROIs panel to automatically set up an ROI grid.
- ✓ *Annotation*—opens the Annotation window and Annotation panel where you can annotate, create custom views, and label your image with data.
- ✓ *Database*—opens the Database panel where you can set search criteria to find images using your project's File Information.

Adjusting Magnification

Drag the slider to the left to decrease the magnification and to the right to increase the magnification of the image.



Magnification levels are 0.25X, 0.33X, 0.5X, 1X, 1.5X, 2X, 4X, 6X, 8X, 16X, and 32X in all panels except the Annotation panel. The Annotation panel levels are 0.25X, 0.33X, 0.5X, 0.75X, 1X, 1.25X, 1.5X, 1.75X, 2X, 2.5X, 3X, 4X, 6X, and 8X.

 **NOTE:** The Magnification slider maintains the center of the image. This differs from the Zoom tools, which maintain the center position based on where the tool is clicked.

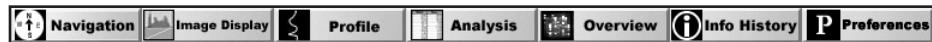
The Status Bar

The Status bar provides easy access to useful functionality as you work through your project.



The Quick Access Bar

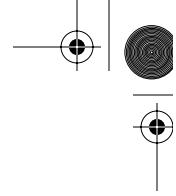
The Quick Access bar is designed to provide easy navigation to commonly used windows and is located above the Image Section.



- ✓ *Navigation*—displays the Navigation panel that guides you through the analysis of images.
- ✓ *Image Display*—opens the Image Display window where you can adjust the appearance of the on-screen and printed image.
- ✓ *Profile*—displays the Profile window (median profile of the lanes) if lane analysis has been completed. Use the profile to compare experimental and standard lanes, or edit the bands defined by the software.
- ✓ *Analysis*—opens the Lane/ROI Analysis windows displaying analysis results for the current image.
- ✓ *Overview*—displays the scaled version of the entire acquired image. A red box identifies the area shown in the Image Section.
- ✓ *Info History*—opens the File Info dialog box and displays the history tab.
- ✓ *Preferences*—opens the Carestream MI Preferences window. The Preferences are divided into five tabs. You can set a preference for an individual project or can apply a set of preferences to any new projects.

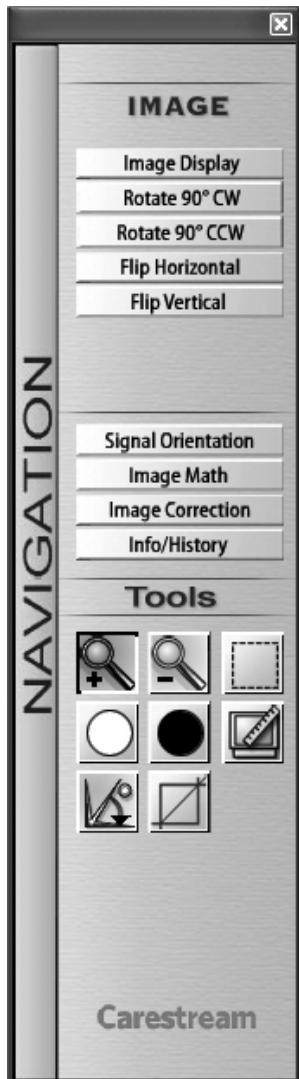
To set the Preferences for all new projects—choose New Project Preferences from the Edit menu or click on the Preferences button on the Quick Access bar when no projects are open.

To change the Preferences for the open project—choose Project Preferences from the Edit menu or click on the Preferences button on the Quick Access bar with the project open and in the active window.

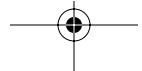
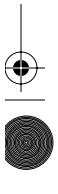
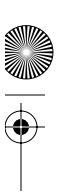


Optimizing Images Using the Image Panel

Once an image is acquired, a new project opens and the image is displayed in the Project window. From within the Project window you can crop, rotate, or adjust how the image is displayed using the Image panel. Let's Review the Image panel by clicking Image from the Navigation panel. The Image panel appears.



- ✓ *Image Display*—opens the Image Display window where you can adjust the appearance of the on-screen and printed image.
- ✓ *Rotate 90° CW*—rotates the image 90° clockwise.
- ✓ *Rotate 90° CCW*—rotates the image 90° counter-clockwise.
- ✓ *Flip Horizontal*—flips the image horizontally.
- ✓ *Flip Vertical*—flips the image vertically.
- ✓ *Signal Orientation*—defines the feature of interest as either white or black. This preference affects the finding algorithms.
- ✓ *Image Math*—opens the Image Math dialog box. You can perform complex calculations on a single or pair of images. The resulting image becomes a new project, with Image History documenting how you created the image. Image Math has three different types of options—Tasks, Formula, and Image Processing Filters.
- ✓ *Image Corrections*—applies a lens or illumination correction to an Image Station 1000, 2000 or 4000 image.
- ✓ *Info/History*—opens the File Information/History window, which stores archival information concerning the project. In addition, a History file tracks any changes that are destructive to the image file, e.g., cropping.
- ✓ *Tools*—provides you the tools you need to perform functions within the Image panel.

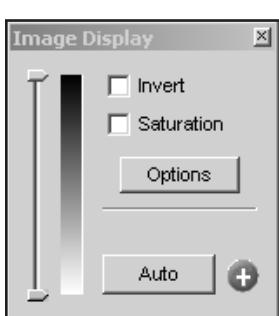


Adjusting Image Display

The Image Display window is available as either a Basic Image Display window or an Advanced Image Display window. The Basic Image Display window, the default option, is designed to provide the most commonly used functions including brightness/contrast, inverting of the image, and saturation display. The Advanced Image Display window provides more comprehensive set of tools. The +/- icon toggles to the Advanced Image Display window. The Options button allows you to change the default option.

 NOTE: The adjustments made in the Image Display window affect the on-screen, exported, and printed image only. These adjustments do not affect the acquired image data.

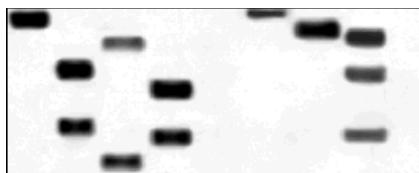
The Basic Image Display Window



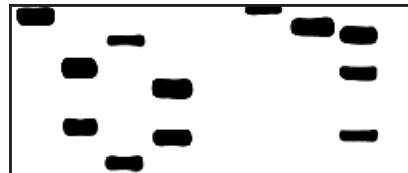
- 1 Select the +/- icon to toggle to the Basic Image Display window. If the Image Display window is not displayed on-screen, choose Image Display from Image panel, click on the Image Display button on the Quick Access bar, or click Image Display from the Show menu.

 NOTE: Options offers preferences for using the Image Display window.

- 2 To adjust the brightness and contrast of the image, use either using the Contrast sliders or the Auto (Contrast) button.
 - ✓ *Contrast sliders*—the top slider adjusts the minimum display value and the bottom slider adjusts the maximum display value.



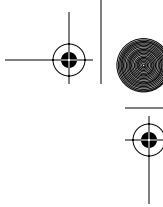
Low Contrast Image



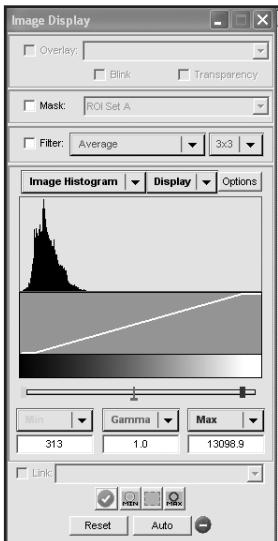
High Contrast Image

 *Auto (Contrast) button*—chooses optimal white and black points that maximize the appearance of the image using the Image Histogram.

- 3 Select Invert to reverse the intensity values; for example whites become black.
- 4 Select Saturation to show any saturated pixels in the image in red.



The Advanced Image Display Window

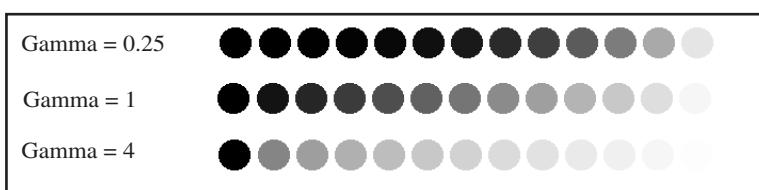


The Advanced Image Display window provides a comprehensive set of options, including brightness/contrast, gamma adjustment, filtering, and pseudocolor. The +/- icon toggles to the Basic Image Display window (default option). The Options button allows you to change the default option.

Contrasting the Image

- 1 Select the +/- icon to toggle from the Basic Image Display window to the Advanced Image Display window. If the Image Display window is not displayed on-screen, choose Image Display from Image panel, click on the Image Display button on the Quick Access bar, or click Image Display from the Show menu.
 - 2 You can adjust the white, black, and gamma points in the image in several ways:

- ✓ Use the *Contrast sliders*—the left slider adjusts the minimum display value, the right slider adjusts the maximum display value, and the center slider adjusts the gamma. Adjusting the gamma of an image disproportionately skews the gray level distribution; higher gamma values lighten the image and lower gamma values darken the image.

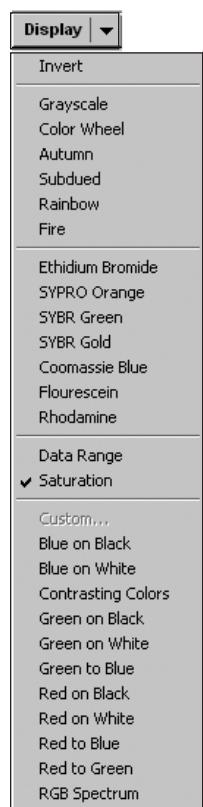


- ✓ Use the *Black Point*, *Gamma*, and *White Point* text edit boxes—to assign numerical values.
 - ✓ Use the *Min*, *Max*, and *Gamma*—pop-up menus to select preset choices.
 - ✓ Use *Auto (Contrast)* button—to choose optimal white and black points that maximize the appearance of the image.
 - ✓ Use the *White Point* and *Black Point Dropper tools*—to assign the values by clicking on a pixel in the image—these new values are used to contrast the image.
 - ✓ Use the *Black Point/White Point Selection tool*—to select the areas to use as the min/max of the selection to contrast the image.

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Guided Tour



Adjusting the Display—Pseudocolor



- 1 Choose the Display pop-up menu to alter the appearance of on-screen, exported, and printed image.
- ✓ *Invert*—reverses the intensity values; for example whites become black.
 - ✓ *Grayscale*—displays the image in grayscale.
 - ✓ *Color Wheel*, *Autumn*, *Subdued*, *Rainbow*, and *Fire*—assign a pseudocolor palette to the intensities in the image.
 - ✓ *Ethidium Bromide*, *SYPRO Orange*, *SYBR Green*, *SYBR Gold*, *Coomassie Blue*, *Fluorescein*, and *Rhodamine*—assign a pseudocolor palette based on their respective color.
 - ✓ *Data Range*—highlights regions of the image that may be over-exposed (saturated) or under-exposed (data lost in background). Information in either of these regions may not be accurate.
 - ✓ *Saturation*—displays saturated pixels in the image in red.
 - ✓ *Blue on Black*, *Blue on White*, *Contrasting Colors*, *Green on Black*, *Green on White*, *Green to Blue*, *Red on Black*, *Red on White*, *Red to Blue*, *Red to Green*, and *RGB Spectrum*—assign a pseudocolor palette based on their respective color.

 NOTE: The pseudocolor adjustment affects the on-screen, exported, and printed image only. These adjustments do not affect the acquired image data.

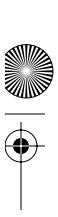
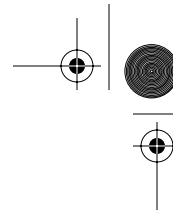
Filtering Images

To apply a filter to an image using the Image Processing Options:

- 1 Click the *Filtering Checkbox* to apply filters.
- 2 Choose the filter type using the *Filter Pop-up Menu*.

Over 30 filters can be applied. Let's review.

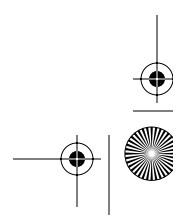
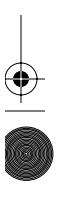
- ✓ *Average*—filter determines the average pixel intensity within a neighborhood and assigns the averaged value to the pixel, resulting in a smoother image.
- ✓ *Row* and *Column Average*—filters average a row or column of pixels within the neighborhood and assigns the averaged value to the pixel smoothing the image.



- ✓ *Low Pass*—filter smooths the appearance of the image by removing the high contrast edges (blurring the edges). This filter is useful for filtering out or reducing noise in the image.
- ✓ *Gaussian*—filter applies a weighted average to the pixels; adding low frequency detail.
- ✓ *High Pass*—filter enhances the high contrast edges in an image; high frequency values in the image brighten, while lower frequency portions darken resulting in a sharper image. This filter may also introduce more noise in your image.
- ✓ *Mean*—filter calculates the mean pixel value within a neighborhood and assigns the mean value to the pixel.
- ✓ *Unsharp Mask*—filter gives added emphasis to the edge detail. This filter can help improve an image that appears out of focus.
- ✓ *Laplacian*—filter enhances all edges within the image and is well suited for looking at the noise of an image.
- ✓ *Horizontal, Vertical, or Diagonal Edge*—filters look for horizontal or vertical edges. The filter takes an image and shifts it by one pixel over and subtracts it from the original.
- ✓ *Gradient North, NE, East, SE, South, SW, W, or NW*—filters are designed to highlight one of eight compass directions—creating edge line drawing.
- ✓ *Emboss North, NE, East, SE, South, SW, W, or NW*—filters enhance the edge, making the image features appear as raised 3D objects. The eight different directions indicate of angle for embossing.
- ✓ *Median*—filter determines the median value within a neighborhood and assigns the median value to the pixel. The median filter despeckles an image and is good for reducing noise but also causes some blurring of the image.
- ✓ *Minimum*—filter determines the darkest pixel value within a neighborhood and assigns the darkest value to the pixel.
- ✓ *Maximum*—filter determines the brightness pixel value within a neighborhood and assigns the brightest value to the pixel.
- ✓ *Normalize Histogram*—(Histogram Equalization) uses a histogram of pixel values to produce a filtered image that has more gray levels for regions of the histogram (peaks) which have the greatest number of pixels.

- 3** Choose how many pixels are used in each filtering operation using the Filter Kernel pop-up menu. The options are 3 x 3, 5 x 5, and 7 x 7 pixels. Increasing the Filter Kernel size potentiates the effect of the filter.

 NOTE: These adjustments affect the on-screen, exported, and printed image only. These adjustments do not affect the acquired image data.



Rotating Images

Use the Rotation tool when you need to straighten less than 90°. If you are analyzing lanes on a gel or blot, rotate your image so that the lanes are vertical. Any curvature to the lanes can be later adjusted using the curved lane option. Once analysis has begun, the rotation functions become inactive.

- ✓ Choose Rotate 90° CW or Rotate 90° CCW using the buttons on the Image panel to rotate the image.
- ✓ Using the Rotation Tool to straighten less than 90°

1 Select the Rotation tool from the Image panel.

2 Click and drag the Rotation tool to draw a line parallel to any feature in the image. When you release the mouse button, the image rotates to the nearest 90° axis. The degree of rotation is displayed in the Status bar.

 NOTE: If you are not satisfied with the rotation, select Undo Rotate in the Edit menu and try again.

Flipping Images

You can flip images using the buttons on the Image panel tools.

- ✓ Choose Flip Horizontal or Flip Vertical using the buttons on the Image panel.

 NOTE: If you are not satisfied with Flip, select Undo Flip in the Edit menu and try again.

Cropping Images

You can crop your image to a selection. Cropping permanently removes a portion of your image. The History records any cropping information within the File Information window.

To crop an image:

- 1** Choose the Crop tool from the Image panel tools. The cursor changes to a crosshair.
- 2** Click and drag to select the region of interest of the image. When you release the mouse button, the selected area appears. You can adjust the positioning of the selection or the size of the selection.
- 3** To crop, move the cursor to the inside of the selection and double-click or click on the Crop tool.

 NOTE: If you are not satisfied with the crop, select Undo Crop in the Edit menu and try again.

 NOTE: Once you have cropped your image and saved the project, you will not be able to undo the crop. You may want to save the cropped image as a new project, using the Save As command.

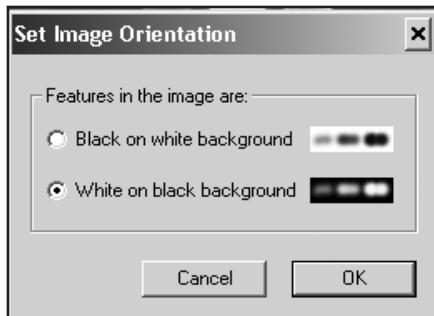
 NOTE: This tool becomes inactive if you have performed any analysis on the image. Cropping the image is destructive to the image file and is recorded in the history.

11

Guided Tour

Signal Orientation

When an image is captured with a Carestream Imaging System, your image capture selections are used by the software to determine whether the features on the image have a white or black signal. If an image is captured with a TWAIN device or is a TIFF or JPEG image file, the program reviews the image histogram to determine signal type. If the image is not well contrasted, the software may have difficulty identifying the signal type. Use the Set Image Orientation on the Image panel to manually define the signal type.

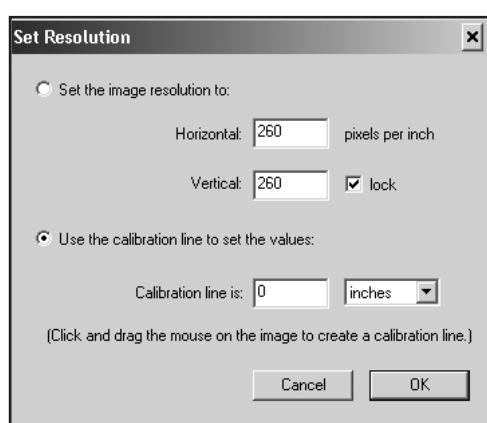


- 1 Click Signal Orientation from the Image panel. The Set Image Orientation window opens.
- 2 Select Black on white background or White on black background depending upon on the signal features.

