Image Lab Software

User Guide

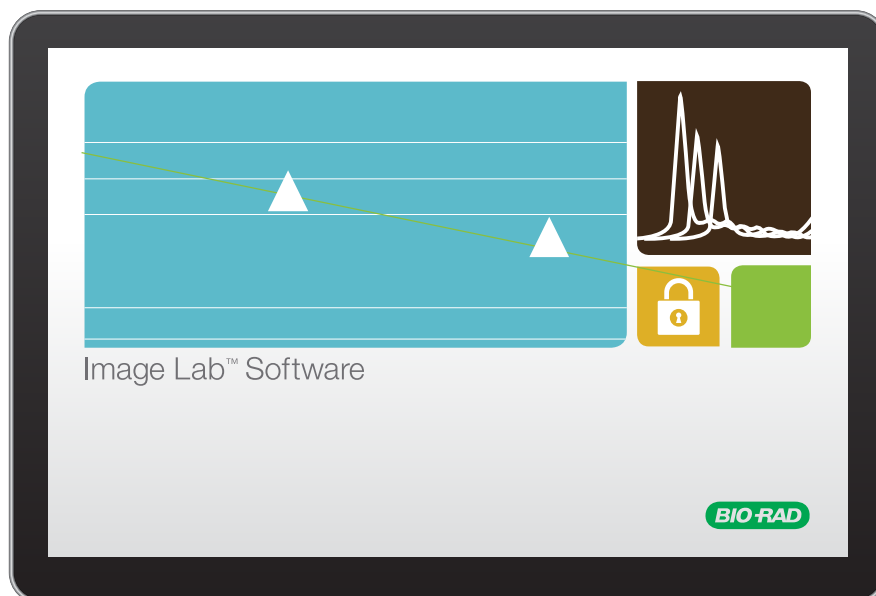**Version 6.1**

Image Lab™ Software

BIO·RAD

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User Guide

Version 6.1

BIO-RAD

Legal Notices

Technical Support

The Bio-Rad Technical Support department in the U.S. is open Monday through Friday, 5:00 AM to 5:00 PM, Pacific time.

Phone: 1-800-424-6723, option 2

Web: www.bio-rad.com

Email: Support@bio-rad.com (U.S./Canada Only)

For technical assistance outside the U.S. and Canada, contact your local technical support office.

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Chapter 1 Introduction

Image Lab software works with your imaging system to create a protocol — a reproducible, automated, and time-saving workflow for imaging and analyzing gels and blots. You can create protocols to ensure repeatable results, capture optimized image data, edit the analysis, and produce customized reports.

On the Windows operating system, this version of Image Lab supports image acquisition on the following instruments:

- ChemiDoc MP

- ChemiDoc XRS+

- Gel Doc EZ

- Gel Doc XR+

- GS-900 Calibrated Densitometer

On Mac operating systems, this version of Image Lab supports image acquisition only on Gel Doc EZ and Gel Doc XR+ instruments. For detailed procedures about image acquisition settings and running protocols, see the instrument guide for your imaging system.

Note: Bio-Rad standalone imagers acquire images using Image Lab Touch software, which resides on the imagers. You can export images from these imagers and analyze them using Image Lab on your computer.

Image Lab Security Edition is available to help organizations comply with US FDA 21 CFR Part 11 regulations.

Chapter 2 Setting Up Image Lab Software

Image Lab Software can be installed on a computer running Microsoft Windows or a Mac operating system. The amount of memory required for running Image Lab depends on the size of the images you scan and analyze. Images scanned at high resolution can be quite large. For this reason, Bio-Rad recommends that you archive images on a network file server or on removable storage media.

Tip: Bio-Rad can provide an appropriate computer for use with your imaging system. Contact your local Bio-Rad representative for details.

System Requirements

System Element	Minimum Requirement
Operating system	Microsoft Windows 7 (64-bit) Microsoft Windows 10 (64-bit) Mac OS X 10.14 or 10.15 **Note:** Applies only to the standard edition of Image Lab Software. The security edition is not supported on Mac operating systems.
Processor	Intel Core i3 or equivalent
Hard disk space	60 GB
Memory (RAM)	4 GB
Ports for connecting instrument	1 USB 2.0 port

Before You Install Security Edition

During Image Lab installation, you can choose to install the standard edition or the security edition Security Edition requires a license, which you enter as part of the installation.

Note: Security Edition is available for Windows computers only.

Important: To enable secure mode, Image Lab Administrator role privileges must be in place. You must create the required groups and assign the Image Lab roles to users in those groups before you install Image Lab software.

■ For information on required Image Lab roles, groups, and users, see Configuring Users and Groups on a Local Computer on page 184.

■ For information about setting up groups, user names, and passwords, see Configuring Users and Groups on a Network Domain on page 188.

Installing Image Lab Software

The standard edition of Image Lab Software can be installed on a computer running the Windows or Mac operating system. The security edition can be installed only on a computer running Windows

Installing Image Lab on a Mac

You can install Image Lab Software from a USB flash drive or from the Bio-Rad website.

To install Image Lab on a Mac from a USB drive

1. Insert the Image Lab software USB flash drive into a USB port on the computer.

2. When the contents appear, double-click the icon.

   Two folders appear, Mac and Windows.

3. Open the Mac folder.

4. Double-click the Image Lab.dmg file.

5. Drag the Image Lab application icon into the Applications folder.
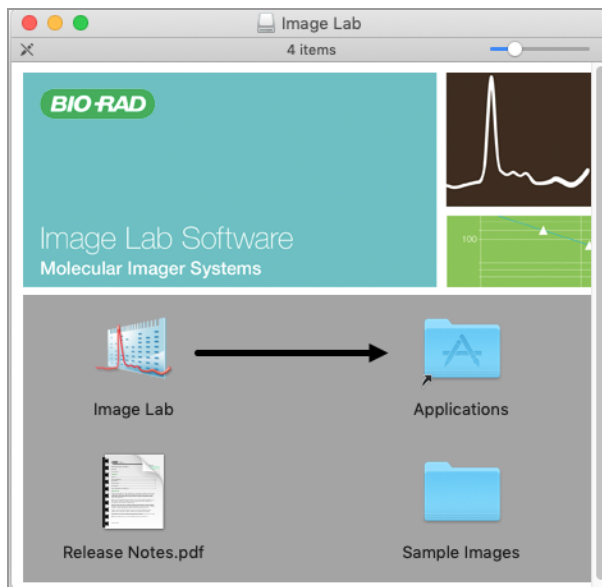
To install Image Lab on a Mac from the website

1. Enter the following URL into a web browser:

   https://www.bio-rad.com/ImageLab

2. When the Image Lab page appears, click Log In/Register in the upper-right corner.

3. Do one of the following:

   ■ If you have an account, enter your user name and password, and then clickd
   Sign In.

   ■ If you do not have an account, click Register Today and complete the fields under
   Registration and Contact Information. When your account is verified, enter your user name
   and password, and then click Sign In.