Set Resolution

Set resolution resizes your image by choosing a dpi or by using a calibration line that you define. This is especially useful if you did not record your zoom settings in the Acquire window during capture with a Carestream Imaging System. This tool becomes inactive if you have performed any analysis on the image.

- 1 Click Set Resolution from the Image panel. The Set Resolution window opens.

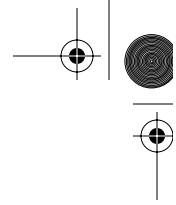


✓ *Set the image resolution to* sets the horizontal and vertical resolution (pixels per inch) of the image.

☞ NOTE: If you specify resolution, you can lock it so that the horizontal and vertical is the same by clicking the checkbox.

✓ *Use the calibration line to set the values* resets the resolution based on a calibration line.

- 2 Click OK.



Merging Two Images Using Image Math

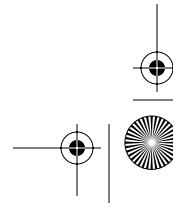
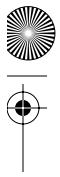
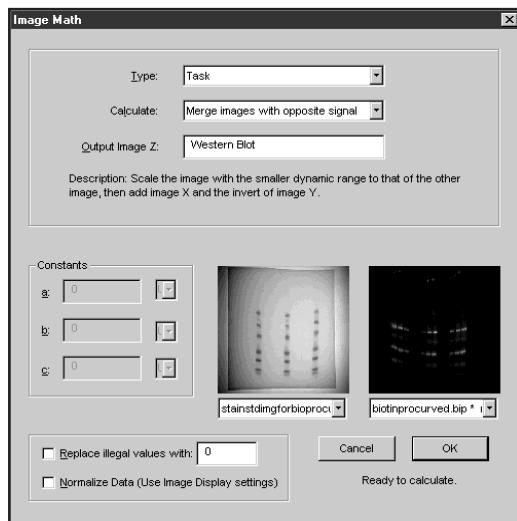
Using Image Math, you can perform complex calculations on a single or pair of images. The resulting image becomes a new project, with Image History documenting how you created the image. Image Math has three different types of options—Tasks, Formula, and Image Processing Filters.

- 1** Open the input images chemiblotXYZ and GelXYZ from the Demo Images Folder.
- 2** Choose Image Math from Imaging panel. The Image Math dialog box appears.
- 3** Choose Task using the Type pop-up menu.

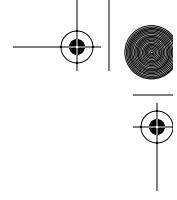


- ✓ **Formula**—displays a list of formulas in the Calculate menu.
- ✓ **Task**—displays a list of common tasks that show in the Calculate menu (i.e., average two images, add two images).
- ✓ **Image Processing Filters**—provides a list of filters that can be permanently applied to the image.

- 4** Using the Calculate pop-up menu choose Merge images with opposite signal.



- 5 Define the Input Images X and Y as chemiblotXYZ and GelXYZ from the Demo Images folder, respectively using the pop-up menus. The files must be open prior to opening the Image Math dialog box to be available for use. When input images are selected, a thumbnail of each appears in the Image Math dialog box.
- 6 Type Merged Test in the Output Image Z text edit box. Click OK.
- 7 The resultant image is opened as a new project. The history records how the image was generated.



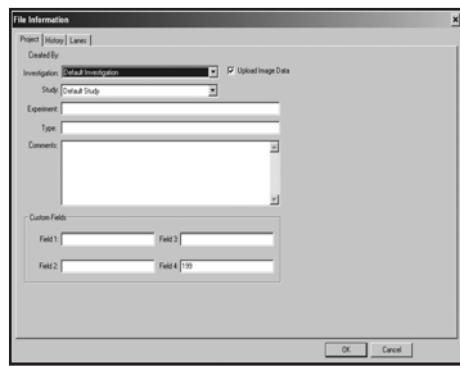
Using the File Information Window

The File Information window is divided into three tabs—Project, History and Lanes.

The Project Tab

Use the Project Tab to enter archival information concerning the project

- 1 View the File Information by choosing Info/History from the Image panel or by clicking the Info History on the Quick Access bar.



- 2 Click on the Project tab.

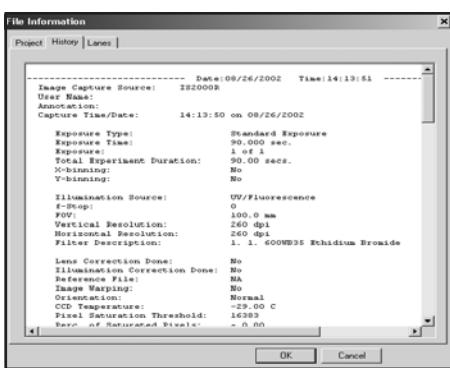
- 3 Enter the project information in the various text edit boxes in the Projects tab.

NOTE: If you use the database feature, the information can be used to retrieve or sort saved projects.

- 4 Click OK to save changes.

The History Tab

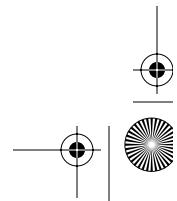
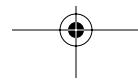
The History tab provides capture information and tracks changes made to the image.



- 1 View the File Information by choosing Info/History from the Image panel or by clicking the Info History on the Quick Access bar.

- 2 Click History tab to review the history of the image.

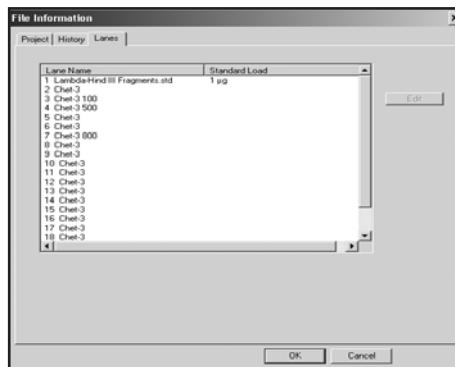
NOTE: The History records image capture conditions. In addition, it tracks any changes that are destructive to the image, i.e., cropping, rotating. This dialog box cannot be edited.



3 Click OK

Lanes Tab

The Lanes tab records lane assignments if you have performed Lane Analysis on the image.



- 1 View the File Information by choosing Info/History from the Image panel or by clicking the Info History on the Quick Access bar.
- 2 Click Lanes tab to review designation of lanes, or use the Edit button in this tab to designate or rename lanes.
- 3 Click OK. The file information is saved.

Tour Stop 2: Lane and Band Analysis

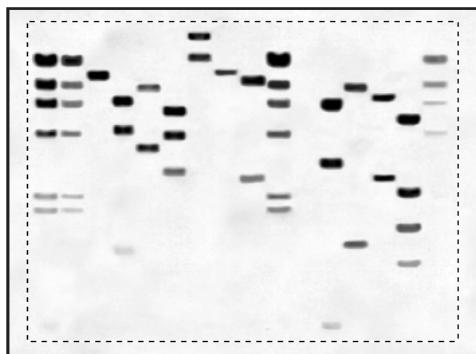
Carestream MI Software allows you to analyze gels and blots for molecular weight and mass. Let's walk through a sample project. If you haven't already done so, open the TIFF image called Tutorial from the Demo Images folder.

Defining Lanes



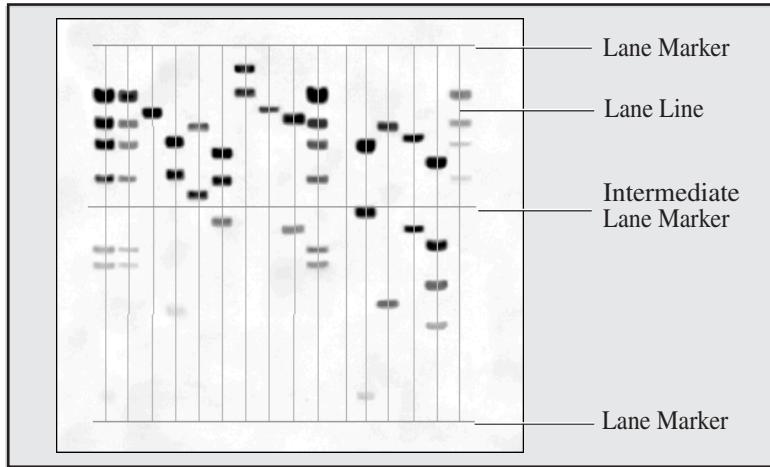
When selecting the area for analysis, be sure that the borders of the selection area are representative of the overall background and do not include the wells or artifacts. The selection is critical in defining lanes and calculating molecular weight and band mass. The upper and lower borders are used in calculating the background level for the analysis, and therefore, their placement is critical.

- 1 Select Lanes from the Navigation panel.
- 2 Choose the Set Search Area from the Lanes panel. The Selection Rectangle tool is activated and a default selection rectangle is displayed.
- 3 Adjust the selection rectangle or redraw the rectangle by clicking and dragging to select the area of the image to be analyzed.



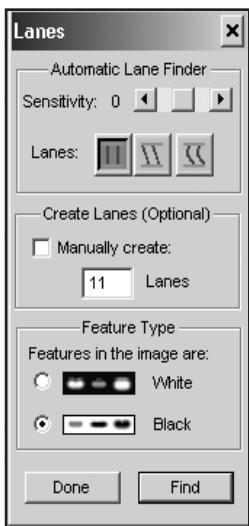
NOTE: Draw the line below the wells and avoid any artifacts, since this will be where the first background reading is taken. Also, it's best to make your selection approximately 1 to 2 mm on either side of the lanes.

- 4** Click New Lane Set from the Lanes panel. Two horizontal Lane Markers appear and are connected with vertical Lane Lines designating the lanes.

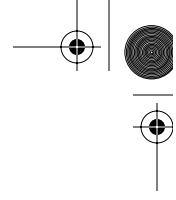


 NOTE: Intermediate Lane Markers are automatically placed on your image. These Intermediate Lane Markers are important in adjusting for the skew or curvature of your image.

- 5** Click Adjust Lanes from the Lanes panel. The Lanes dialog box appears. Note the various options that can be used to edit how the lanes are defined. The Lane Line should pass directly through the center of the lane.



- ✓ *Automatic Lane Finder*—uses a multiple pass algorithm to determine the lanes on the image.
- ✓ *Sensitivity*—changes the lane finding sensitivity. Clicking the right arrow increases the sensitivity of the lane finding process, while clicking the left arrow decreases the sensitivity of the lane finding process.
- ✓ *Lanes*—chooses between three methods of finding the centers of the lanes: straight lanes, slanted lanes, and curved lanes.
- ✓ *Create Lanes (Optional)*—provides the tools to manually create lanes.
- ✓ Click the *Manually Create* checkbox—to ignore the Automatic Lane Finder and allow you to manually enter the desired number of lanes. Enter the number of lanes in the *Manually Create* text edit box.



 NOTE: This number is used by both manual and automatic lane finding. For manual, this sets the number of lanes and spaces them equally across the selection. For Automatic, the program uses this value to determine the best placement of Lane Lines.

- ✓ The *Feature Type buttons*—are used to define the band type as either white or black. This preference affects the lane finding and band finding algorithms.

 NOTE: If you are using a Carestream Imaging System, the selection you make is used by the software to determine signal orientation based on the selections you make in the acquire window.

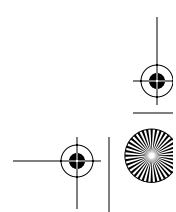
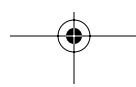
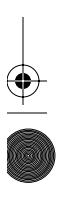
 NOTE: Brightness/contrast adjustments using the Image Display window are dynamically linked to the lane-finding algorithm. To increase sensitivity, adjust the image to best visualize the bands of interest.

- ✓ *Find*—initiates the lane finding process using the defined settings.
- ✓ *Done*—exits the Lanes dialog box and returns to the Project window without any changes.

- 6 Use the Pointer tool to select the Lane Lines and Lane Markers to adjust for the skew of the gel.

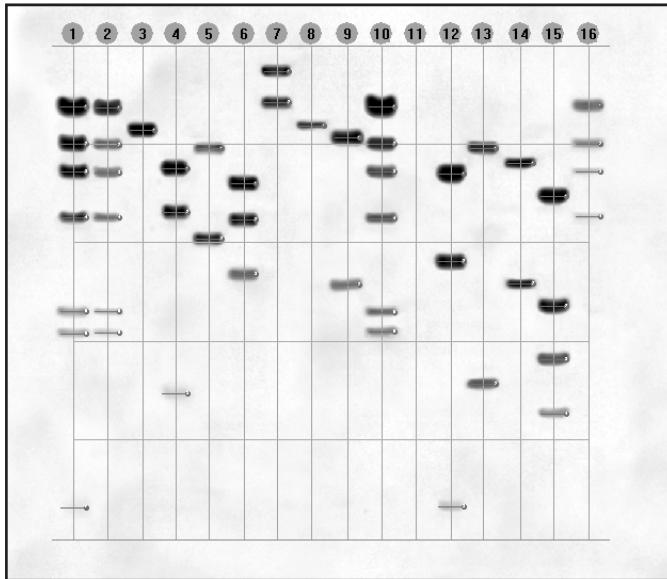
 NOTE: You can move, angle, stretch, and shrink the horizontal Lane Markers. Try to make adjustments that mimic the width of the image and the curve of the lanes.

 NOTE: The top and bottom Lane Markers identify the original selection and have limited movement. For especially warped or skewed gels, the Intermediate Lane Marker(s) can be curved using Carestream MI Iso Molecular Weight Line function. This function allows you to indicate bands of the same molecular weight.



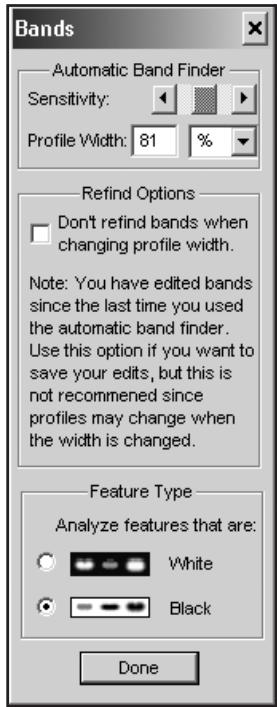
Finding Bands

- 1 Click the Find Bands button on the Lanes panel. Band Labels appear on the image when the analysis is complete. Each band is numbered sequentially from the top of the image.



 NOTE: When an image is low contrast, Carestream MI Software may have difficulty determining whether the band signal is black or white. The correct signal can be manually selected using the Feature Type option in Adjust Bands on the Lanes panel.

- 2 Review the image to determine if the parameters for band finding are set correctly. Look for extraneous bands or bands that have not been defined.



- 3** Click the Adjust Bands on the Lanes panel. The Bands (Adjust Bands for Macintosh) dialog box appears.

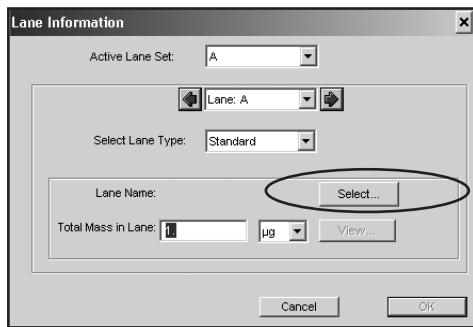
- ✓ The *Sensitivity arrows*—adjusts the band finding sensitivity. The highest sensitivity (+3) finds the most bands, while the lowest sensitivity (-3) finds only well resolved bands. As you adjust the sensitivity, Carestream MI Software updates the number of bands on the image.
- ✓ Adjust the percentage of the Profile Width to encompass the band width. Ideally, you want to encompass the entire band with little or no background within the band rectangle. Click on a band and view the Profile window to see the band rectangle. If the *Refind Options* checkbox is checked bands, only the profile would be adjusted but no new bands will be found.
- ☞ NOTE: A setting of 99% may be necessary for bands which are bleeding into the next lane. A smaller setting may improve accuracy of bands that are curved or nonuniform. Consider using ROI analysis for bands of this type.
- ✓ The *Feature Type buttons*—are used to define the band type as either white or black. This preference affects both the lane finding and band finding algorithms.
- ☞ NOTE: Brightness/contrast adjustments using the Image Display window are dynamically linked to the band and lane finding algorithms. To increase sensitivity, adjust the image to best visualize the bands of interest.

- 4** When all the bands on the image have been found, click Done.

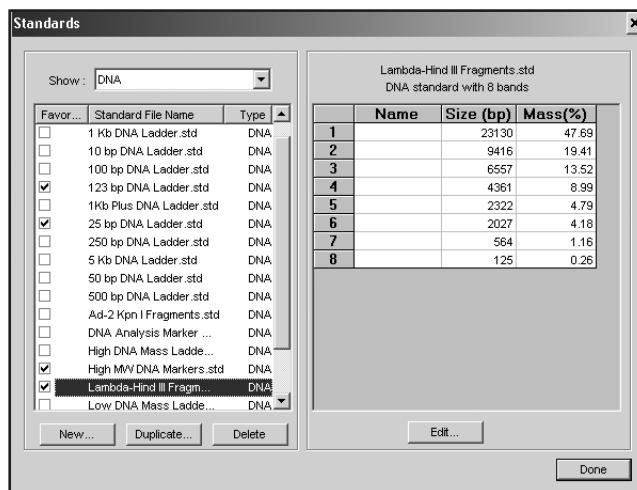
Labeling Lanes

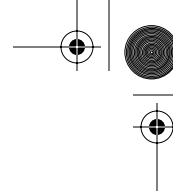
You can define lanes as standard, experimental, or inactive for subsequent analysis. Lanes can be labeled before or after finding bands.