4. Click the Image Lab Software link, and then click Learn More.

5. Select the Download tab, and then click the Software Download button for Image Lab Software for
   Mac.

6. Follow the prompts to install the software.

Installing Image Lab on a Windows PC

Image Lab does not require a license code. However, Image Lab Security Edition does require you to enter a license code during installation.

Important: Before you install Security Edition, locate the 18-digit license code in the Image Lab product folder pocket. If you do not have the license code, obtain it from your Bio-Rad customer service representative before attempting to install the software.

When deciding whether to install Image Lab or Image Lab, Security Edition, consider the following:

■ If you are licensed to install the Security Edition but choose to install the Standard Edition, you must first uninstall the Standard Edition and then install the Security Edition before you can use it.

■ If you are licensed to install the Security Edition and choose to install it, note that only a user assigned the role or group of Image Lab Administrator can enable and disable secure mode.

   Note: A user assigned the role of Image Lab Administrator does not have to be the network or IT administrator.

For more information, see Setting Up Image Lab Security Edition on page 23.

To install Image Lab on a Windows PC

1. Do one of the following:

   ■ **To install from a USB flash drive**

   a. Insert the Image Lab software USB flash drive into a USB port on the computer.

   b. When the contents appear, double-click the icon.

      Two folders appear, Mac and Windows.

   c. Open the Windows folder and double-click ImageLabSetup.exe.

   ■ **To install from the website**

   a. Enter the following URL into a web browser:

      https://www.bio-rad.com/ImageLab

   b. When the Image Lab page appears, click Log In/Register in the upper-right corner.

      Do one of the following:

      ☐ If you have an account, enter your user name and password, and then click Sign In.

☐ If you do not have an account, click Register Today and complete the fields under Registration and Contact Information. When your account is verified, enter your user name and password, and then click Sign In.

c. Click the Image Lab Software link, and then click Learn More.

d. Select the Download tab, and then click the Software Download button for Image Lab Software for Windows.

The Image Lab installer wizard opens.
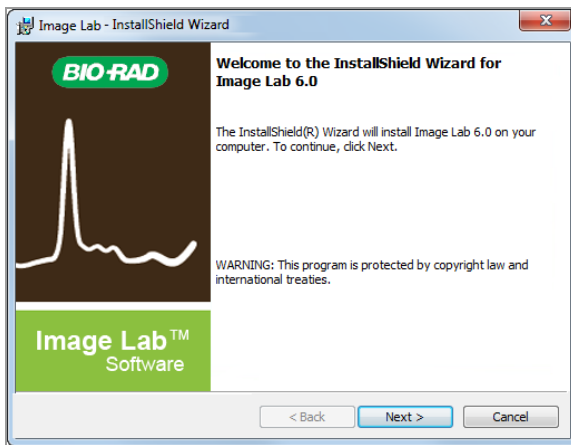
2. On the Welcome screen, click Next.

3. Accept the license agreement and click Next.

The Edition Selection dialog appears.
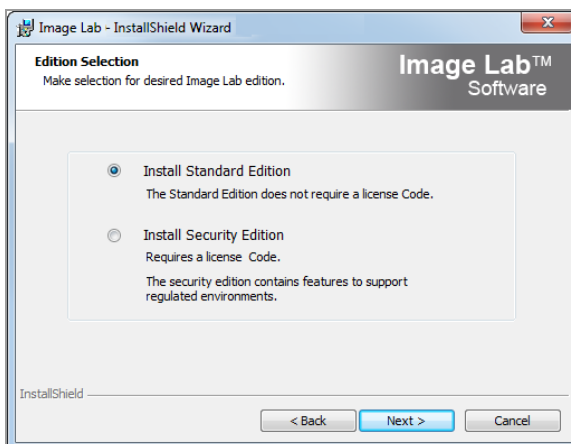
4. On the Edition Selection screen, do one of the following:

    ■   Install Standard Edition. Skip to Step 7.

    ■   Install Security Edition. Continue to Step 5.

5. (Optional) If you specified the Security Edition, the Validate Code screen appears.
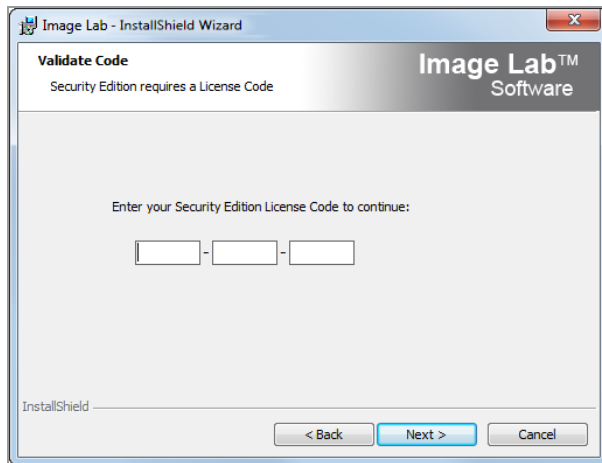
6. Enter your 18–digit license code in the Validate Code screen.

    If you are unable to enter a valid license code during installation, you can

    ■   Return to the previous screen and select Standard Mode.

    ■   Enter any 18 characters to serve as a placeholder. The Security Edition will be installed, but its features remain disabled until you activate them with a valid code. For information, see Activating Image Lab Security Edition after Installation on page 23.

7. Click Next.

    The Destination Folder screen appears.
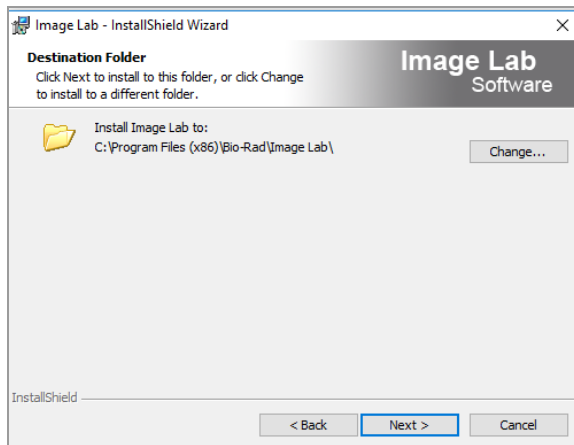
8. Accept the default location or click Change and browse to the folder you want.

9. Click Next.

   The Ready to Install the Program screen appears.
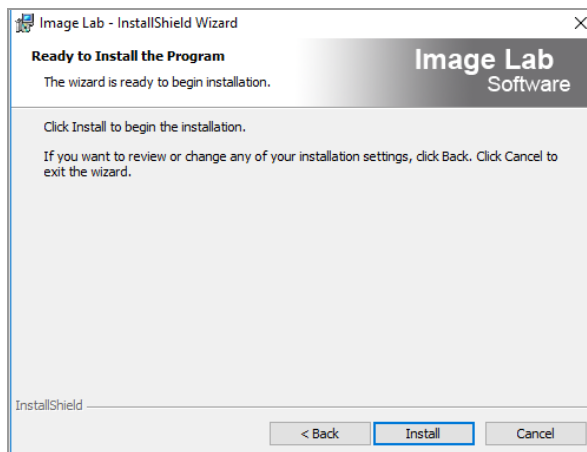
10. Click Install.

    The wizard installs Image Lab.

11. When the installation finishes, a final wizard screen appears, in which you can choose to display the Release Notes, the Windows Installer log, or both.
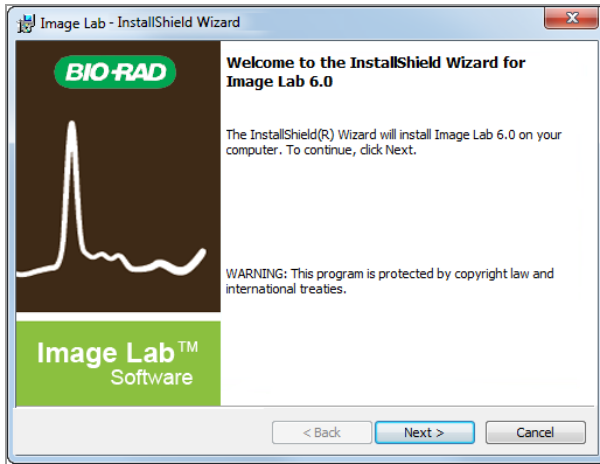
12. Click Finish to exit the wizard.

    The Image Lab icon appears on your desktop.

Windows 7 Drivers

On Windows 7, the device driver is installed during Image Lab installation. After installation, a message confirms that the device driver software installed successfully.

Note: You can ignore the error message below if it appears during installation. The error is more likely to occur if the instrument is plugged in, but the driver is installed despite the message.
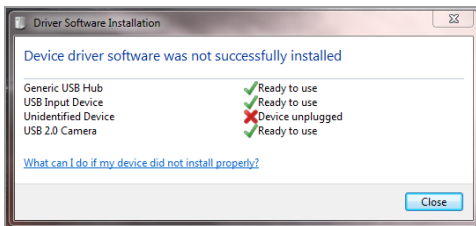
Setting Up Image Lab Security Edition

When you install the Security Edition, a Security menu is included on the menu bar. From the Security menu, Image Lab Administrators can do the following:

■ Activate Security Edition, if it was not activated during installation

■ Enable or disable Secure Mode in Security Preferences

■ Rename security groups

■ Enable write once support

■ Deactivate Security Edition

Activating Image Lab Security Edition after Installation

If you have an unactivated or deactivated version of Security Edition installed, an Activation dialog box appears each time you open the software.
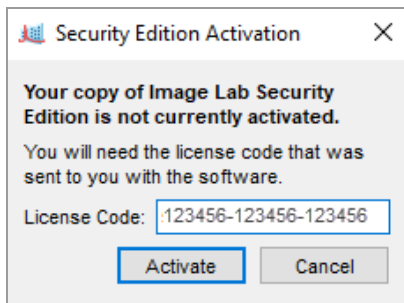
If you click Cancel, a message appears, advising that Security Edition is not activated.

You can continue to use Security Edition in Standard Mode, but you are prompted to activate the software each time you open it until Security Edition is activated with a valid license code.

Note: If you need a new license code, contact Bio-Rad Technical Support for assistance.

To activate Security Edition

1. From the Security menu, select Activate Security Edition.
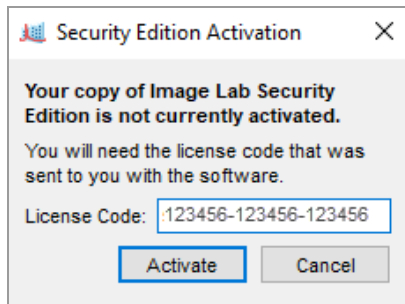
2.   Enter a valid license code in the License Code field.

3.   Click Activate.

A message appears, indicating that Security Edition is activated.

4.   Click OK.

Security features are now available in the software.

Enabling and Disabling Image Lab Secure Mode

You must have Image Lab Administrator role privileges to switch between Image Lab secure and standard modes.

To enable secure mode

1.   On the Security menu, click Security Preferences to display the Security Preferences dialog box.
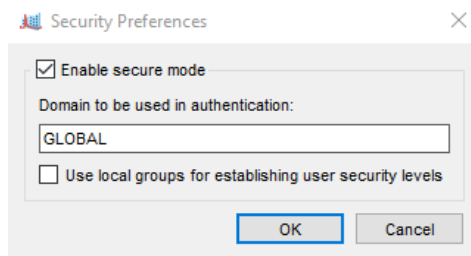
2.   Select Enable secure mode.

3.   Click OK to display the Enable Secure Mode dialog box.
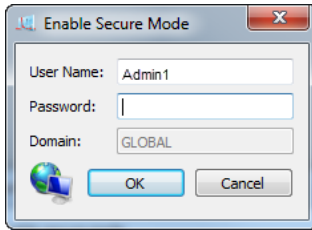
4. Enter your Image Lab Administrator user name and password.

5. (Optional) If you are working on a Windows network server, type the name of the Windows domain in the Domain box.

    Note: By default the name of the domain on which the current Windows user is located appears in the Domain box.

6. Click OK to save your changes.

    A message instructs you to restart Image Lab.

7. Click OK.

Important: For more information on setting security preferences, see Setting Security Preferences on page 27.

To disable secure mode

1. On the Security menu, click Security Preferences to display the Security Preferences dialog box.
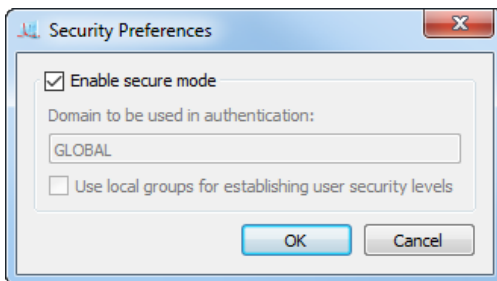
2. Clear the Enable secure mode checkbox.

3. Click OK.

    The Admin Authentication dialog box appears.
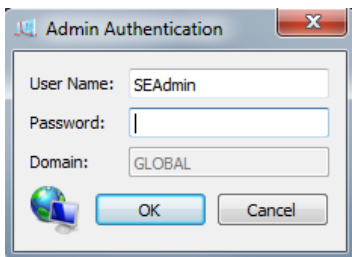
4. Enter your Image Lab Administrator user name and password.

5. If you are working on a Windows network server, type the name of the Windows domain in the Domain box.

    Note: By default the name of the domain on which the current Windows user is located appears in the box.

6. Click OK to save your changes.
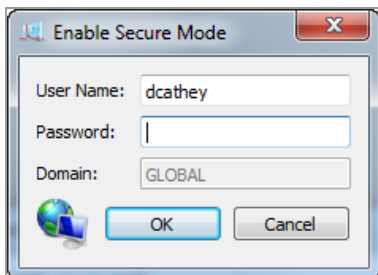
    A message instructs you to restart Image Lab.

7. Click OK.

Setting Security Preferences

You must know your network domain name in order to set security preferences. If you do not know this name, see Finding Your Authentication Domain Name on page 183 and follow the instructions to obtain your network domain name.

To set security preferences, you specify the security settings described below.