- 1 Click the Set Standards button from the Lanes panel or double-click a vertical Lane Line with the Pointer tool.
- 2 The Lane Information dialog box appears.
- 3 Choose the Active Lane Set in which you would like to label.
- 4 Designate Lane A as a standard by choosing Standard from the Select Lane Type pop-up menu. The Lane Information dialog box is modified for a standard lane.



- 5 Click Select. The Standards dialog box appears and displays the list of the available standards.





NOTE: The Show pop-up menu sorts the standards by type.

- All Standards*—displays all the standards that were pre-loaded with the software and any standards that you have added.
- Favorite Standards Only*—displays your commonly used standards. You can designate a favorite by clicking the standard in the Favorite column.
- DNA*—only DNA standards are displayed.
- RNA*—only RNA standards are displayed.
- Proteins*—only protein standards are displayed.

6 Choose Lambda Hind III Fragments from the standards list and click Done.

NOTE: You can also add your own standards or customize any existing standards using the New or Duplicate button.

7 Enter “1” as the Total Mass in Lane text box and choose units as “ μg ” using the pop-up menu.

8 Using the Next/Previous arrows, label the remaining standard lanes as follows:

Lane	Standard Name	Total Mass
2	Lambda Hind III Fragment	0.5 μg
10	Lambda Hind III Fragment	1 μg
16	Lambda Hind III Fragment	0.2 μg

9 Shift-click to highlight lanes 3 through 9 and 12 through 16.

10 Click OK.

11 Choose Experimental using the Select Lane Type pop-up menu.

12 Enter “Sample” in the select Lane Name text box and Select 1, 2, 3 from the pop-up menu.

13 Select Lane 11. Choose Inactive using the Select Lane Type pop-up menu. Lane 11 is now inactive and will not be used in the analysis.

14 Click OK. All the lanes are labeled.

NOTE: When assigning molecular weights to standard bands, MI assumes that the first band found is the first band in the standards band list. If some of the bands are unresolved, you can manually assign a molecular weight to a band. You can also merge two bands together—MI averages their molecular weights and adds their masses. To access these dialog boxes, double-click on the band or click on Re-Map Standard from the Lanes panel.

Viewing Profiles and Editing Bands

Carestream MI Software creates a median profile of pixel intensities for each lane. Let's view the profiles for Lane 1 and Lane 3.

- 1** Click the Profile on the Quick Access bar located across the top of the Project window. The Profile window appears on the right side of the Project window.
- 2** Click the Pointer tool on the Lanes panel.
- 3** Select a lanes to view by clicking on Line 3 or choosing the Lane 3 from the Exp pop-up menu at the bottom of the Profile window. To display a Standard, select the Standard (Std) pop-up menu at the bottom of the Profile window.
- 4** Click a band in the active lane. A rectangle appears around the band in the Image section.



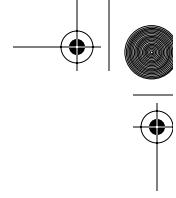
This rectangle represents the image data Carestream MI Software uses during the analysis process. Three red lines appear in the profile to indicate the position of the band's vertical center and upper and lower boundaries. Drag the grab handles to adjust these positions.

NOTE: Increase the image magnification to better view the lane median profile.

Deleting Selected Band Label(s)

- 1** Select the Pointer tool from the Lanes panel.
- 2** Click the band labeled 1 in Lane 6 (Sample F) with the Pointer tool. If more than one band needs to be deleted, Shift-click to select multiple objects with the Pointer tool or click and drag the mouse over an area of the image containing more than one object.
- 3** Press the Delete key to remove the band information from the Image section and the Lane Analysis Data window. The remaining bands are automatically renumbered sequentially.

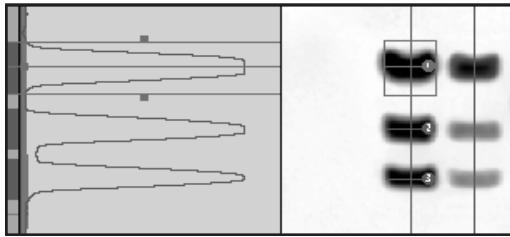
NOTE: To delete all the bands, click Delete Bands from the Lanes panel.



Adding a Band Label

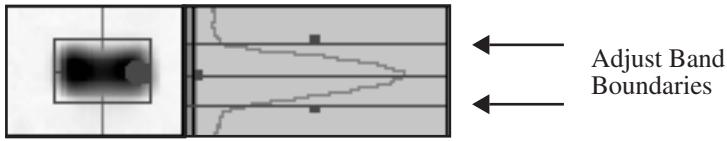
You may need to add Band Labels if all bands of interest were not found automatically.

- 1** Choose the Band Label tool from the Lanes panel.
- 2** Click the center of the band you just deleted in Lane 6 (Sample 4).

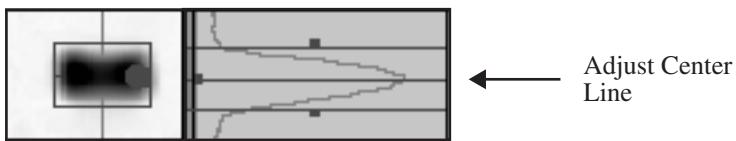


A Band Label appears and all subsequent Band Labels are renumbered. The band is selected and the band editing lines in the Profile window are active.

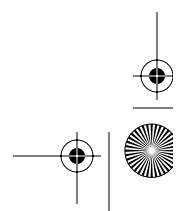
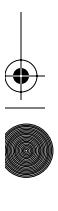
- 3** In the Profile window, drag the top and bottom lines to adjust the band boundaries. This defines the band rectangle used to determine net intensity, and therefore, band mass.



- 4** In the Profile window, drag the center line to position it at the point of peak intensity. Alternatively, drag the Band Label in the Image section into position.



The center line defines the band mobility, which is used to calculate molecular weight if a standard is used.



Adjusting Background Points

You can edit how the background is defined within the lane profile by adding background control points. Backgrounds can be further improved by combining the manual adjustments with fitting bands (which applies Gaussian or asymmetrical Gaussian fitting to the band data).

Adding these points forces the software to adjust the background to:

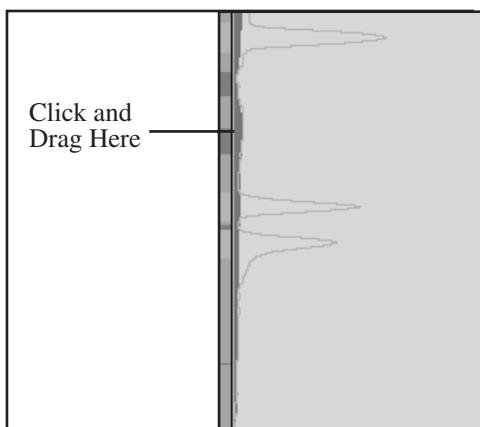
- ✓ Go through local profile points for non-Gaussian fitted bands.
- ✓ Refit the Gaussian model in the localized region using a 10X weight for a single point and a 1X weight for a region of control points.

To add background points along the profile display:

1 Click the Profile on the Quick Access bar located across the top of the Project window. The Profile window appears on the right side of the Project window.

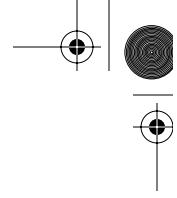
2  Click the Pointer tool in the background portion of the lane profile. The cursor changes to a red line.

3 Click and drag the cursor over the area in which you would like to call the background, or simply click to add individual points.



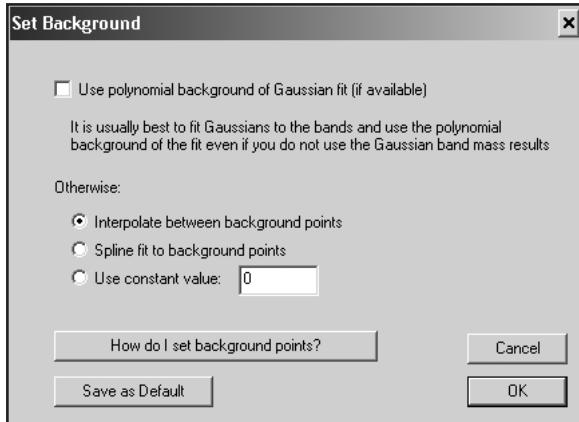
4 The control points are added and the background is refitted (represented in the window in gray). Next to the lane profile, a solid red line denotes the area in which background measurements are taken. You cannot, however, add background points where the signal is saturated.

 NOTE: To remove, repeat the click and drag over area.



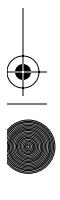
Setting the Background Method

- 1 Click Set Background on the Lanes panel. The Set Background appears.



- ✓ Choose the Background method you'd like to use. A *Polynomial Background*—best represents the background intensity in your lane profiles because a Gaussian fit considers the profile as a composite of the background and the emission from all the bands at every point in the profile.
- ✓ *Interpolate between background points*—useful when you have well separated bands. Each background is estimated by interpolating between background points along the background.
- ✓ The *Spline*—provides more “manual” control over the shape of the background. A spline is a mathematical function that will go exactly through each of the background control points that you specify while at the same time generating a background curve varies smoothly between all the other points that are not marked as background control points. When you add background control points to a spline curve, it is generally best not to add too many and be careful not to add them too close to one another as this can cause the spline to behave erratically as it tries to go through each point in the profile.
- ✓ *Constant Value*—you can enter any value in the text edit box. The number (gray levels) that you enter is subtracted from each pixel within the band rectangle as background.

- 2 Click OK.





Viewing Lane Analysis Data

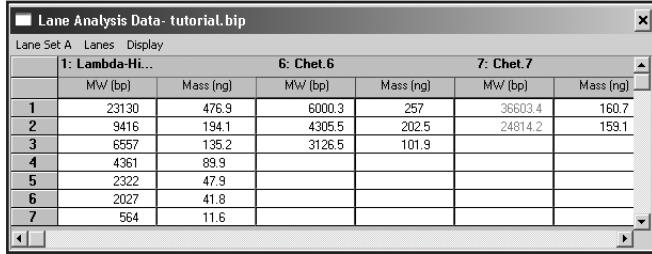
The Lane Analysis Data window can display the following information for each band:

- ✓ *Molecular Weight*—for an experimental band, is the mobility relative to one or more standards.
- ✓ *Mobility*—measured location of the band from the top Lane Marker/Iso Molecular Weight Line relative to the length of the lane. Mobility is reported in pixels, inches, or centimeters. You can select units in the Preferences dialog box.
- ✓ *Mass*—for an experimental band, is determined by comparing the sum of the background subtracted intensities (Net Intensity) of all the pixels in the band with the background subtracted intensities of all pixels in the standards.
- ✓ *Band Area*—the area of the band in pixels², inches², or cm². You can select units for area in the Preferences dialog box.
- ✓ *Band Name*—displays the name of the band, if assigned.
- ✓ *Band Peak Intensity*—provides the intensity value at the peak of the profile.
- ✓ *Net Intensity*—the sum of the background-subtracted pixel values in the band rectangle.
- ✓ *Sum Intensity*—the sum of all the pixel intensities in the band rectangle.
- ✓ *Relative to*—offers two options:
 - other bands in the same lane* which calculates the percent intensity contribution of a band within a lane.
 - in the same bands* in which the same band number across lanes is compared. The reference band is entered in the text edit box.
- ✓ *Mean Background Intensity*—the average background intensity in the band rectangle.
- ✓ *Mean Intensity*—the average intensity of the pixels in the band rectangle.
- ✓ *Maximum Intensity*—the maximum pixel intensity in the band rectangle.
- ✓ *Model Net Intensity* (Only available for Gaussian Analysis)—the mathematical approximation of the Net Intensity using a Gaussian or asymmetric Gaussian model.
- ✓ *Model Mass* (Only available for Gaussian Analysis)—the mathematical approximation of the mass using a Gaussian or asymmetric Gaussian model.



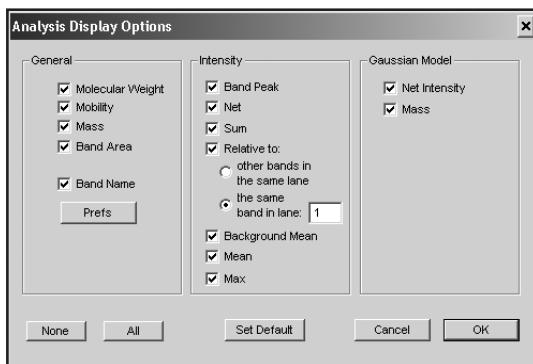
To view lane analysis data:

- 1 Click Analysis on the Quick Access bar. The Lane Analysis Data window appears.



NOTE: If the Profile window is displayed, only the data associated with the Profile window is displayed in the Lane Analysis Data window. If the Lane Profile window is not open, the Lane Analysis Data window displays the results for all active lanes in the Image section.

- 2 Use the Lane Set pop-up menu to choose the Lane Set data you want to view. The active lane set is the default display.
 - 3 Select the lanes you want displayed using the Lanes pop-up menu in the Lane Analysis Data window. Choose All, None, or select the lanes you want displayed.
- NOTE: The defaults selection is All. When you want to display a single lane, choose None to deselect all lanes, and then select the lane you want displayed.
- 4 Choose Display to select the analysis variables you want shown in the Lane Analysis Data window.

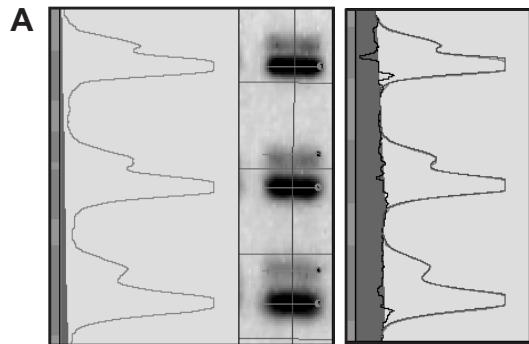


 NOTE: The units of your data are defined in the Preferences. Access the Preferences by choosing Preferences from the Quick Access bar.

- 5 Click OK. The Lane Analysis Data window updates to reflect the variables you have selected.

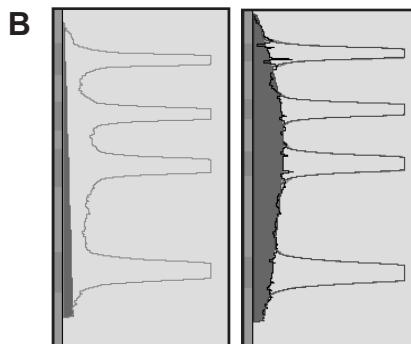
Fitting Bands

In some cases, you may want to fit your bands to more accurately predict the mass and molecular weight of bands. Some instances are unresolved bands (A), an image with an uneven or high background (B) or over-saturated bands (C).

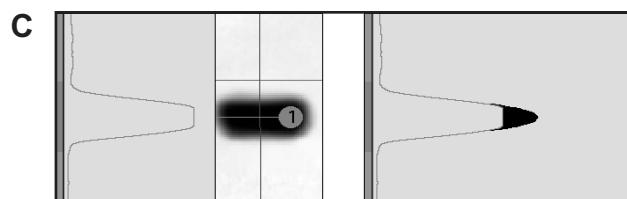


✓ Gaussian's can be useful for estimating poorly resolved bands.

✓ The first gel image has unresolved bands which can be seen in the Profile. The second image shows the Profile after modeling.



✓ When a profile has uneven background as shown in B and/or saturated bands, a Gaussian curve should be used.



✓ Notice the flat peak of the profile on the first image labeled C. This indicates over-saturation of the band.

✓ Gaussian approximation of the Profile—the black region represents the enhanced dynamic range of Gaussians.

Tour Stop 3: Analyzing Spots Using Manual ROI Tools

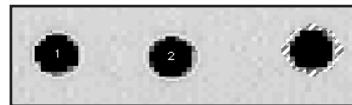
You can select regions of interest (ROIs) to take intensity, size, and volume measurements. There are two basic methods to generate these ROIs within Carestream MI Software—either manually or automatically.



To manually draw ROIs, MI provides tools that allow you to make both area and volume measurements. Once drawn, these ROIs are editable, movable, and can be duplicated. Let's use the manual method to analyze spots.

- 1 Open FluorescenceTMRdots.bip in the Demo Images folder.
- 2 Click Manual ROIs on the Navigation panel. The Manual ROI panel opens.
- 3 Use the Magnification slider or the Zoom tools to maximize the size of the on-screen image.
- 4 Click New ROI Set to record that a new set of ROIs are to be recorded.
- 5 Draw a circle using the Ellipse tool from the Manual ROI panel. Position the cursor in the center of the circle you want to draw, click and drag out to increase the size of the circle. Release the mouse and the drawn ROI is represented by moving red and white dashes (active ROI).

NOTE: By double-clicking on the Ellipse tool, you can choose a preference between drawing from the center or drawing from the corner.



- ✓ To draw a rectangle, select the Rectangle tool from the Manual ROI panel. Position the cursor at the starting point, one corner of the rectangle and click and drag diagonally across to the ending location of the rectangle.

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Guided Tour

✓ To draw a Polygon or polyline, select the Polygon tool from the Manual ROI panel. Position the cursor at the starting location of the first segment. Click and drag to the end of the first segment.

✓ To draw a free form object, select the Free Form tool from the Manual ROI panel. Click and drag the mouse to outline the region of interest.

✓ To draw a line, select the ROI Line tool from the Manual ROI panel. Position the cursor at the starting location of the line, click and drag to the end location.

✓ To use the Magic Wand tool to automatically define an ROI shape, click and drag along a small portion of the edge of the ROI that you want to define. The gray level information from these pixels is used to define the boundary. Alternately, you can click in the center of an ROI and the finder determines the edge using the threshold level value selected in the Magic Wand options.

6 Release the mouse and the new ROI is represented by moving red and white dashes (active ROI).

7 Place your cursor in the center of the active ROI; the cursor changes to a hand.

8 Click and drag the ROI to a new location. A new ROI is created. This may be done in succession to create a series of the same ROI.

9 To record the ROI analysis data, press the Enter key (Return key for Macintosh).

 NOTE: To create a new ROI Set, click the New ROI Set on the Manual ROI panel. The new ROI set becomes the active set.

10 You can choose to hide or show ROI boundaries and ROI labels.

✓ Click ROI Boundaries from the Options pop-up menu in the ROIs tab or choose ROI Boundaries from the Show menu and the ROI submenu

✓ Click the ROI ID Numbers from the Options pop-up menu in the ROIs tab or choose ROI ID Numbers from the Show menu and the ROI submenu

11 Edit ROIs using the tools provided in the Manual ROIs Panel.

✓ Delete a specific ROI by selecting the ROI with the Pointer tool and pressing the Delete key.

✓ Use the Center ROIs button to automatically centers ROIs based on the centroid (center of mass or the 2nd moment of the intensity distribution).

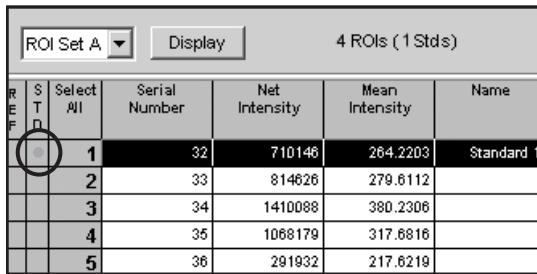
✓ Use the Pointer tool to move or edit an ROI.

✓ The Reactivate ROI tool reactivates an ROI so that it can be used to make multiple recordings of the same shape.

Setting Standards

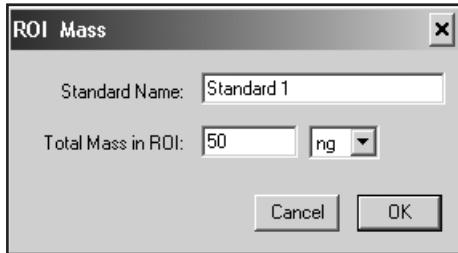
You can designate ROIs as standards and use these standards to generate a mass curve to quantitate unknown ROIs.

- 1** Select Set Standards from the Manual ROI panel. The ROI Analysis window opens.
- 2** Click in the Standard (STD) column to select an ROI as a standard.



REF	S	Select All	Serial Number	Net Intensity	Mean Intensity	Name
			32	710146	264.2203	Standard 1
			33	814626	279.6112	
			34	1410088	380.2306	
			35	1068179	317.6816	
			36	291932	217.6219	

- 3** The selected ROI is added to the standards list and ROI Mass dialog box appears.

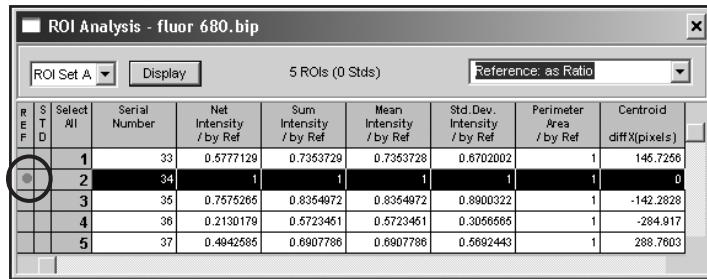


- 4** Enter the standard name and the total mass in the Standard Name and the Total Mass in ROI text edit boxes, respectively.
 - ✓ The maximum number of characters in a standard name is 32.
 - ✓ The units in the Total Mass in ROI pop-up menu corresponds to the options in the Preferences menu.
 - ✓ Each ROI set, must have its own mass curve and set of standards.
- 5** Click OK. The ROI is assigned a mass value and is used in the mass calculation.
- 6** Repeat steps to assign all the standards.

Using References

The Reference Selector in the ROI Analysis Data window designates a single ROI as a reference to measure against all other ROIs.

- Select a row 2 in the ROI Analysis Data window as a reference by clicking in the adjacent Reference (REF) column.



The screenshot shows a software interface titled "ROI Analysis - fluor 680.bip". At the top, there are tabs for "ROI Set A" and "Display", and a dropdown menu set to "5 ROIs (0 Stds)". Below this is a dropdown menu set to "Reference: as Ratio". The main area is a table with the following data:

R	S	Select	Serial Number	Net Intensity / by Ref	Sum Intensity / by Ref	Mean Intensity / by Ref	Std.Dev. Intensity / by Ref	Perimeter Area / by Ref	Centroid	diffX(pixels)
E	T	All								
F	D									
		<input checked="" type="checkbox"/>	1	33	0.5777129	0.7363729	0.7363728	0.6702002	1	145.7256
		<input type="checkbox"/>	2	34	1	1	1	1	1	0
		<input type="checkbox"/>	3	35	0.7575265	0.8354972	0.8354972	0.8900322	1	-142.2928
		<input type="checkbox"/>	4	36	0.2130179	0.5723461	0.5723461	0.3056666	1	-284.917
		<input type="checkbox"/>	5	37	0.4942585	0.6907786	0.6907786	0.5692443	1	288.7803

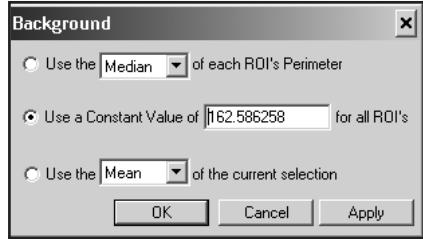
- Choose how you want your data to be displayed using the Reference pop-up menu.
 - Don't Use*—is the default and will not compare the data against the reference set.
 - Reference as % Difference*—displays the ROI data as a percent difference between the reference and the experimental ROIs.
 - Reference as Ratio*—displays the ROI data as a ratio between the reference and the experimental ROIs.

Setting Background

You can define how background for an ROI is calculated. Options include using the perimeter intensity values (median, mean, minimum, or maximum) around each ROI, using any constant value, or using the median, mean, minimum or maximum intensity of a selection. The default is median of each ROI of the perimeter since it is less susceptible to single pixel noise.

- Select Set Background from the Manual ROIs panel. The Background dialog box appears.

2 Select from one of the three options:



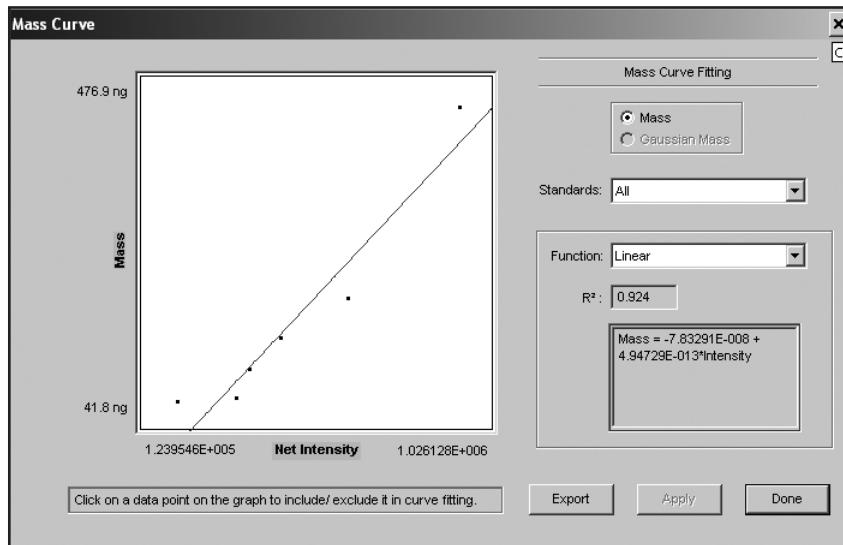
- ✓ Use the perimeter of each ROI—each ROI uses perimeter intensity information to calculate a local background. Use the pop-up menu to choose Median, Mean, Minimum, or Maximum of the each ROI's perimeter.
- ✓ Use a constant value for all ROIs—you can enter any value in the text edit box. The number (gray levels) that you enter are subtracted from each pixel within the ROI as background.
- ✓ Use the current selection to generate a background for all the ROIs—allows you to define a selection using the Selection Rectangle tool. The intensity value from this selection are used to calculate the background. Use the pop-up menu to choose Median, Mean, Minimum, or Maximum of the selection as the background.

3 Click Apply to update the background.

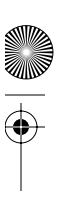
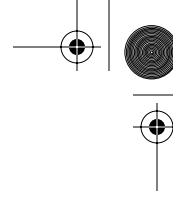
Reviewing the ROI Mass Standard Curve

Once you have defined standards. The standard data is plotted on a graph which is used to calculate the experimental mass values (net intensity versus standard mass). You can optimize the fit function to the data and choose which ROIs to include in the determination of mass.

- 1 Access the Mass Curve dialog box by selecting Mass Curve from Manual ROIs panel. The Mass Curve dialog box appears.



- 2 To maximize the accuracy of your mass determination, review the curves to make sure that the appropriate points are included.
- 3 Choose the fitting function that best represents the data using the Function pop-up menu. The R^2 value aids you in determining the best fit.



Viewing the ROI Analysis Data

The ROI Analysis Data can display the following information for each ROI.

ROI General Data

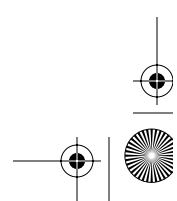
- ✓ *Serial Number*—is a unique identifier for ROIs. If the ROI is deleted, the serial number is never reused. To reset the serial numbers, all ROIs need to be deleted.
- ✓ *ROI Type*—specifies the type of ROI (Rectangle, Ellipse, Shape, or Line).
- ✓ *Comments*—allows you to type information (up to 255 characters) about the ROI.
- ✓ *Name*—assign a name to the ROI (up to 255 characters).
- ✓ *Mass*—for an experimental ROI, is determined by comparing the sum of the background subtracted intensities (Net Intensity) of all the pixels in the ROI with the background subtracted intensities of all pixels in the standard ROIs.

ROI Intensity Data

- ✓ *Net*—is the sum of the background-subtracted pixel values within the ROI.
- ✓ *Sum*—adds together all the pixel intensities within the ROI (includes background).
- ✓ *Mean*—is the average intensity of the pixels within the ROI.
- ✓ *Background*—provides the background value. This is defined within the ROI Analysis Display dialog box.
- ✓ *Maximum*—provides the maximum pixel intensity within the ROI.
- ✓ *Minimum*—provides the minimum pixel intensity within the ROI.
- ✓ *Standard Deviation*—is the square root of the sum of the squared deviation of each pixel value from the mean pixel value. The standard deviation is a useful measure of the statistical error or noise in your data. To find the noise level of your image, create an ROI that includes only background pixels typical of the image. The standard deviation for this ROI is a good indicator of the random variations you can expect for other pixels in the image.

ROI Geometry Data

- ✓ *Width/Height*—for all ROIs except the rectangle and oval, are the horizontal and vertical distances between the centers of the *top left* and *bottom right* pixels described above. For rectangular ROIs, width and height are the number of interior pixels in the horizontal and vertical direction when the ROI is in its unrotated position. Carestream MI Software makes this special case so that area equals width x height for unrotated rectangles, as expected. For ellipses the width and height are always the width and height of the major and minor axes of the interior pixels in





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the unrotated ellipse. The width and height values of rectangles and ovals do not change when they are rotated. Width and height may change for other ROIs when rotated, due to changes in the bounding rectangle.

- ✓ *Area*—for each ROI is calculated by counting the number of interior pixels. This is done so you know exactly how many pixels were used to calculate other statistics, such as the mean and r.m.s. or the interior pixels. If an ROI has no interior pixels (e.g., a line or an open polygon) the area is zero. Note that the area is not calculated from geometry (e.g., $W \times H$ for a rectangle) because when ROIs are rotated the number of interior pixels may change due to the nature of a digital imaging.
- ✓ *Angle*—(for all ROIs except the line ROI) is the current counter clockwise rotation angle of the ROI. For the line ROI the angle is reported as the angle from the +x axis to the line. Note that it makes a difference which end of the line is the starting point and which is the end point of the line. If you click and draw a line ROI up, the angle is $+90^\circ$. If you click and draw the line down, the angle is -90° although the two line ROIs appear the same on the image.
- ✓ *Perimeter Length*—for all ROIs, except the Polygon and ellipse ROI, the perimeter length is calculated by summing the Pythagorean distances between the centers of the vertices (nodes) of the ROI and/or the centers of the end points. For the free form ROI, which has no control points, the perimeter length is the length of the line that connects the centers of the perimeter pixels in the original order that the perimeter points were drawn. When ROIs are rotated their perimeter lengths may change as a result of the new locations of the perimeter pixels, end points or vertices. For oval ROIs, a formula is used to calculate the perimeter of the ellipse that passes through the centers of the perimeter pixels. This is more accurate than using the techniques for arbitrary shapes and polygons because continuous curves and like ovals are not as well represented in digital imaging.

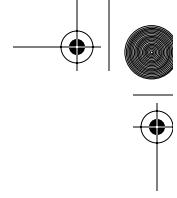
NOTE: The centers of the perimeter pixels is used to calculate the perimeter lengths because this is less ambiguous for very complicated shapes or unclosed figures like open polygons and lines that have no “inside” or “outside.”

- ✓ *Perimeter Area*—is the area that is designated by the perimeter.

ROI Position Data

- ✓ *Centroid*—for all ROIs, the (x,y) location of the “center of mass” or the 2nd moment of the intensity distribution. You can calculate the geometrical center of the ROI from the *top left* and *bottom right* values. The centroid is calculated because it is a better indicator of the position of the ROI; the 2nd moment finds the position of the feature inside the boundary of the ROI even if the boundary is not well centered on the object.

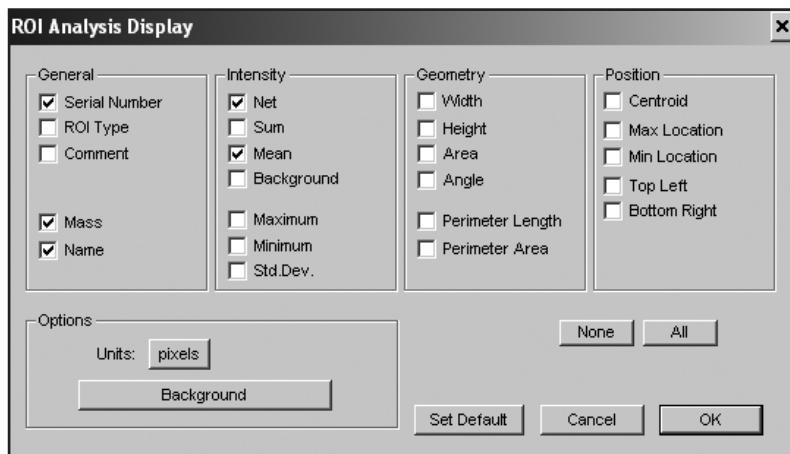




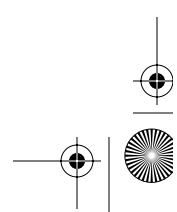
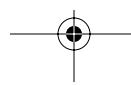
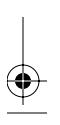
- ✓ *Max Location*—defines the pixel that has the highest intensity value.
- ✓ *Min Location*—defines the pixel that has the lowest intensity value.
- ✓ *Top Left/Bottom Right*—are determined by finding the smallest rectangle that completely encloses the perimeter pixels of the ROI. The (x,y) coordinates of the pixels in the *top left* and *bottom right* corners of the bounding rectangle are what is reported for *top left* and *bottom right*. When you rotate an ROI, its bounding rectangle may change, therefore, the *top left* and *bottom right* values for the ROI may change after it is rotated.

You can choose the ROI set and the analysis data type to be displayed.

- 1 Click Analysis from the Quick Access bar or choose ROI Analysis Data from the Show menu. The ROI Analysis window appears.
- 2 Choose the ROI Set you want to display using the ROI Set pop-up menu.
- 3 Select Display button. The ROI Analysis Display box appears.



- 4 Select the variables that you want to display for all ROIs.
 - 5 Click OK. The data appears in the ROI Analysis Data window.
- ☞ NOTE:** As you click on an ROI analysis data field, that ROI is selected in the Image section. As you select an ROI in the Image section, the data for that ROI is highlighted.

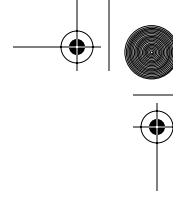


Sorting the ROI Analysis Data

As ROIs are created, ROIs are added to the bottom of the list. You can, however, sort the data by any column.

- ✓ Click any column heading, this sorts the data in descending order.
- ✓ Double-click any column heading to sort in ascending order.
- ✓ To sort in original order, click on the Serial Number heading.

Congratulations, you have successfully learned the basics on Manual ROIs.



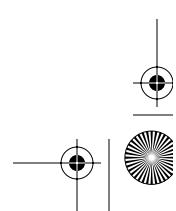
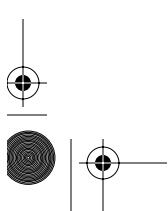
Tour Stop 4: Counting Colonies Using the Automated ROI Finder

Carestream MI Software provides several automated boundary detection methods—Edge Detection, Density Slice, and Threshold. The Edge Detection method is typically not biased by intensity variations or background in the image—therefore, it can be useful for analyzing blots, colonies, and plaques. Density slice is useful when counting blue/white colony assays, gels that are stained with different dyes, or images with uneven backgrounds. Similarly, the Threshold method is sensitive to uneven backgrounds and is useful when your image contains objects that are well separated (i.e., dot and slot blots).



Let's use Edge Detection to count colonies.

- 1 Open the yeast plate image from the Demo Images folder. The yeast plate will be displayed in the Image window.
- 2 Use the Magnification slider or the Zoom tools to maximize the size of the on-screen image.
- 3 Click on the Set Search Area. Using the Ellipse Selection tool, draw a circle to best represent the plate.
- 4 Use the Search pop-up menu to define what part of the image you want to find ROIs.
 - Entire Image*—searches the entire image for ROIs.
 - Selection Only*—searches the interior of the selection. The selection is defined by the Rectangle or Ellipse Selection tools. This option is only available when a selection is defined prior to entering the dialog box.