Setting	Description
Network domain	A remote domain-controlling computer or system, which ensures that only authorized users with valid credentials can access and run Image Lab. **Note:** By default, the Security Preferences dialog box displays the name of the domain on which the current user logged in.
Local domain	Local computer on which Image Lab is running, which ensures that only authorized users with valid user credentials can access and run Image Lab.
Credentials	Valid user name and password that allows or prohibits specific user actions

To enable only network domain users to run Image Lab

▶ In the Domain to be used in authentication box, enter the name of your network domain.

Leave the Use local groups for establishing user security levels checkbox unselected.
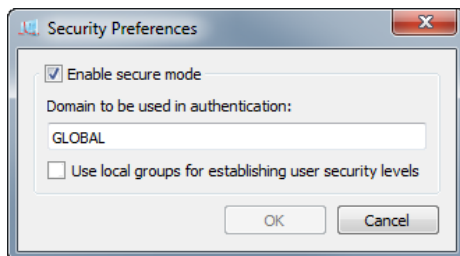
To enable only domain users who are also valid members of specific local groups to run Image Lab

1. In the Domain to be used in authentication box, enter the name of your network domain.

2. Select the Use local groups for establishing user security levels checkbox.
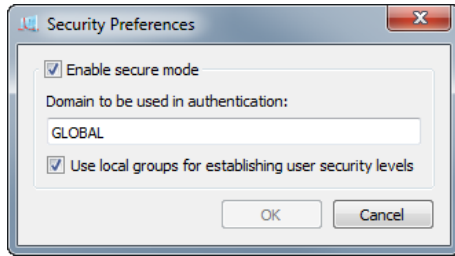
To enable only local users to run Image Lab

▶ In the Domain used in authentication box, enter the local computer name.

The Use local groups for establishing user security levels option becomes inactive.

Renaming Security Groups

To rename any of the default Security Groups

1. From the main menu, select Security > Rename Security Groups.

    This menu option is visible only if the person logged into the local computer is logged in as a member of the Windows Administrators group.
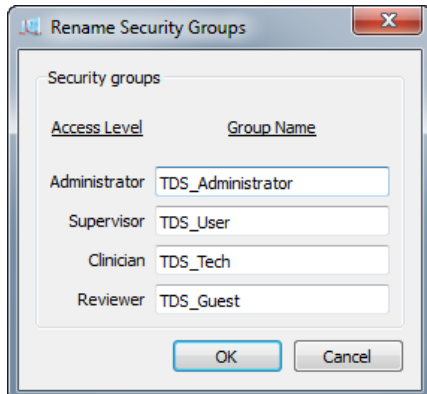
2. Click in any of the Group Name fields.

3. Enter a new name.

4. Click OK to save your changes.

Note: The new user group name must comply with standard Windows Local Users and Groups user names rules.

For more information on setting up security groups, see Setting Up Users and Groups on page 181.

Using Groups on a Local Domain

If you choose not to create or use groups on the network domain, set up local groups. Add the authorized users to the groups on the local domain. In the Security Preferences dialog box, select Use local groups for establishing user security levels.

For information about setting up users and groups for Image Lab Security Edition, see User Names, Groups, and Roles on page 172.

Changing Security Preferences

Changing the domain that is used to authenticate users is a two-step process. You first authenticate on the first domain, then authenticate on the second domain. This change in domains can be performed in either of two ways. It can be performed by one individual assigned the Image Lab Administrator role on both domains, or it can be performed by two individuals, one with the administrator role on the first domain, and the other with the administrator role on the second domain. See User Authentication and Group Membership on page 182 for more information about using this dialog box.

Deactivating Image Lab Security Edition

You must have a license for each instance of Image Lab that you install. You can load Image Lab on a second computer only if you deactivate it on the initial computer first.

You can deactivate Security Edition using the option in the Security menu.

To deactivate Security Edition

1. From the Menu bar, select Security, and then click Deactivate Security Edition.

    The Deactivate Security Edition dialog box appears.
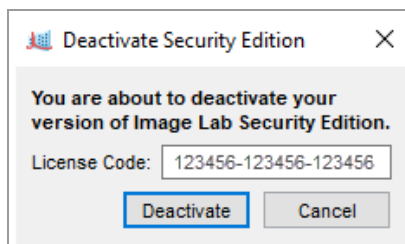
2. Click Deactivate.

    Security Edition is immediately deactivated, and a success message appears.
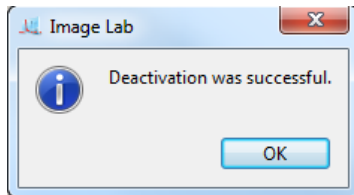
3. Click OK.

Note: To reactivate Security Edition, you must reenter your license code.

Chapter 3 Image Lab Software Overview

Image Lab Software controls image acquisition and analysis, and saves your settings in protocols you can reuse for repeatable results.

Main Window

When Image Lab starts, the main window appears.
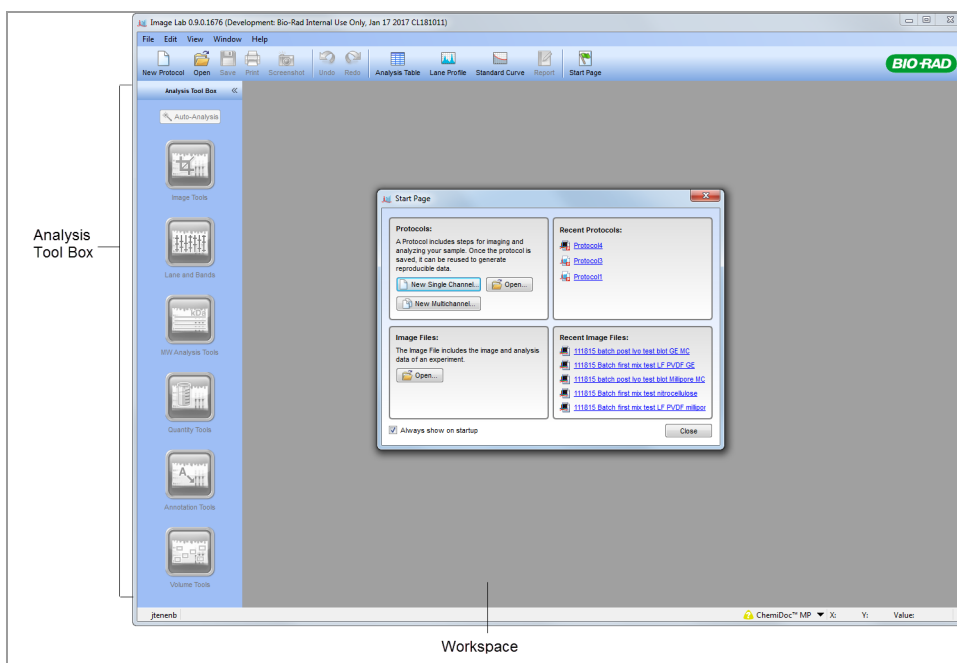
Workspace

Main window menu commands enable you to review and arrange multiple screens in the workspace to facilitate comparison of imaging results.

Names of open protocols and image files appear in the Window menu.

▶   Click a protocol or image file name to make it active.

Start Page

By default, the Start Page dialog box appears when Image Lab Starts. You can

- Select the type of protocol to create

- Open a protocol or image file

- View lists of recently saved protocols and image files

- Open external 8-bit and 16-bit, grayscale images stored in .tif format
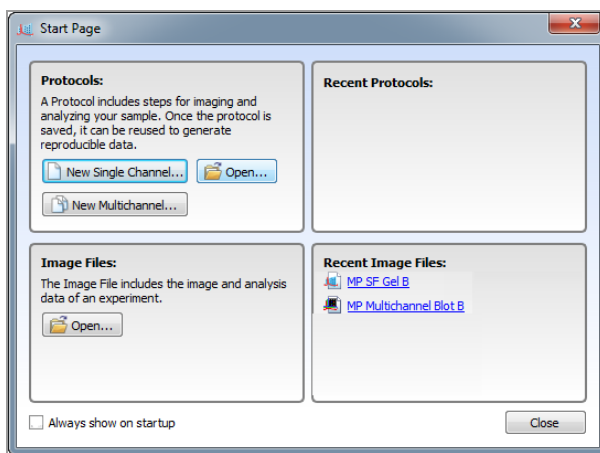
To start Image Lab without displaying the Start Page

▶ Clear the checkbox in the bottom left corner of the dialog box.

Main Toolbar

You can access most Image Lab tools by clicking buttons in the main toolbar.
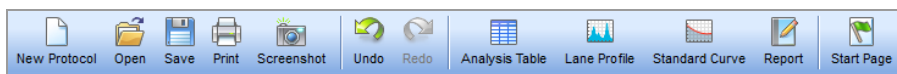
The main toolbar includes buttons that access different views of results data.

Clicking Screenshot sends a screen capture of the current image to the clipboard or saves it as a file. Unlimited Undo and Redo actions make it easy to correct missteps.

Results Data

You can view image analysis results data as an analysis table, a lane profile, a standard curve, or a report, and you can display all views at the same time.

Buttons in this section of the main toolbar access the different views of results data.

For information about each view, see Chapter 5, Viewing Images and Image Data.

Display Toolbox

The Display Toolbox, located below the main toolbar and above every image provides a variety of options to display your images.

See Chapter 5, Viewing Images and Image Data for a description of each option.

Analysis Tool Box

Analysis tools enable you to analyze and image, and are active when an image is selected. For detailed information about using these tools, see Chapter 6, Analyzing Images.

1. Click Auto-Analysis to analyze an image quickly.

2. Click a button to select the tool you want to use.

Image Tools — flip, rotate, and crop images and transform image files.

Lane and Bands — detect, resize, adjust, and bend lanes; detect, adjust, add, or delete bands. Normalize volume data in multichannel images to correct for sample loading errors.

MW Analysis Tools (Molecular Weight Analysis) — choose standard samples, assign standard lanes, and choose a regression method.

Quantity Tools — automatically quantify bands using relative or absolute values.

Annotation Tools — add formatted text and arrows to an area of a gel.

Volume Tools — manually quantify an object inside a boundary that you define, and choose background subtraction method.

Status Bar

The status bar at the bottom of the main window displays the imager type and the X and Y values at the cursor position on the image. The status bar also displays the intensity (Int) or optical density (OD) values for the image at the cursor position. The maximum data range is 0–65,535 (int) or 0.0–4.0 (OD). However, the actual range varies depending on the values contained within each image.

Tip: (Multichannel images only) Move the pointer over the composite image to display color-coded intensities for all channels.
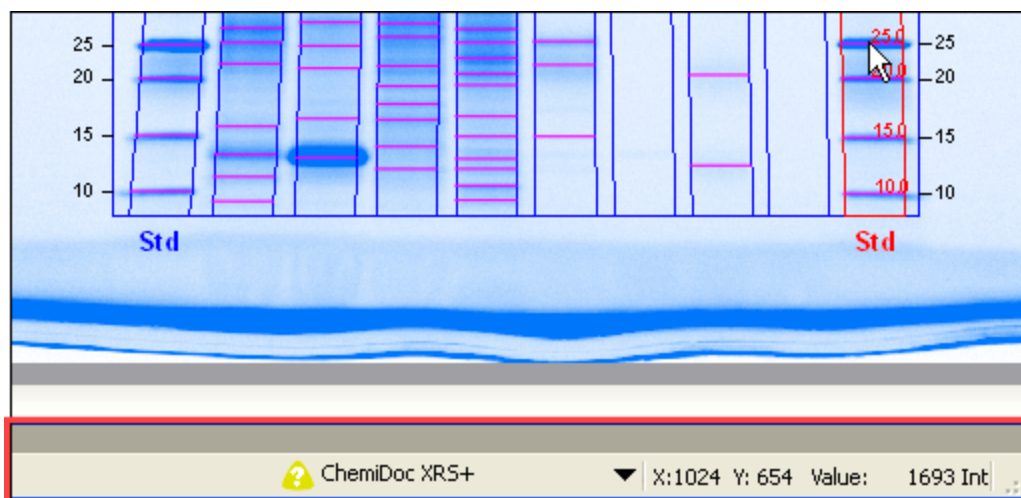
Main Window Menu Commands

This section describes all menu commands in the File, Edit, View, Window, and Help menus. Many commands are also available on the toolbar or the Start Page.

File Menu Commands

▶ From the menu bar, select File to display the options described in Table 1.

Table 1. File options

Command	Description
New protocol	Create a new protocol, which is a file that stores your settings for acquiring images, analyzing them, and creating customized reports. **Note:** This applies only to imaging systems that are controlled by Image Lab Software.
Open	Browse the file system to retrieve a protocol or image file. **Note:** You can open most external 16- or 8-bit TIFF images that are stored in strip or tile format and are either uncompressed or compressed with LZW. Most grayscale TIFF images are stored this way.
Recent images	Open a recent image file.
Recent protocols	Open a recent protocol. **Note:** This applies only to imaging systems that are controlled by Image Lab software.
Save	Save a named protocol or image file.
Save as	Name and store a protocol or image. **Note:** Protocols are stored with a .ptl or .sptl extension. Image files are stored with an .scn or .sscn extension.
Close	Close the active window.
Close all	Close all windows.

Table 1. File options, continued

Command	Description
Export	Export gel images or analysis tables using the following options: ■ Export for publication — export a displayed image to a file. You can select from .bmp, .png, .jpg, and .tif formats. The gel displays with any lanes, bands, and annotations that appear on the screen. ■ Export for analysis — create a TIF file that retains all gel image data. Analysis data are not included. Use this option to analyze the image in other software. See Exporting Gel Images for Publication on page 156 ■ Export for PulseNet— reduce the image to an 8-bit .tif file. Resolution is limited and file size is restricted to 300 dots per inch (dpi). ■ Lane and band table to Excel — export the lane and band table data to an Excel (or Numbers on a Mac) spreadsheet (Excel or Numbers must be installed on your computer). ■ Lane and Band table to file — export as a comma-separated values (CSV) file so that the lane and band table can be opened in a database application. ■ Volume table to Excel — export volume table data to an Excel (or Numbers on a Mac) spreadsheet (Excel or Numbers must be installed on your computer). ■ Volume table to file — export as a CSV file so the volume table can be opened in a database application. **Note:** See Exporting Results on page 155 for more information about exporting files.
Image info	Display information about individual gel and blot images, such as acquisition date and data range, and image capture detail, such as exposure time and illumination source used. **Note:** Click the Image Details, Analysis Settings, and Notes tabs to display these properties. See Image Info on page 76 for more information.
Page setup	Access print settings such as orientation (landscape or portrait), margins, printer used, and paper size.
Print	Display a preview of the gel and header information, which includes the file name of the image, the user's name, and the date and time of printing. **Note:** Click Print in the Print Preview dialog box to select a printer and the number of copies to print.
Exit	Close Image Lab.