- ✓ *Oval Quadrant 1, 2, 3, 4*—uses the selection borders to create a circle within the box (the selection is defined by the Ellipse Selection tool). The circle is then divided into four equal quadrants. This option is only available when a selection is defined prior to entering the dialog box.

5 Choose Edge Detection from the Search Method pop-up menu.

 NOTE: *Edge Detection* searches for the edges of features. This method looks for gradient edges and is usually not biased by intensity variations or background in the image.

6 Use the Gradient text edit box or slider to define a gradient setting to 20%.



 NOTE: This value is used by the algorithm to determine how steep of a slope the edge must have (rate of change of intensity across the edge) before it is defined as a new ROI object. The higher the percentage, the sharper the edge and more intense the transition.

- ✓ For low contrasted images (low signal to background), lower the gradient value.
- ✓ For well contrasted images, increase the gradient value.

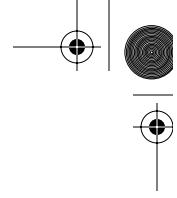
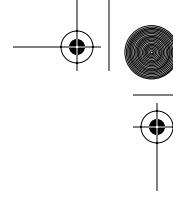
7 Choose the Smooth Edges checkbox to remove noise artifacts that appear around the edge of the ROIs. Boundary detection does not always create smooth edges around the object.

8 Use the Grow ROIs by checkbox and text edit box to define the number of pixels you want to grow the edge off the feature edge. The Grow feature should be used when:

- ✓ The gradient is set too low and large number of artifacts are found. When a larger gradient doesn't find the edge—use the Grow command to find proper edges.
- ✓ Two or more ROIs are connected. In this case setting the gradient higher separates the ROIs.

9 Use the Minimum (Min) and Maximum (Max) checkboxes and text edit boxes to define the minimum and maximum sizes of the ROIs found. ROIs found below the minimum or above the maximum values are automatically removed.

 NOTE: If you have drawn a Reference ROI, the information from the reference is automatically entered.



10 Verify that the signal of the feature you are searching for is accurately defined as either as either white or black. This preference affects the Auto Find algorithms.

NOTE: If you are using a Carestream Imaging System for image capture, the selection you made when acquiring images is used by the software to determine signal orientation.

11 Click Find. Carestream MI uses these settings to find ROIs.

12 Review the ROIs found and refine the search, as needed.

- ✓ Use the Gradient text edit box or the slider to adjust the Find parameters. Click Replace.
- ✓ Separate closely spaced or touching ROIs using the Separate ROIs tool. See *Editing Automatic ROIs*, later in this chapter. Using ROI tools, you may also manually define ROIs that were not found.
- ✓ The Add button allows you to add selection results together. This can be useful in finding ROI in different regions of the image using different boundary detection techniques. To use this option, make a selection, find ROIs and then exit the Find ROIs dialog box. Define another selection, and begin the Find ROIs process again. Add becomes an available option in the dialog box.

13 When you are satisfied with the results, choose Done to exit the dialog box. You can create an unlimited number of ROI Sets by repeating the above steps.

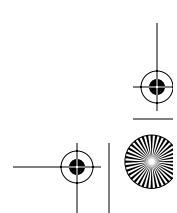
14 To separate two connected ROIs, click and drag across the boundary of two connected ROIs with the Separate ROIs Tool.

NOTE: The Separate ROIs tool only works with free form ROIs drawn by the ROI Free Form tool, the Magic Wand tool or the Auto ROI methods.

15 Use the Magic Wand tool to define an ROI not found. To use, click and drag along a small portion of the edge of the ROI that you want to define. The gray level information from these pixels is used to define the boundary.

Alternately you can click in the center of an ROI and the finder determines the edge using the threshold level value selected in the Magic Wand options. You can adjust how close the Magic Wand tool sets the threshold level with respect to its estimate of the background. To access the Magic Wand tool options, double-click on the tool.

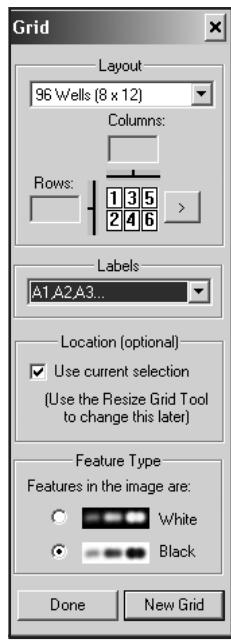
16 You can view the ROI Analysis Data window as described in Tour Stop 3 or you can get a count of ROIs from the Status bar. The first value identifies the specific ROI that has been selected. The second value reports the total number of ROIs found.



Tour Stop 5: Analyzing Microtiter Plate Assays Using the Grid ROI Tools

You can perform measurements by creating grids of regions of interest (ROIs) on the image for analyzing slots, spots, arrays, or microplates that are regularly spaced in rows and columns. The Grid ROIs panel guides you through making an ROI Grid. You can make a grid containing rectangles or ellipses. Once drawn, these ROIs are editable, movable, and can be duplicated.

You can choose to apply a pre-set grid or create your own grid. Once created, you can rotate, reposition or resize your grid and ROIs. If you are routinely performing similar experiments, you can save the grid as a template. When you apply a template to an image, an exact copy of the previously defined ROIs is applied.

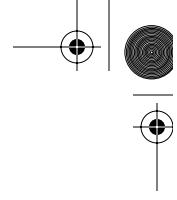


- 1 Open the GL 96 well plate from the Demo Images folder. The 96 well microtiter plate will be displayed in the Image window.
- 2 Select the Grid ROIs panel from the Navigation panel.
- 3 Choose the Set Search Area from the Grid ROIs panel. The Selection Rectangle tool is selected and a default selection rectangle is displayed.
- 4 Adjust the selection rectangle or redraw the rectangle by clicking and dragging to select the area of the image to be analyzed.
- 5 Choose Set Reference ROI—the ROI Ellipse or ROI Rectangle will become the active tool. Choose the Ellipse tool.

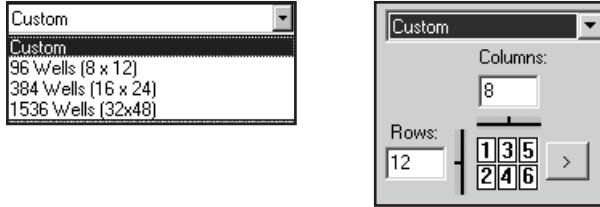
NOTE: The software remembers the last ROI type drawn in Grid ROIs.
- 6 Draw an ROI to encompass the object you want to measure.



- 7 Click Make New Grid on the Grid ROIs panel. The Grid dialog box appears.

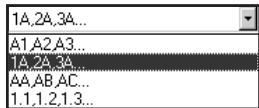


- 8** Choose the size grid you want drawn using the Grid pop-up menu. Several common grid sizes are available for selection or choose Custom to define your own.



NOTE: Once you have created a custom grid, you can save the grid as a template. Refer to *Using ROI Templates*, later in this chapter.

- 9** Use the Label pop-up menu to define how you want the analysis data to be labeled.



NOTE: The labels appear in the comments section of the ROI Analysis Data window.

- 10** Verify that the signal of the feature you are searching for is accurately defined as either as either white or black. This preference affects the Auto Find algorithms.

NOTE: If you are using a Carestream Imaging System, the selection you made when acquiring images is used by the software to determine signal orientation.

- 11** Click Make Grid. A grid is drawn.

NOTE: If you have made Set Grid Area with the Rectangle Selection tool, the Location checkbox is automatically checked. The grid will be drawn within the selection.

NOTE: Click Center ROIs from the Grid ROIs panel to automatically center the ROI based on the centroid (center of mass or the 2nd moment of the intensity distribution).

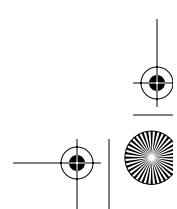
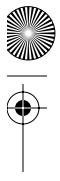
- 12** To edit the ROIs and the grid:

NOTE: Magnify the image if you have trouble selecting the control point.

The Move ROI tool is used to move or adjust the position of an ROI within the grid. You can select multiple objects clicking and dragging the mouse over an area of the image containing more than one object.

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The Resize All ROIs tool is used to edit the shape of all ROIs within a grid. Click on the tool and then select an ROI to resize. Position the tool over a control point. Click and drag the control point in the direction that you want to resize the object. All ROIs within the grid will be resized.



The Rotate All ROIs tool is used to rotate all ROIs within a grid. Click on the tool and then select a control point on the ROI and rotate in the any direction. All ROIs within the grid will be rotated.



The Resize Grid tool resizes the spacing of the grid. Click on the tool and then select an ROI to reposition. Click and drag the ROI into position. The grid spacing will change, but the ROI size is not altered.



The Rotate Selected ROI tool is used to rotate a selected ROI within a grid. Click on the tool and then select a control point on the ROI and rotate in the any direction. Only the selected ROI will be rotated.



NOTE: You can select multiple ROIs by Shift-clicking objects or by dragging the Resize Selected ROI tool over the ROIs. When you resize on ROI, all selected ROIs will resize.

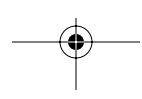


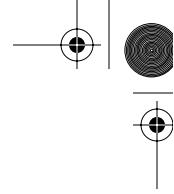
Delete a specific ROI by selecting the ROI with the Pointer tool and then pressing the Delete key. Delete all the ROIs in a Set by choosing one of the ROIs in the set and clicking on Delete ROI Set from the Grid ROIs panel.



NOTE: You can select multiple objects by Shift-clicking the ROIs, by dragging the Pointer tool over the ROIs, or by selecting them in the ROI Analysis Data window.

13 You can set standards, references and view mass curve and analysis as described in Tour Stop 3

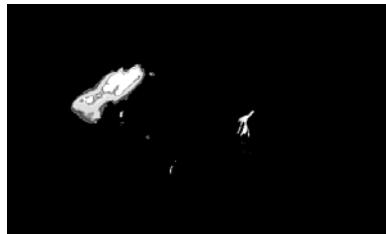




Tour Stop 6: Overlaying Two *in vivo* Images

Using the Advanced Image Display window, you can display one image on top of another image. You may also want to pseudocolor one or both of your images prior to overlaying.

- 1 Open the mouse X-ray and mouse CY5 files from the Demo Images folder.
- 2 Use the contrast features in the Image Display window to best display the features of interest.

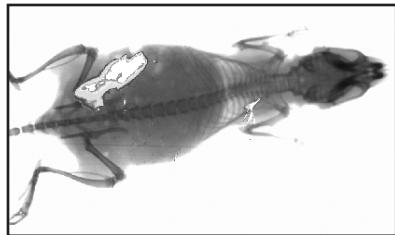


NOTE. Use the contrast features in the Image Display window to best display the features of interest. You can use pseudocolor to better display the features that you are overlaying. See Tour Stop 1: *Software Overview*.

- 3 Click on the background X-ray image to make that project the active window.
- 4 Open the Advanced Image Display window by choosing Image Display from Imaging panel, clicking on the Image Display button on the Quick Access bar or clicking Image Display from the Show menu. Use the +/- icon to show the Advanced Image Display window.
- 5 Click Overlay and use the pop-up menu to select the overlay image (mouse CY5).

NOTE: The Overlay pop-up menu is populated with the list of all currently open documents.

- 6 Click Transparency or Blink.





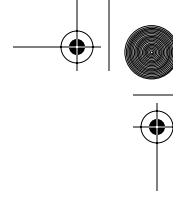
- ✓ *Blink checkbox*—when selected, the overlay image is shown and hidden at approximately 1 second intervals. This is functionally the same as selecting and deselecting the *Overlay checkbox* once every 1 second. This function is particularly useful if you are looking for change between two images (e.g., an object that changed position or brightness).
 - ✓ *Transparency checkbox*—when selected, the overlay image is shown on top of the active document image, but wherever the overlay image has a value far below the minimum display value for the overlay image, the overlay image appears transparent to allow the active image to be seen. The transparent color could be black or white, depending on the invert setting.
- 7 You can print and export the overlay image.
- ✓ Overlays in “Blink” mode will not print.
 - ✓ For “Transparency” mode, the transparent image and the active document image will be printed as they are displayed. On a color printer the images should print with their respective color tables.
 - ✓ Export Images—Windows Users can select metafile format (.emt) to export the image using the current overlay display settings and with separate color tables. Macintosh Users can export PICT files, 32-bit color image with the colors of the two images properly rendered. If greater resolution is required, color output files at full image resolution can be generated.

Overlays an ROI Set

If you do not want to overlay all the features in an image, you can use an ROI set to mask out the features that you do not want displayed. This is similar to Overlay except that you are only overlaying the feature(s) which are interior to an ROI in the ROI set.

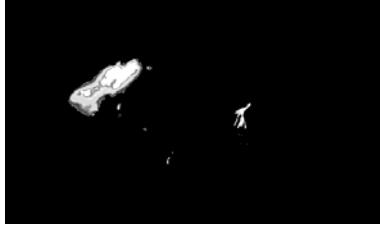
- 1 Open the project which contains the background image and adjust the display to maximize contrast.



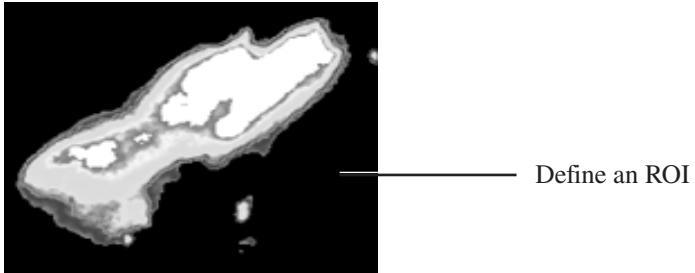


- 2** Open the project you want to be the overlay image and adjust the display to maximize contrast.

NOTE. Use the Image Display window to best display the features of interest. You can use pseudocolor to better display the features that you are overlaying. See *Adjusting the Display—Pseudocolor* in Tour Stop 1.

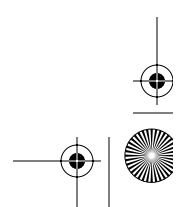
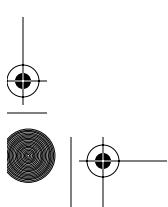


- 3** Define one or more ROIs on the overlay image using Manual ROIs, Auto ROIs or Grid ROIs. The ROI boundaries defines what will be displayed. Do not close the project.



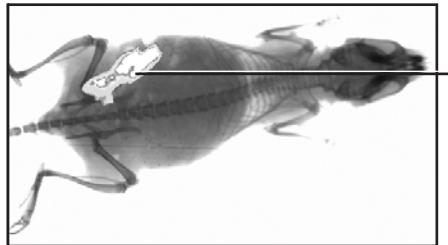
- 4** Open the Advanced Image Display window by choosing Image Display from Imaging panel, clicking on the Image Display button on the Quick Access bar or clicking Image Display from the Show menu. Use the +/- icon to show the Advanced Image Display window.
- 5** Click on Mask and choose the ROI set you created above using the pop-up menu.
- 6** Click on the background image to make that project the active window.
- 7** Select Overlay in the Advanced Image Display window Overlay and use the pop-up menu to select the overlay image.

NOTE: The Overlay pop-up menu is populated with the list of all currently open documents.





8 Click Transparency or Blink.



Only the ROI is overlaid

- ✓ *Blink checkbox*—when selected, the overlay image is shown and hidden at approximately 1 second intervals. This function is particularly useful if you are looking for change between two images (e.g., an object that changed position or brightness).
- ✓ *Transparency checkbox*—when selected, the overlay image is shown on top of the active document image, but wherever the overlay image has a value at far below the minimum display value for the overlay image, the overlay image will be transparent to allow the active image to be seen. The transparent color could be black or white, depending on the invert setting.

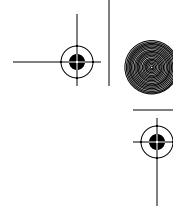
9 The ROI's from the overlay will appear on the background image.

10 You can print and export the overlay image.

- ✓ Overlays for “On” and “Blink” modes will not print.
- ✓ For “Transparency” mode, the transparent image and the active document image will be printed as they are displayed. On a color printer the images should print with their respective color tables.
- ✓ Export Images—Windows Users can select metafile format (.emf) to export the image using the current overlay display settings and with separate color tables. Macintosh Users can export PICT files, 32-bit color image with the colors of the two images properly rendered. If greater resolution is required, color output files at full image resolution can be generated.

Congratulations, you have successfully learned how to overlay two images

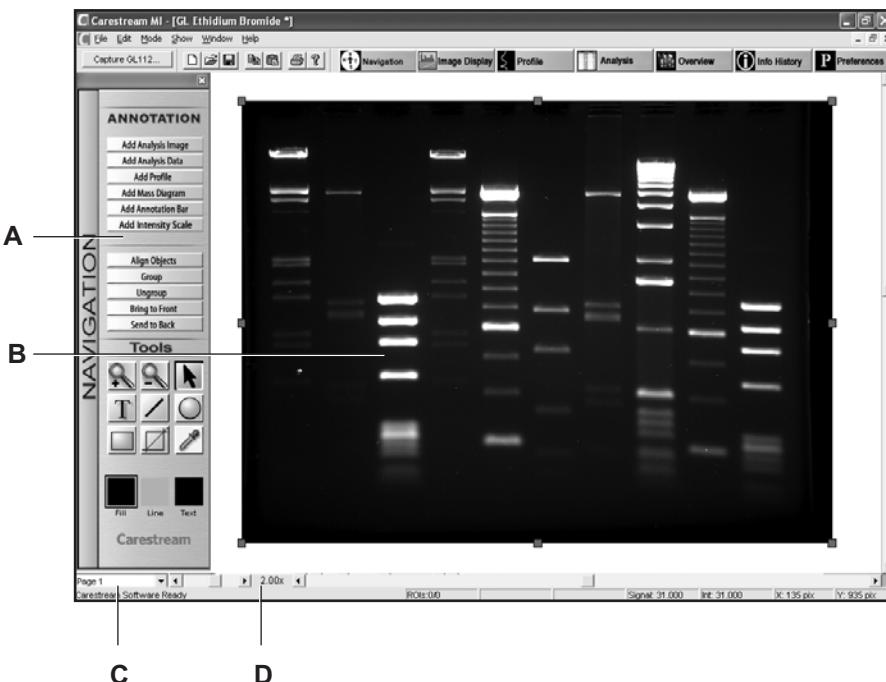




Tour Stop 7: Annotating the Image

The Annotations window provides you with a copy of the image for adding comments, labeling, or preparing the image for publication. You can choose to show or hide Lane Markers, Lane Lines, Band Labels, or ROI Labels. We will use the Tutorial image used in Tour Stop 2.

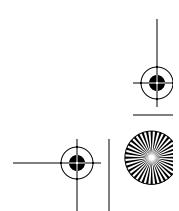
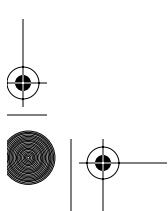
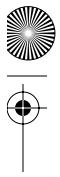
- 1 Choose Annotation from the Navigation panel. The Annotation window appears.



- A *Annotation panel* provides you with the features and tools preparing your images and data for presentation and publication.
- B The *Annotation window* displays a printable area that corresponds to the default printer and Page Setup selections.
- C *Page pop-up menu*—create up to 10 pages of annotations per project allowing you to store detailed information about your experiment in your project.
- D The *Magnification slider* provides digital magnification from 0.25X to 8X. The Magnification slider maintains the center of the image. This differs from the Zoom tool which shifts the center of the image to wherever the tool is clicked.

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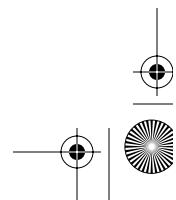
The Annotation Panel

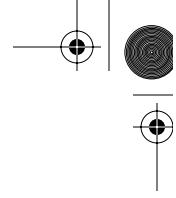
The Annotation window provides you with a copy of the image for adding comments, labeling, or preparing the image for publication.

The Annotation Panel Buttons



- ✓ *Add Analysis Image*—adds an image to the Annotation window. When selecting Add Analysis Image, the Annotation Image Layout window opens. You can customize the size, zoom in on features of interest, and choose the portion of the image you want to display.
- ☞ NOTE: You can cut and paste images from other projects or sources, i.e., Powerpoint, Photoshop, JPG or TIFF).
- ✓ *Add Analysis Data*—opens the Analysis window and allows you to copy the data from the Analysis worksheet as individual values or as columns of data.
- ✓ *Add Profile*—appends a lane profile to the image. The lane profile is only available if the image has been analyzed for lanes and bands. The profile that is appended to the image is the lane(s) that is displayed in the Profile window prior to entering the Annotation mode.
- ✓ *Add Mass Diagram*—displays the mass standard curve in your annotations, if you analyzed your image and assigned mass standards.
- ✓ *Add Annotation Bar*—When acquiring images using Carestream Imaging Systems, you have the option to append an Annotation bar to your image. Once you have made the selection to append an Annotation bar, the information can be edited in the Annotation window.
- ✓ *Add Intensity Scale*—provides a visual index of intensities. The color index is defined in the Advanced Image Display window.
- ✓ *Align Objects*—lines up selected objects precisely along a horizontal or vertical axis, as well as distributes them evenly across a horizontal or vertical axis. If you are aligning to the right, all selected objects align to the right edge of the selected object furthest to the right.





- ✓ *Group*—takes individual objects and fuses them into a single unit that can be moved as one object.
- ✓ *Ungroup*—separates “fused” objects that had been grouped. Each individual object can be edited independently.
- ✓ *Bring to Front*—you can change the stacking order of objects that are overlapped. The Bring to Front button rearranges the stacking order, placing the selected object on top of the stack.
- ✓ *Send to Back*—you can change the stacking order of objects that are overlapped. The Send to Back button rearranges the stacking order, placing the selected object behind all other objects in the stack.

The Annotation Panel Tools

Tools in the Annotation panel are specific to this mode and are designed to help you annotate your image.



Use the Zoom tools to adjust the size of the image through levels of magnification (0.25X, 0.33X, 0.5X, 0.75X, 1X, 1.25X, 1.5X, 1.75X, 2X, 2.5X, 3X, 4X, 6X, and 8X). To increase the magnification, click the image with the + Zoom tool. To reduce magnification, click the image with the - Zoom tool.

When you change the magnification with the Zoom tools, the software positions the point where you clicked in the center of the Image section. This differs from the Magnification slider, which maintains the center of image in the window.



Use the arrow-shaped Pointer tool to select objects to move or resize. Shift-click on multiple objects with the Pointer tool or drag the mouse over an area of the image containing more than one object.



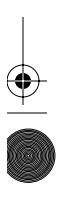
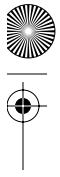
Use the Text tool to annotate with image using text. You can select custom fonts, sizes, styles, and rotation of the text. To use, select the Text tool, define the area in which you want the label to appear by clicking and dragging on the image.



The Line tool is designed to draw lines, arrows, or brackets on the image.



The Ellipse tool is designed to draw circles or ovals which can either be filled, framed, or filled, and framed. Double-click on the Ellipse tool to access drawing options.

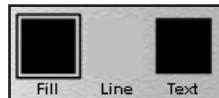


 The Rectangle tool is designed to draw boxes which can either be filled, framed, or filled and framed. Double-click on the Rectangle tool to access drawing options.

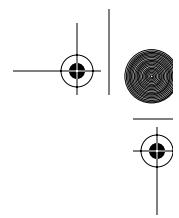
 The Crop tool masks part of the object using a rectangle to trim the edges of the image so they are not displayed.

Use the Dropper tool to select a new foreground or background color by clicking the Dropper tool on the object.

 NOTE: The currently selected color (as shown by the frame) corresponds to whether or not the Dropper tool selects a foreground or background color.



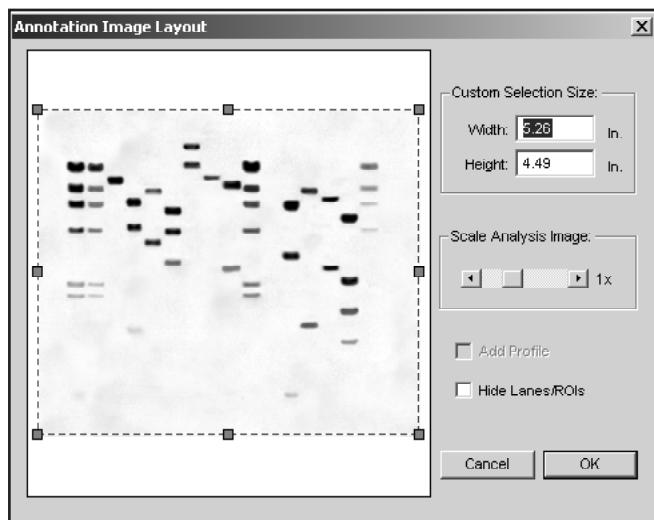
Use the Fill, Line or Text Color option buttons to define color. Click the Color Selector to adjust the color. For color reproducibility, use the More Choices button to access the color wheel.



Adding an Analysis Image

If you are preparing a presentation, you can add multiple copies of your experimental image to highlight important features. This enables you to show specific bands at different magnifications or eliminate lanes that are not of interest.

- 1 Choose Add Analysis Image from the Annotation panel. The Annotation Image Layout window appears.



- 2 Use the Custom Selection Size text edit boxes to define the size (in inches) of the image you want the image to appear on the Annotation page.
- ✓ Adjust the Scale Analysis Image slider to magnify or reduce the image size between 0.25X and 8X. The image will not resize in the Annotation Image Layout window, however, the portion of the image that will be displayed is bounded in a red Selection Rectangle. The magnification factor appears to the right of the Scale Analysis Size slider.
- ✓ Click and drag the Selection Rectangle to the region of the image you want to display. The region inside the selection is displayed in the main Annotation window.
- ✓ To show the Lane/ROI or Profile, select the Hide Lanes/ROI or Profile checkboxes.
- 3 Click OK (Set for Macintosh).

NOTE: You can add multiple copies of the image to highlight important features. This enables you to show specific features at different magnifications or eliminate parts of the image that are not of interest.

Cropping the Image Using the Grab Handles

 1 Using the Crop tool, click once on the image to select the object. Nine grab handles appear.

2 When you drag the cursor over a grab handle, the cursor changes into one of three different icons.

 Change size vertically.

 Change size horizontally.

 Change both vertical and horizontal size of the image.

 NOTE: This operation is not a true cropping of the image. It merely moves a mask over the image so as to display only the region of interest defined by the selection window. The underlying image remains intact and can be viewed, whole or in part, by readjusting the grab handles or by moving the entire selection window.

3 Click OK when finished.

Repositioning the Image

You can move the image anywhere within the Annotation window. If you drag the cursor over the image, the cursor changes to an open hand.

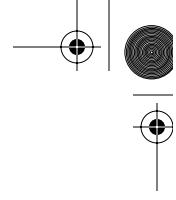
- ✓ To change the image placement on the page, click and drag. As you move the cursor, the image can be repositioned.
- ✓ Click and drag to move the entire image. Click and drag with the Shift key (Options key in Macintosh) depressed to reposition the image within the region outlined by grab handles.

Importing Image Objects into Annotations

If you are preparing a presentation, you can import objects via the clipboard using the copy and paste functions. The imported object is a separate item within the Annotation window.

Images of any color type can be imported—full color, 256 index color, grayscale, and black and white.

 NOTE: If you want to copy images between projects, make a selection, copy from within the Image window, and paste into the Annotation window. The image is pasted as an object.



Adding Analysis Data

Lanes, bands, and ROI data can be labeled by dragging and dropping text from an Analysis Data window.

- 1** Open Analysis data set by selecting Analysis from the Quick Access bar.
- 2** Display all columns and values needed for annotation.
- 3** Choose Annotation from the Navigation panel.
- 4** Select a cell using the Pointer tool. Click and press until a light blue line or gray background appears around the selected cell.

NOTE: Any cell from the Analysis Data windows can be moved to the Annotation window. When you drop a text object, the text object maintains the attributes of the previous text object, i.e., font, size, style, and rotation.
- 5** Adjust the label position using the Pointer tool. Click and drag to reposition the label.
- 6** Adjust label size with the Pointer tool. Double-click on a label to adjust the format using the Text Options dialog box.

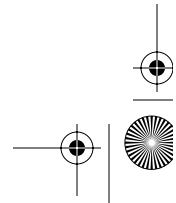
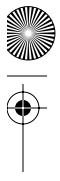
Dragging and Dropping a Column from an Analysis Data Window

A column of data can be dragged and dropped from an Analysis window.

- 1** Open Analysis data set by selecting Analysis from the Quick Access bar.
- 2** Display all columns and values needed for annotation.
- 3** Choose Annotation from the Navigation panel.
- 4** Move an entire column by clicking and dragging the column label to the Annotation window.

The Lane Number Label moves all data points (Lane Analysis only).
 The column heading moves a column of data without a table.

NOTE: The graphic retains its shading and gridlines. You cannot edit these text fonts.
- 5** Use the Pointer tool to adjust the label position. Click and drag to reposition the label.
- 6** To adjust label size, select any of the grab handles to resize the text block or double-click to access the Annotation Image Layout window.
- 7** Use the Custom Selection Size text edit boxes to define the width and height of an image.

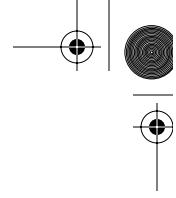


- 8** Adjust the Scale Analysis Image slider to magnify or reduce the image size between 0.25X and 8X.

 NOTE: The image will not resize in the Annotation Image Layout window, however, the portion of the image that is displayed is bounded in a red Selection Rectangle. The magnification factor appears to the right of the Scale Analysis Size slider.

- 9** Select the region of interest. If you have selected an image size which is too large, you may not be able to view the entire image in the Annotation window. Reposition the selection region by clicking and dragging the red selection rectangle in the window. The region inside the selection is displayed in the main Annotation window.

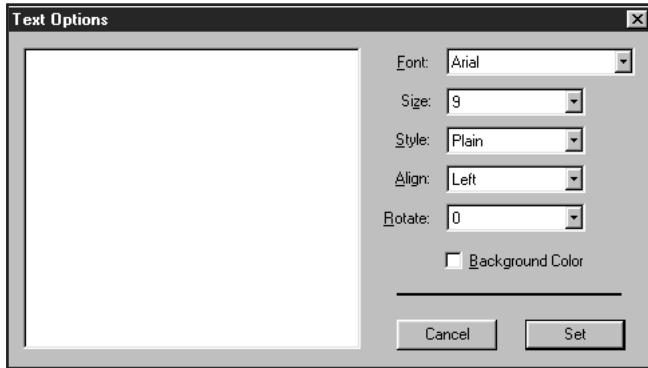
 NOTE: Manually adjust the image view, in the Annotation window, by pressing down the Shift key (Option key for Macintosh) while clicking and dragging.



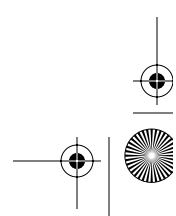
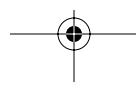
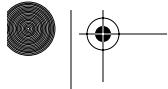
Text Formatting Options

Add titles or other labels to the image using the Text tool.

- 1** Select the Text tool from the Annotation panel.
- 2** Using the Text tool, click and drag on the image where the label is desired. The Text Options dialog box appears.



- 3** Type text and then set the format with the pop-up menus.
 - ✓ *Font*—use any available font on your computer.
 - ✓ *Size*—use default sizes or use the other option to define size.
 - ✓ *Style*—choose from Plain, Bold, and Italics.
 - ✓ *Align*—align text to the right, center, or left.
 - ✓ *Rotate*—orient text at 0°, 45°, and 90°.
 - ✓ *Background color*—define a background color using the Color Selector.
- 4** Click Set. The text appears in the Annotation window.
- 5** Using the Pointer tool, click and drag to position the text box.
- 6** To adjust the format, double-click on the Text Tool or the Text. The Text Options dialog box appears.



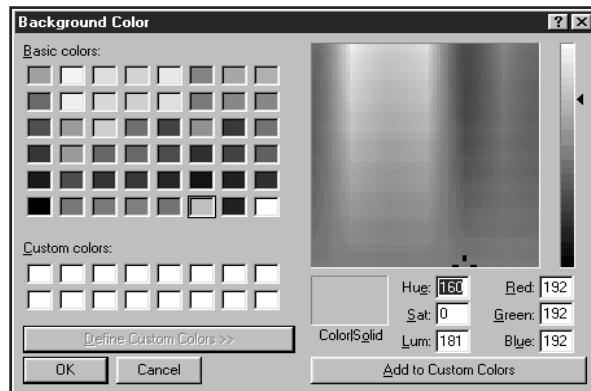
Using the Color Selector

The Color Selector sets the foreground and background color for text and objects.

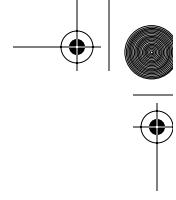
Using the Color Selector—Windows

The Color Selector sets the foreground and background color for text and objects. You can set the color for and object either before or after the object is created or placed.

- 1 If the text or object is already placed, click and drag to highlight the text or click to select the object using the Pointer tool.
- 2 Double-click on the type of object that you would like to adjust color (Fill, Line or Text). The Windows Color Selector appears.



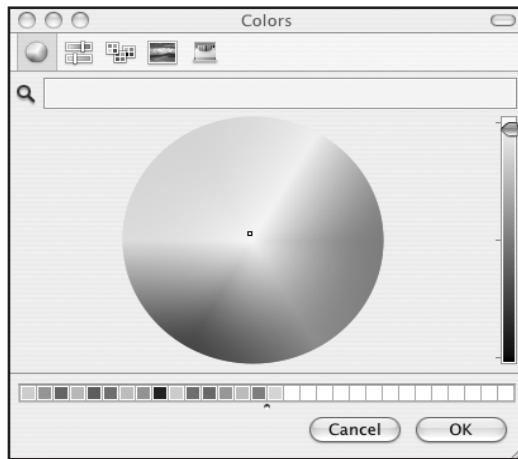
- 3 Select a color from the color palate or click on the Color Selector to apply a custom color. Click on either the foreground or the background to select the current color setting with the Dropper tool.
- 4 Click OK when done.



Using the Color Selector—Macintosh

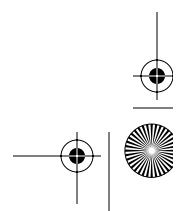
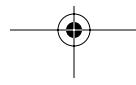
The Color Selector sets the foreground and background color for text and objects. You can set the color for and object either before or after the object is created or placed.

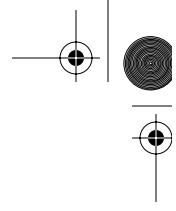
- 1 If the text or object is already placed, click and drag to highlight the text or click to select the object using the Pointer tool.
- 2 Double-click on the type of object that you would like to adjust color (Fill, Line or Text). The Macintosh Color Selector appears.



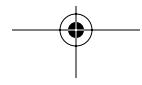
- 3 Select a color from the color palate or click on the Color Selector to apply a custom color. Click on either the foreground or the background to select the current color setting with the Dropper tool.
- 4 Click OK when done.

Congratulations, you have completed the Annotations overview!





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Angle

Angle (for all ROIs except the line ROI) is the current counter clockwise rotation angle of the ROI. For the line ROI, the angle is from the +x axis to the line. Note that it makes a difference which end of the line is the starting point and which is the end point of the line. If you click and draw a line ROI up the angle is +90°. If you click and draw the line down the angle is -90° although the two line ROIs look alike on the image.

Annotation Window

The window that allows you to annotate project images using a copy of the image. The annotations are nondestructive objects that can be later edited. Annotations can be printed or exported for publication.

Area

For each ROI, area is calculated by counting the number of interior pixels and is used to calculate statistics such as the mean and root mean square and the interior pixels. If an ROI has no interior pixels (e.g., a line or an open polygon) the area is zero. Note that the area is not calculated geometrically (e.g., W x H for a rectangle) because when ROIs are rotated the number of interior pixels may change due to the nature of a digital imaging.