Edit Menu Commands

▶ From the menu bar, select Edit to display the options described in Table 2.

Table 2. Edit options

Command	Description
Undo	Undo the last action.
Redo	Restore the last action after an Undo.
Screenshot	Capture an image of the Lane Profile Window, the Standard Curve Window, or Current Image View.
	You can include the image name in the screenshot, and you can send the screenshot to the clipboard or save it in a file.
Default imager	Connect two or more Bio-Rad imaging systems to the same computer, allowing users to switch between them..
Instrument setup	Display information about the instrument, including its name, serial number, camera serial number, illumination options, and last calibration.
	If you add accessories to the instrument, you can reset the system calibration in this dialog box.
Report settings	Select or clear checkboxes to configure reports from different tabs. Selections apply to all reports.
	■ The General tab has options for excluding or reporting information about your gel image.
	■ The Lane and Band Table tab enables the researcher to choose whether to include all lanes or selected lanes, with appropriate identifiers. Lane profiles can also be included.
	■ The Volume Table tab enables the researcher to choose appropriate identifiers for the volume table and provides the option of excluding the table from reports.
	■ The Lane Statistics tab enables the researcher to change the statistical categories that are displayed in reports.

Table 2. Edit options, continued

Command	Description
Preferences	Set naming and color preferences for image files.

■ The Protocol tab display presets for naming image files. You can include a designated Prefix, User Name, Date, and Time in the name of your image files.

■ The Colors tab allows you to choose colors for the graphic elements in gels, such as Lane Frame, Lane, Band, Band Attribute, and MW Legend to ensure that the elements are visible, regardless of the gel color.

Note: This dialog box has two tabs and can also be accessed from the Image Lab menu in a Mac OS.
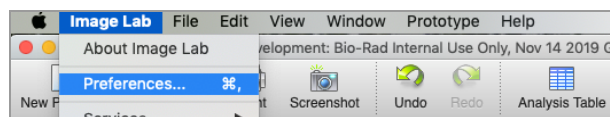
View Menu Commands

▶ From the menu bar, select View to display the options described in Table 3.

Table 3. View options

Command	Description
Image overview	Display the gel image with a red rectangle outlining the area visible in the larger main window. This is useful when you zoom in to a small section of an image.
Image transform	Display a histogram that enables you to adjust the light and dark values of a gel image. This adjustment does not change your data, only the way the data display on your monitor.
Operations history	Display the sequence of actions performed by both the user and the software.
View system log	Display a log of events related to running Image Lab software, including enabling or disabling secure mode, and the users who log on to or log off of the software.
View instrument log	Display events related to the instrument, including calibrating the instrument and the success or failure of the calibration. This log file is visible only if Image Lab is used to control an instrument.
View document log	When Security Edition is enabled, display events related to creating and modifying secure protocol and image files.

Security Menu Commands

▶ From the menu bar, select Security to display the options described in Table 4.

Important: This menu is visible only in Image Lab Security Edition. For more information, see Chapter 10, Using the Security Edition.

Table 4. Security options

Command	Description
Security preferences	Allows the Image Lab Administrator to enable and disable secure mode, choose the domain to be used for authentication, and choose whether to use local groups for security levels.
Rename security groups	Allows a user logged in as a member the Windows Administrators group to change any of the four default Image Lab security group names (TDS_Administrator, TDS_User, TDS_Tech, and TDS_Guest).
	Note: This command is visible only if the user logged into the local computer as a member of the Windows Administrators group. Only users logged in as a member of that group maintain the authority to change any of the four default Image Lab security group names. Any changes made to these Security Group names must match the names your Windows system administrator has given those groups.
Sign document	Enables users to sign images and protocols. Users enter their user name and password and provide a reason for signing. When the document is signed, the reason is saved in the System Log file.

Window Menu Commands

Window menu commands enable you to show and hide multiple open image files in the workspace. This menu also lists all currently open images and protocols.

▶ From the menu bar, select Window to display the options described in Table 5.

Table 5. Windows options

Command	Description
Tile	Align all open image files so they are visible at the same time.
Tile horizontal	Align all open image files from top to bottom.
Tile vertical	Align all open image files from left to right.
Cascade	Stack all open image files and protocols with overlapping title bars, so each one can be easily chosen for viewing.
Imitate zoom	Change the zoom setting of all open images to the zoom setting of the current image file.
Imitate transform	Change the brightness and contrast of all open images to the transform settings of the current image file.
Next	Cycle through all open image files from oldest to newest.
Previous	Cycle through all open image files from newest to oldest.

Help Menu Commands

▶ From the menu bar, select Help to display the options described in Table 6.

Table 6. Help options

Command	Description
Image Lab User Guide	Display this guide.
Instrument Guides	Display a list of instument guides for imaging systems that work with Image Lab Software.
Register Image Lab	Display the Image Lab Registration Form.
About	Display Image Lab software version, release date, and copyright information.

Chapter 4 Protocols

Image Lab Software runs configurable, application-based protocols for sample imaging.

A protocol includes the settings for acquiring images, analyzing them, and creating customized reports. You can retrieve, revise, and reuse protocols.

Image Lab supports single channel and multichannel protocols.

You can use Image Lab to analyze images acquired by any Bio-Rad imaging system.

Important: This chapter describes the major steps of creating and running protocols. Because protocol settings are specific to each imaging system, more detailed procedures about image acquisition settings and running protocols appear in the instrument guide for your imaging system.

View Screens Designed for Your Imager

When you turn on an imager connected to the computer running Image Lab software, Image Lab detects the imager type and displays screens relevant to its use. To see if your computer is connected to your imager, check the imager status. The name of the selected imager appears on the status bar in the right corner of the Image Lab screen:
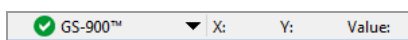
The green check mark next to the instrument name indicates that the instrument is connected to the computer running Image Lab software and is communicating with Image Lab.

Tip: To create protocols for another imager, choose a different imager name in the dropdown list.

The Protocol Setup Window

Note: The images in this section show a protocol for the GS-900 Calibrated Densitometer. If you are using a different imager, the screen might look slightly different.

In the left pane, numbered Protocol Setup steps appear under different headings.