Asymmetrical Gaussian

An analytical technique used to model data to a bell-shaped curve. The modeled data will be used to determine molecular weight and mass. Asymmetrical Gaussian should be used to model gels that do not have a Gaussian profile.

Autoradiography

A method of capturing radioactively labeled signals by superimposing film over a labeled sample, typically used in blotting and sequencing applications.

Average Filter

Average filter determines the average pixel intensity within a neighborhood and assigns the averaged value to the pixel, resulting in a smoother image.

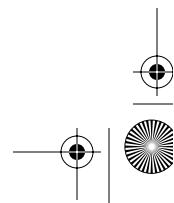
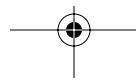
Band Area

The area of a band is the total number of pixels inside the band rectangle. The units of area (pixels², inches², or cm²), are defined in the Preferences dialog box.

Band Labels

Band labels indicate the position of a band on an image. The vertical position of a band indicates its relative mobility. The horizontal length of the band label indicates the portion of the lane used to generate the median profile and calculate the band mass.

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Band Mass

For an experimental band, band mass is determined by comparing the sum of the background subtracted intensities (Net Intensity) of all the pixels in the band with the background subtracted standards information to generate a mass curve.

BIP

BIP is a proprietary file format for saving MI projects. The BIP format saves the image as acquired, annotations, analysis data, preferences, and standards data of the project.

Bit (Binary Digit)

The smallest amount (unit) of information, measured on a binary scale (integer exponents of 2), where combinations of 0's and 1's are used to code information. Refer to Bit Depth. Eight bits of information is called one byte.

Bit Depth (Bits-Per-Pixel or Pixel Depth)

The number of intensity values that can be assigned to each pixel. Images usually fall between 8- and 24-bits.

Black Point

Corresponds to an intensity value in the image that represents pure black in the screen image. The black point can be adjusted using the histogram sliders to help visualize different features in the image. Features with intensity values below the black point can no longer be seen in the screen image. Adjustments to the black point alter the screen image, but not the data for analysis.

Blot

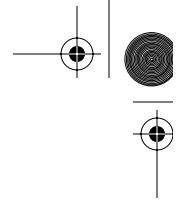
A methodology in which molecules/particles of interest are transferred and affixed to a solid support (membrane), usually for the sake of detection and imaging. A liquid sample may be applied to a membrane as a spot, dot or slot (band), defining a formatted sample array as a Dot or Slot Blot. Electrophoresis gels used to length-resolve DNA, RNA or protein molecules may be similarly transferred to membranes by mechanical extrusion or electrophoresis; such blots are respectively called Southern, Northern or Western Blots.

Brightness

A relative measure of light associated with a pixel representing its gray level from black to white, through intermediate levels of gray. Perceived brightness increases from dark to bright, or black to white through intermediate levels of gray. However, the convention of quantitative imaging is quite the opposite, wherein the grayscale is increased from white to black.

Byte

Equals 8 bits. This is the fundamental quantity of information assigned to pixel areas. A byte can represent any value between 0 to 255.



Centroid

Centroid for all ROIs the (x,y) location of the “center of mass” or the 2nd moment of the intensity distribution. You can calculate the geometrical center of the ROI from the Top Left and Bottom Right values. The centroid is useful because it is a better indicator of the position of the ROI; the 2nd moment will find the position of the feature inside the boundary of the ROI even if the boundary is not well centered on the object.

Chemifluorescence

Chemically mediated production of a fluorochrome. Fluorescent molecules may be produced by the chemical (enzymatic) conversion of a non-fluorescent molecule (substrate), upon excitation (laser or UV illumination).

Chemiluminescence

Chemically mediated production of light. Luminescence or light emission may be produced by a chemical reaction or an enzyme operating on a substrate.

Color Palette

The color palette can be changed for standard or experimental lanes. Colors affect the appearance of the Lane Lines and Band Labels.

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Color Selector

Use the Dropper tool to change the foreground and background color. Click the Dropper tool on an object and the color of the selection will change, either foreground or background.

Column Average Filter

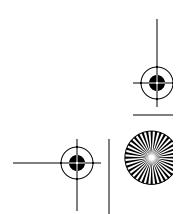
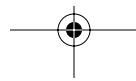
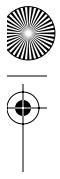
Row Average filters average the row or column pixels within the neighborhood and assigns the averaged value to the pixel smoothing the image.

Compression

Compression algorithms are proprietary mathematical techniques to reduce the image information, and thereby reduce the file size.

Contrast

The contrast of an image represents the intensity of the dark and light areas within the image. A low contrast image contains gray levels that are similar in intensity, whereas a high contrast image contains extreme differences in intensity. The amount of the intensity scale used by the image is referred to as the dynamic range of the image.





Convolution Kernel

Convolution kernels perform mathematical calculations on a group of pixels surrounding the current pixel. The resulting mathematical value is used to change the appearance of the output image. Some types of image processing filters use convolution kernels to enhance or manipulate image data.

Crop

Removing or editing part of an image that is not wanted. To crop an image, select the area you want to save by using the Crop tool. Then click on the Crop tool or the center of the selection.

Default Preferences

The start-up mode setting. Preferences are used to tailor the software to your needs.

Density Slice

Density Slice segments the image into three discrete density ranges. This technique is useful when counting blue/white colony assays or gels that are stained with different dyes. Like the Threshold technique this technique is also sensitive to background variations in the image. ROIs are formed from those groups of pixels that are in the middle range.

Using this technique you can set the maximum and minimum signal value to only find and analyze lighter or darker objects.



Dialog Box

A window in a computer application that allows you to make specific selections.

Digital Camera

A camera that uses an electronic sensor to record an image in a digital binary format.

Dot Blot

A technique in which nucleic acid or protein is spotted onto a membrane and then hybridized to a probe for detection.

Driver

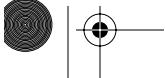
Software designed to interface with a hardware device.

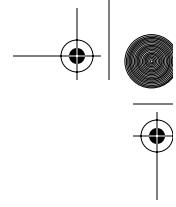
Dropper Tool

In the Annotation window, the Dropper tool changes the foreground and background color.

Dynamic Range

The maximum range of significant digits over which system hardware performs electronic imaging, usually expressed as a signal-to-noise ratio.





Edge Detection

This boundary detection technique searches for the edges of features. This method looks for gradient edges and is usually not biased by intensity variations or background in the image (relative to the background). The program determines the rate of intensity changes a boundary. This is accomplished by searching for gradient or slope variations. The number of ROIs found is controlled by the gradient setting (0.1%–100%). This value is used by the algorithm to determine how steep of a slope the edge must have (rate of change of intensity across the edge) before it defined as a new ROI object. The higher the percentage chosen, the sharper the edges that are searched for and more intense the ROI.

Edge Filters

Edge filters look for horizontal or vertical edges. The filter takes an image and shifts it by one pixel over and subtracts it from the original.

Electrophoresis

Separation of molecules on the basis of charge and size.

Ellipse Tools

The ROI Ellipse tool measures elliptically shaped objects. The Ellipse Selection tool defines an area for analysis.

Emboss Filter

Emboss North, NE, East, SE, South, SW, W, or NW filters enhance the edge, making the image features appear as raised 3D objects. The eight different directions indicate of angle for embossing.

Export Data

The Export Data command in the File menu exports the analysis, profile, or histogram data to a tab-delimited text file (analysis data). Text files can be imported into many Macintosh or Windows spreadsheet programs.

Find Bands

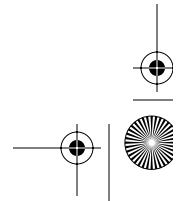
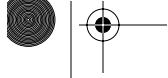
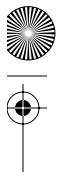
The Find Bands command locates and labels bands within the area of the image designated by Lane Markers.

Flash Memory

RAM chip embedded in a small credit card sized holder for storage of data from digital cameras and computers.

Floating Point

A means of representing a signal as real numbers (fractions or decimals). Floating point calculations are important in maintaining the accuracy of analysis data since data is not truncated or clipped during mathematical operations. The Carestream MI Software maintains floating point numbers when saved in the MI file format (.bip).





Free Form Tool

The ROI Free Form tool measures irregularly shaped objects.

Gamma

A mathematical transformation function that can be used to improve image appearance by decreasing or increasing the contrast of an element of interest in an image. Adjusting the gamma of an image disproportionately skews the gray level distribution, higher gamma values (>1) lighten the image and lower gamma values (<1) darken the image. A gamma of 1 represents the image file unaltered. Adjusting the gamma does not alter the image data file and is only used to enhance the viewing of the image.

Gaussian

An analytical technique used to model data to a bell shape. The modeled data will be used to determine molecular weight and mass.

Gaussian Filter

Gaussian filter applies a weighted average to the pixels—adding low frequency detail.

Gel

A separation matrix which is typically agarose or acrylamide and is used for electrophoresis.

Grab Handles

Grab handles are the small boxes that appear when an object is selected. Drag on a grab handle to stretch, shrink, or angle an object. Grab handles are available when drawing Lane Markers and adjusting Lane Lines. In addition, grab handles are used in the Profile window to adjust profiles. Annotation objects also use grab handles.

Gradient Filter

Gradient North, NE, East, SE, South, SW, W, or NW filters are designed to highlight one of eight compass directions—creating shadows next to objects.

Gray Level

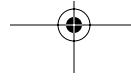
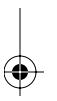
The digital signal assigned to a pixel associated with a level of light (from black to white). For example, an 8-bit and a 16-bit system includes gray level values between 0–255 and 0–65,535, respectively.

Grid

The ROI Grid analyzes a group of ROIs that are regularly shaped and spaced.

High Pass Filter

High Pass filter enhances the high contrast edges in an image—high frequency values in the image will brighten, while lower frequency portions will darken resulting in a sharper image. This filter may also introduce more noise in your image.



Histogram

The graphical representation of the distribution of different intensity pixels within an image. The horizontal axis represents the gray level and the vertical axis represents the number of pixels.

Image Analysis

Numerical tabulation of a digital image. The image analysis data is displayed in worksheet like tables in Carestream MI Software.

Image Section

The Image section is the area of the window that displays the largest view of the image and is the active window for analysis. This image area can be magnified to see details.

Information Bar

The Information bar is located below the Main Menu bar. The Information bar changes features and displays image analysis information depending on which tool you have selected.

Interpolation

A numerical estimate of a value within a range of empirical data, based on the mathematical trend of data. Contrasts with extrapolation, in which a value outside the range of data is estimated.

JPEG

A commonly used file format used for compressing images.

Laplacian Filter

Laplacian filter enhances all edges within the image and is well suited for looking at the noise of an image.

Landscape

Image/data that is oriented on a horizontal axis.

Lane Analysis Data Window

This window displays the analysis results including molecular weight, mass, mobility, mean intensity, net intensity, sum intensity, maximum intensity, mean background, and band area.

Lane Line

A line that passes vertically through each band in a lane. A Lane Line should be positioned as close as possible to the middle of the bands in order to obtain the most accurate determination of mass and molecular weight.

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Lane Marker

Lane Markers identify the section of an image for analysis. Two horizontal Lane Markers are connected by vertical Lane Lines that pass through the bands of each lane. Each Lane Marker is positioned and sized to adjust the vertical Lane Lines as close to the centers of the lanes as possible.

Lane Profile

The Lane profile is a graph of the median of the intensities along a lane line. Use the Profile to check the accuracy of the Band Label and Band Width.

Line Tool

The ROI Line tool provides lines for analysis, In the Annotation mode the Annotation Line tool draws lines of different types in the Annotation window.

Low Pass Filter

Low Pass filter smooths the appearance of the image by removing the high contrast edges in an images and blurring the edges. This filter is useful for filtering out or reducing noise in the image.

Magnification Tool/Slider

Lets you enlarge or reduce images. You can set the magnification to 1X (actual size) or levels between 0.25X to 32X except in the Annotation panel. The Annotation panel provides magnification of 0.25X to 8X.

Mass Standards

The associated mass values for some molecular weight standards.

Maximum Filter

Maximum filters determines the brightness pixel value within a neighborhood and assigns the brightest value to that pixel.

Maximum Intensity

The maximum intensity of a band is the maximum signal measured within a band area. The band area used to determine the maximum intensity is defined in the Adjust Bands dialog box. The band area can be adjusted by changing the Profile Width.

Mean Background

The mean background is the mean of the background signal calculated from within the band area. Band area is determined by adjusting the Profile Width in the Adjust Bands dialog box.

Mean Filter

Mean filter calculates the mean pixel value within a neighborhood and assigns the mean value to the pixel.



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Mean Intensity

The mean intensity of a band is the mean of the signal measured within the band area. The band area can be adjusted by changing the Profile Width.

Median

The median is a statistical location that is defined as that value of the variable (in an ordered array) that has equal number of items on either side. Therefore, a median divides a frequency distribution into two halves. The profile represents the median values of each row of pixels within the band rectangle. The default background for ROIs is based on the median of the perimeter.

Median Filter

The Median filter reduces noise in the image. The median despeckles an image but also causes some blurring of the image.

Minimum Filter

The Minimum filter determines the darkest pixel value within a neighborhood and assigns the darkest value to that pixel.



Mobility

The mobility of a band is calculated by measuring the relative position of a band between the top and bottom Lane Markers. The unit of measurement (in pixels, centimeters, or inches) is defined in the Preferences dialog box.

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Normalize Histogram (Histogram Equalization)

Normalize Histogram (Histogram Equalization) uses a histogram of pixel values to produce a filtered image that has more gray levels for regions of the histogram (peaks) which have the greatest number of pixels.

Optical Density

Optical Density (OD) measures the amount of light that passes through an object to determine the amount of matter. OD requires light to pass through the object, and therefore, are only meaningful when the light source is positioned behind the object. OD is not a useful measurement for reflected light captures.

Overview Image

The Overview Image is the region of the window that displays a reduced version of the image. The red frame corresponds to the area displayed in the Image section. This is only visible when the lane profile is not displayed.

PICT

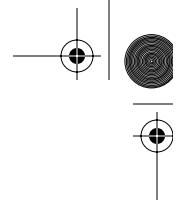
Common Macintosh file format. PICT stores each element as a separate entity.

Peak Finder

The Peak Finder creates oval or rectangular ROIs, not arbitrary shapes, around peaks of intensity. The peak finder can separate peaks that the other finders might group together as one. Also, the peak finder is much less sensitive to variations in the image background than the other auto find ROI methods. You find the peak finder most useful if you are counting, e.g., colony plates or plaque assays

Perimeter Length

This is a data value for all ROIs, except the polyshape and ellipse ROI. The perimeter length is calculated by summing the Pythagorean distances between the centers of the vertices (nodes) of the ROI and/or the centers of the end points. For the free form ROI which has no control points, the perimeter length is the length of the line that connects the centers of the perimeter pixels in the original order that the perimeter points were drawn. When ROIs are rotated their perimeter lengths may change as a result of the new locations of the perimeter pixels, end points, or vertices. For oval ROIs, a formula is used to calculate the perimeter of the ellipse that passes through the centers of the perimeter pixels. This is more accurate than using the techniques for arbitrary shapes and polygons because continuous curves like ovals are not as well represented in digital imaging as well as the straight lines of polygons.



Pixel

The fundamental element in a digital image. In a digital camera, the pixels represents the light sensitive elements on the CCD.

Pointer Tool

Use the arrow shaped Pointer tool to select objects.

Polyline

The ROI Polygon tool allows you to draw segmented lines called polylines. The pixels along the segmented line are analyzed, thus, the polyline is useful in measuring distances of data that do not fall in a straight line and intensity measurements.

Polyshape

The ROI Polygon tool measures irregular geometric shaped objects.

Portrait

Images/data that are oriented on the vertical axis. This is the default printer setting.

Preferences

Program settings adjusted by the user through the Preferences dialog box. Default preferences for new projects are set when all project windows are closed. Preferences for specific projects are set when a project is open. To access the preferences, click Preferences from the Quick Access bar or choose Preferences from the Edit menu.

Project

Each scanned or imported image together with any standard information and analysis data is considered a project.

Pseudocolor

The process of assigning an RGB color palette to replace the gray levels in a grayscale image for display purposes. A series of pseudocolor palette choices are provided in the Image Display window.

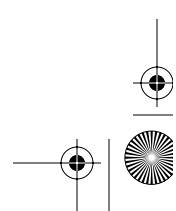
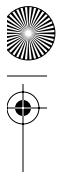
Region of Interest (ROI)

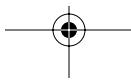
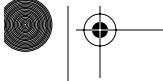
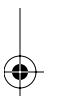
ROIs designate the portion of the image that is of interest for analysis. The ROI for lanes is designated using the Image Selection tool, while the ROI tool provide a series of shapes for measurements.

Relative Intensity

Relative Intensity is the percent intensity contribution of a band within a lane. For example if a band contains 0.5 μg of a total lane mass of 1 μg , the relative mass value would be 0.5 (or 50%).

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Resolution

The capability to distinguish between objects of interest. It is customary, when describing the characteristics of a digital imaging device or image, to describe the resolution by specifying the number of pixels it captures in the horizontal by vertical direction.

Revert to Saved

The Revert to Saved command in the File menu discards current changes and returns to the last saved version of the project.

Row Average Filter

Row Average filters average the row or column pixels within the neighborhood and assigns the averaged value to the pixel smoothing the image.

Saturation

The limitation of signal imposed by either the CCD or the digital scale in which the signal is being represented. If the signal becomes too large the individual pixels on the CCD will be filled and no additional signal can be detected.

Save

Saves project information to a file. Files are saved with image, brightness/contrast settings, standards, analysis data, and annotation objects.

Scale

A geometric operation that reduces or enlarges an image.

Serial Number

This data field is a unique identifier for ROIs. If the ROI is deleted, the serial number is never reused. To reset the serial numbers, all ROIs need to be deleted.

Shape Tool

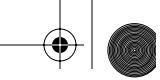
Use the Shape tool to draw boxes, ovals, and circles in the Annotation window.