1. Select or clear checkboxes to enable or disable steps. When you select a step, the right pane displays detailed settings for that step.
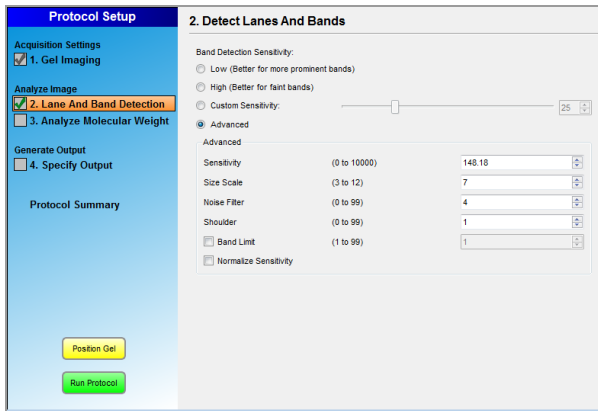
2. Click Protocol Summary in the left pane to review protocol settings. In the right pane, the summary lists the settings for each step.
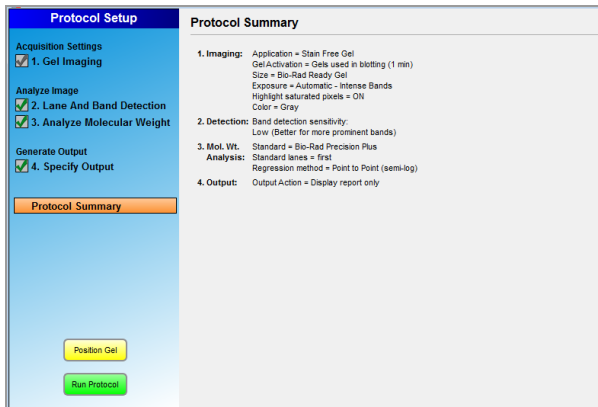
Protocol Setup Steps

You configure the following settings to create a protocol:

■ Acquisition Settings — (required) acquire the image

■ Analyze Image — (optional) detect lanes and bands and analyze the molecular weight

■ Generate Output — (optional) print the image; print or display the report

Image Lab Software presents these steps in a series of Protocol Setup screens. The left pane in each screen lists the tasks of each major protocol step. Protocol Setup screens appear in the order listed in the left pane. After you configure each Protocol Setup step, you can view a summary of your settings and you can return to a previous step and edit its settings.

Note: Example screens in this chapter might look slightly different from protocol setup screens on your imaging system.

Acquisition Settings

Acquisition settings include

■ Selecting an application with predefined image acquisition settings optimal for the sample type

■ Specifying settings for the imaging area

■ Setting the image exposure time and choosing to optimize for faint or intense bands

■ Setting display options to highly saturate pixels and choose a color for the image

Custom Applications

Applications you can select for acquiring images are predefined with image acquisition settings optimal for your sample type. The applications list is specific to your imaging system.

The Application menu also includes a Custom command that enables you to create a custom application. You can also create a custom application identical to a predefined application and rename it. Saved custom applications can be reused or edited.
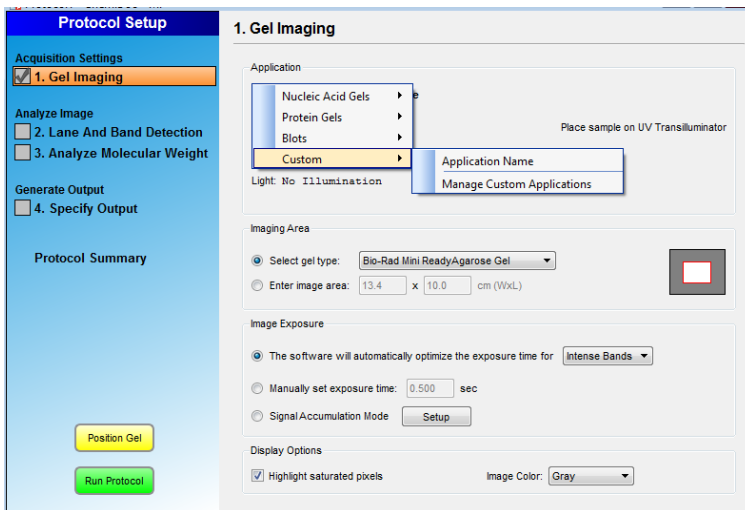
Note: A detailed procedure specific to your imaging system for creating a custom application appears in the chapter Acquiring Images in your imaging system instrument guide.

Multichannel Acquisition Settings

An imager that can acquire images with a multichannel protocol displays acquisition settings for each channel you use. As you configure channels, the software displays available acquisition settings for each channel based on settings already selected for other channels.

Analyze Image

In the Analyze Image protocol setup, you select two steps, Lane and Bands Detection and Analyze Molecular Weight.

Detecting Lanes and Bands

To analyze the gel or blot, Image Lab must detect lanes and bands on the image. Image Lab automatically detects lanes and subtracts the background.
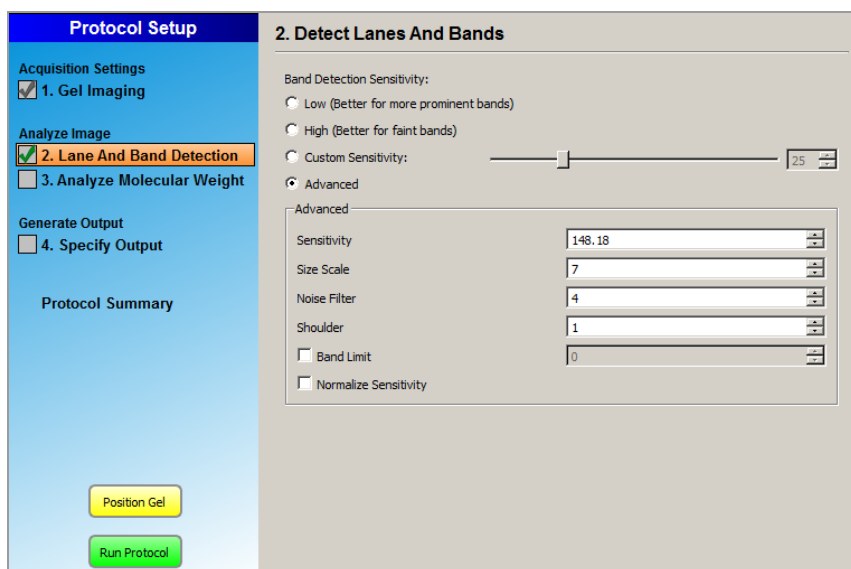
Set detection settings so Image Lab can detect lanes and bands to your requirements:

- Specify sensitivity options for band detection

- Select normalization options for multichannel protocols

Analyzing Molecular Weight

Image Lab calculates the molecular weight for each band based on the standard you specify.

Note: Lane detection works best when standards are placed in the first and last lanes.

Image Lab uses a regression method to calculate the molecular weight of unknown bands.

For information on ordering standards, see Appendix D, Accessories.