Single Pixel Filtering

Single Pixel Filtering performs a mathematical or logical calculation on individual pixels, with the resulting value substituted back into the image. For example, the Invert operation reverses the intensity values of each pixel, i.e. whites become black.

Slot Blot

A process in which a nucleic acid or protein is applied to a membrane using a slotted device which delivers reproducible amounts to a membrane. The samples are hybridized to a labeled probe for detection.



Southern Blot

The process by which DNA is separated by electrophoresis, transferred to a membrane, where it is hybridized to complementary nucleic acid containing a label and detected.

Spline

A spline is a mathematical function that will go exactly through each of the background control points that you specify while at the same time generating a background curve varies smoothly between all the other points that are not marked as background control points. When you add background control points to a spline curve, it is generally best not to add too many and be careful not to add them too close to one another as this can cause the spline to behave erratically as it tries to go through each point in the profile.

Standard Deviation

The square root of the sum of the squared deviation of each pixel value from the mean pixel value. The standard deviation is a useful measure of the statistical error or noise in your data. To find the noise level of your image, create an ROI that includes only background pixels typical of the image. The standard deviation for this ROI is a good indicator of the random variations you can expect for other pixels in the image.

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Standards

A sample containing bands of known size and/or mass that is used for comparison to quantitate an experimental (unknown) sample.

Sum Intensity

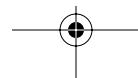
The sum intensity of a band is the total signal of the pixels within the band. The band area used to determine the sum intensity is defined in the Find Bands dialog box. The band area can be adjusted by changing the Profile Width.

Text Tool

Use the Text tool to annotate your image. The Text tool allows you to choose the font, font size, and rotation of the text.

Threshold

Threshold uses a predefined signal threshold value to segregate image objects from the background. This method is useful when your image contains objects that are well separated and have an even background (i.e., dot and slot blots). In this method any groups of pixels above or below the threshold value are grouped together as ROIs (the current state of the Above and Below options will depend on the Black/White features radio button on the ROI tab). This technique will not work well for images with a large variation in the intensity of the background.





TIFF

Tagged Image File Format (TIFF) is an industry standard file format for images. You can export an image in TIFF format for use with other computer programs.

TWAIN

An industry standard protocol for exchanging information between applications and image capture devices such as scanners and digital cameras.

Unsharp Mask Filter

Unsharp Mask filter adjusts the contrast of the image to give added emphasis to the edge detail. This filter can help improve an image that appears out of focus.

Width/Height

Width/Height for all ROIs except the rectangle and oval, are the horizontal and vertical distances between the centers of the top left and bottom right pixels described above. For rectangle ROIs, width and height are the number of interior pixels in the horizontal and vertical direction when the ROI is in its unrotated position. This special case is treated so that area = width x height for unrotated rectangles, as expected. For ovals (ellipses) the width and height are always the width and height of the major and minor axes of the interior pixels in the unrotated ellipse. The width and height values of rectangles and ovals do not change when they are rotated. Width and height may change for other ROIs when they are rotated, however, due to changes in the bounding rectangle described above.

Western Blot

The process by which protein is separated by electrophoresis and then transferred to a membrane and subsequently detected by a labeled probe.

White Point

Corresponds to an intensity value in the image that represents pure white in the screen image. The white point can be adjusted using the histogram sliders to help visualize different features in the image. Features with intensity values above the white point can no longer be seen in the screen image. Adjustments to the white point alter the screen image and do not alter the data for analysis.



A**Keyboard Shortcuts**

Appendix A: Keyboard Shortcuts

Many of the menu commands in Carestream Molecular Imaging Software may be initiated by keyboard commands—pressing designated keys simultaneously. You can use keyboard shortcuts to speed up your work.

Table 1: Keyboard Shortcuts

Command	Windows Shortcut	Macintosh Shortcut
Open	Ctrl + O	Command + O
New Digital Camera Acquire	Ctrl + N	Command + N or V
Close	Ctrl + F4	Command + W
Save	Ctrl + S	Command + S
File Information	Ctrl + I	Command + I
Print	Ctrl + P	Command + P
Quit	—	Command + Q
Undo	Ctrl + Z	Command + Z
Cut	Ctrl + X	Command + X
Copy	Ctrl + C	Command + C
Paste	Ctrl + V	Command + V
Clear	Delete	Delete
Select All	Ctrl + A	Command + A
Duplicate ROI	Alt + D	Command + D
Center Selected ROI	Alt + /	Command + /
Project Preferences	Alt + ;	Command + ;
New Project Preferences	Alt +]	Command +]
Find Lanes	Ctrl + F or U	Command + F
Adjust Lanes	Ctrl + F	Command + F
Lane Information	Ctrl + L	Command + L
Find Bands	Ctrl + B	Command + B
Fit Bands	Ctrl + D	Command + Y
Band Information	Ctrl + H	Command + T
Image Display	Ctrl + 2	Command + 2
Lane Analysis Data	Ctrl + 3	Command + 3

Table 1: Keyboard Shortcuts

Command	Windows Shortcut	Macintosh Shortcut
Profile	Ctrl + G	Command + G
Show /Hide Lane Lines	Ctrl + K	Command + K
Show/Hide Lane Markers	Ctrl + R	Command + M
Show/Hide Iso Molecular Weight Lines	Ctrl + M	Command + H
Show /Hide Band Labels	Ctrl + Q	Command + J
Active ROI Info	Ctrl +X	Command + 4
ROI Analysis Data	Ctrl +A	Command + 5



Appendix B: Software Conventions

The Carestream Molecular Imaging Software User's Guide uses standard terminology. This appendix reviews basic mouse, keyboard, and menu conventions.

Mouse Conventions

Be sure that you are familiar with functions using a mouse:

- ✓ Double-click to quickly access additional functionality
- ✓ Point and click to select an object
- ✓ Click and drag to move an object or draw a selection region
- ✓ Ctrl-click (Shift-click for Macintosh) to select more than one object
- ✓ Drag and drop to move an object to a new location

If you're not sure how to perform these functions with a mouse, refer to your operating system documentation.

B

Conventions

Keys

Although the step-by-step examples in this guide use the menu commands and tools in the Toolbar, you can also use the keyboard shortcuts for these actions. Keyboard shortcuts are listed in Appendix A. To use keyboard shortcuts:

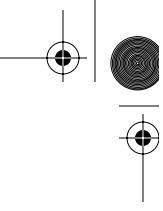
- ✓ Hold down the Ctrl or Alt key and type a letter on the keyboard. For Macintosh operating systems hold down the Command key and type a letter on the keyboard

Menus

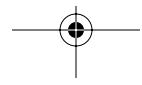
Menu labels are listed across the top of the window.

- ✓ To open a menu item, click on the label and drag down the menu to the desired command and release the mouse button





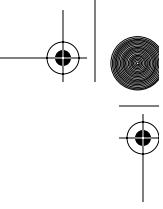
B-2



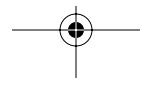
Appendix C: Units of Measurement

This appendix defines many standard units of measurements used in life science laboratories today.

1 milligram (mg)=	$0.001 (10^{-3})$ gram (g)
1 microgram (μ g)=	10^{-6} g
	10^{-3} mg
1 nanogram (ng)=	10^{-9} g
	10^{-6} mg
	10^{-3} μ g
1 xs (pg)=	10^{-12} g
	10^{-9} mg
	10^{-6} μ g
	10^{-3} ng
1 base (b)=	330 g/mole
1 base pair (bp)=	660 g/mole
1 kilobase=	1000 b
	0.66 megadaltons (Md) dsDNA
	0.33 Md ss nucleic acid
1 Md=	106 daltons
1 dalton=	1.65×10^{-24} g
Aver. amino acid=	110 g/mole (MW)
1 OD ₂₆₀ =	50 μ g/mL dsDNA
	33 μ g/mL ssDNA
	40 μ g/mL free nucleotides



C-2



D*Standard Files*

Appendix D: Standard Files

This appendix lists the lane analysis standard files that are shipped with the Carestream Molecular Imaging Software.

Description	Band #	Size	% Mass
0.16-1.77 Kb RNA Ladder (b)	1	1770	—
	2	1520	—
	3	1280	—
	4	780	—
	5	530	—
	6	400	—
	7	280	—
	8	155	—
0.24-9.5 Kb RNA Ladder (b)	1	9490	—
	2	7460	—
	3	4400	—
	4	2370	—
	5	1350	—
	6	240	—

Description	Band #	Size	% Mass
1 Kb DNA Extension Ladder (bp)	1	40000	—
	2	20000	—
	3	15000	—
	4	10000	—
	5	8144	—
	6	7126	—
	7	6108	—
	8	5090	—
	9	4072	—
	10	3054	—
	11	2036	—
	12	1636	—
	13	1018	—
	14	517	—
1 Kb DNA Extension Ladder (bp) cont'd	15	506	—
	16	396	—
	17	344	—
	18	298	—
	19	220	—
	20	201	—
	21	154	—
	22	134	—
	23	75	—

D*Standard Files*

Description	Band #	Size	% Mass
1 Kb DNA Extension Ladder (bp)	1	12216	—
	2	11198	—
	3	10180	—
	4	9162	—
	5	8144	—
	6	7126	—
	7	6108	—
	8	5090	—
	9	4072	—
	10	3054	—
	11	2036	—
	12	1635	—
	13	1018	—
	14	517	—
	15	506	—
	16	394	—
	17	344	—
	18	298	—
	19	220	—
	20	200	—
	21	154	—
	22	142	—
	23	75	—
10 KDa Protein Ladder (d)	1	200000	—
	2	120000	—
	3	110000	—
	4	100000	—

Description	Band #	Size	% Mass
10 KDa Protein Ladder (d) cont'd	5	90000	—
	6	80000	—
	7	70000	—
	8	60000	—
	9	50000	—
	10	40000	—
	11	30000	—
	12	20000	—
	13	10000	—
10 bp DNA Ladder (bp)	1	330	—
	2	320	—
	3	310	—
	4	300	—
	5	290	—
	6	280	—
	7	270	—
	8	260	—
	9	250	—
	10	240	—
	11	230	—
	12	220	—
	13	210	—
	14	200	—
	15	190	—
	16	180	—
	17	170	—
	18	160	—
	19	150	—
	20	140	—
	21	130	—
	22	120	—
	23	110	—
	24	100	—
	25	90	—
	26	80	—

D*Standard Files*

Description	Band #	Size	% Mass
10 bp DNA Ladder (bp) cont'd	27	70	—
	28	60	—
	29	50	—
	30	40	—
	31	30	—
	32	20	—
	33	10	—
100 bp DNA Ladder (bp)	1	2072	—
	2	1500	—
	3	1400	—
	4	1300	—
	5	1200	—
	6	1100	—
	7	1000	—
	8	900	—
	9	800	—
	10	700	—
	11	600	—
	12	500	—
	13	400	—
	14	300	—
	15	200	—
	16	100	—
123 bp DNA Ladder (bp)	1	4168	—
	2	4059	—
	3	3936	—
	4	3813	—
	5	3690	—
	6	3567	—
	7	3444	—
	8	3321	—
	9	3198	—
	10	3075	—
	11	2952	—
	12	2829	—

Description	Band #	Size	% Mass
123 bp DNA Ladder (bp) cont'd	13	2706	—
	14	2583	—
	15	2460	—
	16	2337	—
	17	2214	—
	18	2091	—
	19	1968	—
	20	1845	—
	21	1722	—
	22	1599	—
	23	1476	—
	24	1353	—
	25	1230	—
	26	1107	—
	27	984	—
	28	861	—
	29	738	—
	30	615	—
	31	492	—
	32	369	—
	33	246	—
	34	123	—
1Kb Plus DNA Ladder (bp)	1	12000	—
	2	11000	—
	3	10000	—
	4	9000	—
	5	8000	—
	6	7000	—
	7	6000	—
	8	5000	—
	9	4000	—
	10	3000	—
	11	2000	—
	12	1650	—
	13	1000	—

D
Standard Files

Description	Band #	Size	% Mass
1Kb Plus DNA Ladder (bp) cont'd	14	850	—
	15	650	—
	16	500	—
	17	400	—
	18	300	—
	19	200	—
	20	100	—
25 bp DNA Ladder (bp)	1	500	—
	2	450	—
	3	425	—
	4	400	—
	5	375	—
	6	350	—
	7	325	—
	8	300	—
	9	275	—
	10	250	—
	11	225	—
	12	200	—
	13	175	—
	14	150	—
	15	125	—
	16	100	—
	17	75	—
	18	50	—
	19	25	—

Description	Band #	Size	% Mass
25 bp DNA Ladder (bp)	1	3500	—
	2	3250	—
	3	3000	—
	4	2750	—
	5	2500	—
	6	2250	—
	7	2000	—
	8	1750	—
	9	1500	—
	10	1250	—
	11	1000	—
25 bp DNA Ladder (bp) cont'd	12	750	—
	13	500	—
	14	250	—
5 Kb DNA Ladder (bp)	1	40000	—
	2	35000	—
	3	30000	—
	4	25000	—
	5	20000	—
	6	15000	—
	7	10000	—
	8	5000	—

D*Standard Files*

Description	Band #	Size	% Mass
50 bp DNA Ladder (bp)	1	800	—
	2	750	—
	3	700	—
	4	650	—
	5	600	—
	6	550	—
	7	500	—
	8	450	—
	9	400	—
	10	350	—
	11	300	—
	12	250	—
	13	200	—
	14	150	—
	15	100	—
	16	50	—
500 bp DNA Ladder (bp)	1	8000	—
	2	7500	—
	3	7000	—
	4	6500	—
	5	6000	—
	6	5500	—
	7	5000	—
	8	4500	—
500 bp DNA Ladder (bp) cont'd	9	4000	—
	10	3500	—
	11	3000	—
	12	2500	—
	13	2000	—
	14	1500	—
	15	1000	—
	16	500	—

Description	Band #	Size	% Mass
Ad-2 Kpn I Fragments (bp)	1	7713	21.46
	2	6478	18.03
	3	5758	16.02
	4	5167	14.38
	5	3648	10.15
	6	2339	6.51
	7	2049	5.70
	8	1699	4.73
	9	1086	3.02
Benchmark Protein Unstained (d)	1	220000	—
	2	160000	—
	3	120000	—
	4	100000	—
	5	90000	—
	6	80000	—
	7	70000	—
	8	60000	—
	9	50000	—
	10	40000	—
	11	30000	—
	12	25000	—
	13	20000	—
	14	15000	—
	15	10000	—
Biotinylated Molecular Weight Markers (d)	1	97400	—
	2	69000	—
	3	46000	—
Biotinylated Molecular Weight Markers (d) cont'd	4	30000	—
	5	20100	—
	6	12300	—

D
Standard Files

Description	Band #	Size	% Mass
DNA Analysis Marker System (bp)	1	22621	—
	2	15004	—
	3	11919	—
	4	9416	—
	5	8271	—
	6	7421	—
	7	6442	—
	8	5861	—
	9	5415	—
	10	4716	—
	11	4333	—
	12	3812	—
	13	3397	—
	14	3101	—
	15	2876	—
	16	2650	—
	17	2433	—
	18	2213	—
	19	2015	—
	20	1861	—
	21	1672	—
	22	1568	—
	23	1431	—
	24	1287	—
	25	1176	—
	26	993	—
	27	910	—
	28	784	—
	29	653	—
	30	526	—

Description	Band #	Size	% Mass
High DNA Mass Ladder (bp)	1	10000	38.46
	2	6000	23.08
	3	4000	15.39
	4	3000	11.54
	5	2000	7.69
	6	1000	3.85
High MW DNA Markers (bp)	1	48502	—
	2	38416	—
	3	33498	—
	4	29942	—
	5	24776	—
	6	22621	—
	7	19399	—
	8	17057	—
	9	15004	—
	10	12220	—
	11	10086	—
	12	8612	—
	13	8271	—
Lambda Hind III Fragments (bp)	1	23130	47.69
	2	9416	19.41
	3	6557	13.52
	4	4361	8.99
	5	2322	4.79
	6	2027	4.18
	7	564	1.16
	8	125	0.26
Low DNA Mass Ladder (bp)	1	2000	42.55
	2	1200	25.53
	3	800	17.02
	4	400	8.51
	5	200	4.26
	6	100	2.13

D *Standard Files*

Description	Band #	Size	% Mass
PhiX Hae III Fragments (bp)	1	1353	25.12
	2	1078	20.01
	3	872	16.19
	4	603	11.20
	5	310	5.76
	6	281	5.22
	7	271	5.03
	8	234	4.34
	9	194	3.60
	10	118	2.19
	11	72	1.34
Protein MW Std, High Range (d)	1	200000	—
	2	97400	—
	3	68000	—
	4	43000	—
	5	29000	—
	6	18400	—
	7	14300	—
Protein MW std, Low Range (d)	1	43000	—
	2	29000	—
	3	18400	—
	4	14300	—
	5	6200	—
	6	3000	—
Radiolabeled RNA Ladder System (b)	1	9500	—
	2	7500	—
	3	4400	—
	4	2400	—
	5	1350	—
	6	780	—
Supercoiled DNA Ladder (bp)	1	16210	—
	2	14174	—
	3	12138	—
	4	10102	—
	5	8066	—

Description	Band #	Size	% Mass
Supercoiled DNA Ladder (bp) cont'd	6	7045	—
	7	6030	—
	8	5012	—
	9	3990	—
	10	2972	—
	11	2067	—

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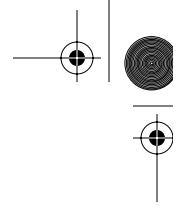
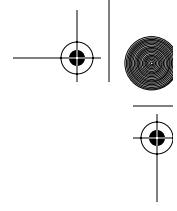
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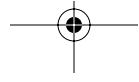
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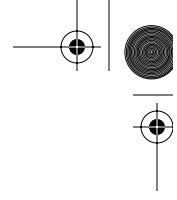
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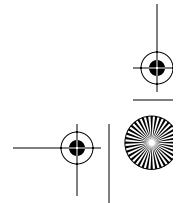


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