Generate Output
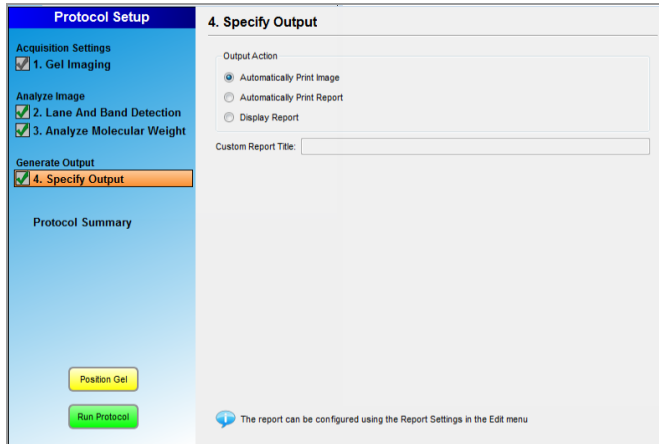
Select the type of output you want for a single image or report:

- Automatically print the image

- Automatically print a report

- Display the report

When you choose Display Report, a scrollable report screen opens (similar to the next image). You can scroll down to view acquisition settings and analysis data.
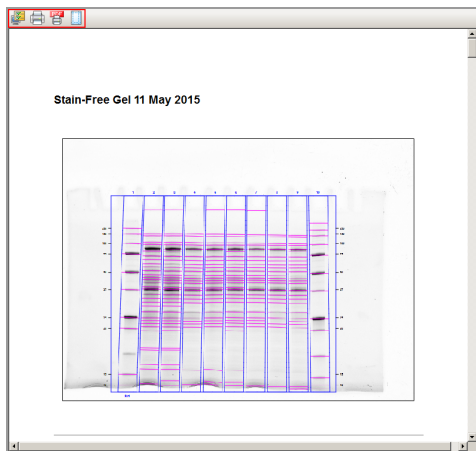
You can select options in the report screen toolbar to customize or print the report, create a PDF file, or adjust the printer settings. For more information, see Report Options

Detect Lanes and Bands

To analyze the gel or blot, Image Lab must detect lanes and bands on the image. You can set band detection to a higher sensitivity level so that the software can detect faint bands on the image. Besides lane and band detection, Image Lab subtracts background automatically. If the sensitivity is set too high, background staining might be detected as bands. If the setting is too low, bands of interest might not be detected. For information about adjusting settings manually after acquiring an image, see Lane and Bands Settings on page 109.

Note: (Multichannel images only) You cannot specify different sensitivity levels for individual channels in a multichannel image. The same sensitivity level applies to all channels. After the image is generated, you can change the sensitivity level for individual channels using Lane and Bands tools.

To configure lane and band detection settings

1. Select the Lane and Band Detection checkbox in the left pane of the Protocol Setup window.

2. In the right pane, select a lane and band detection option.

    - **Low Band Detection Sensitivity** — sets detection at a low level (25) for images with more prominent bands. Faint bands are not detected with this setting.

    - **High Band Detection Sensitivity** — sets detection at a higher level (75) for images that are fainter. To remove extraneous bands later using Band tab settings in the Lane and Bands. See Lane and Bands Settings on page 109.

    - **Custom** — sets a numeric value for Sensitivity from 1 to 100 that you choose by using the slider.

    - **Advanced** — accesses advanced settings. Set more precise values for Sensitivity. Set Size Scale, Noise Filter, and Shoulder values. Turn on Band Limit and Normalize Sensitivity. Advanced settings are defined in the section, Advanced Sensitivity Options, under Detecting Bands on page 118.

Configure the Normalization Channel

(Multichannel images only) You can select and configure any lane in a multichannel image as a normalization channel.
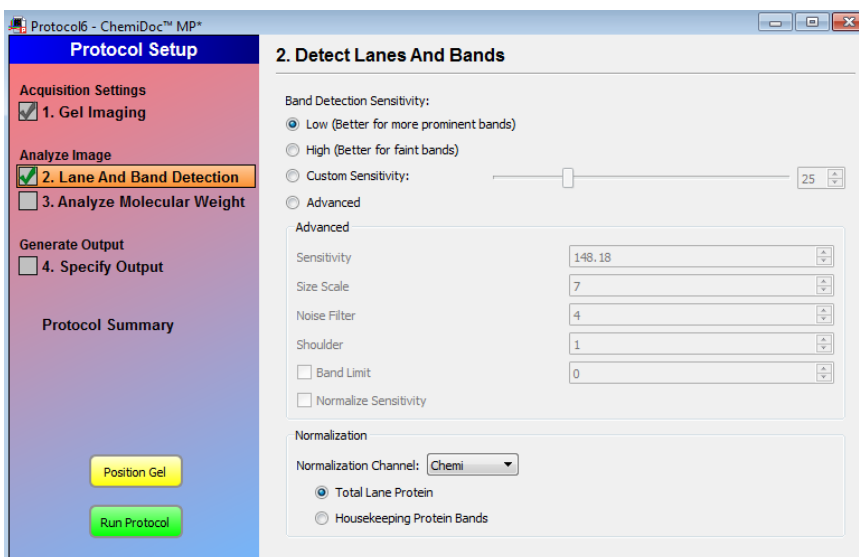
For more information, see Normalizing Volume Data on page 122.

Analyze Molecular Weight

Determining molecular weight depends on selecting the proper protein standards. Image Lab calculates the molecular weight for each band based on the specified protein standard.
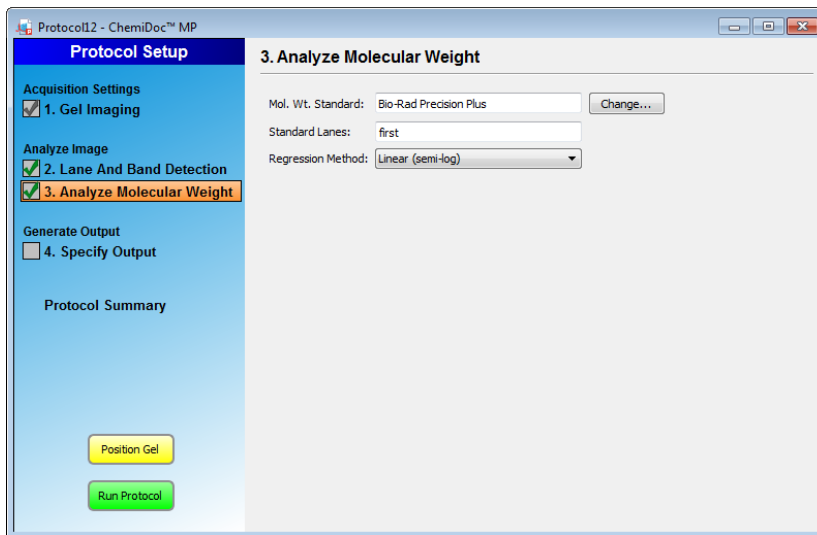
Protein and DNA standards are available from Bio-Rad. For more information, see Appendix D, Accessories.

To analyze the sample molecular weight

1. Select the Analyze Molecular Weight checkbox in the left pane of the Protocol Setup window.

2. Click Change to view a list of predefined standards.

    The Manage Standards dialog box appears.
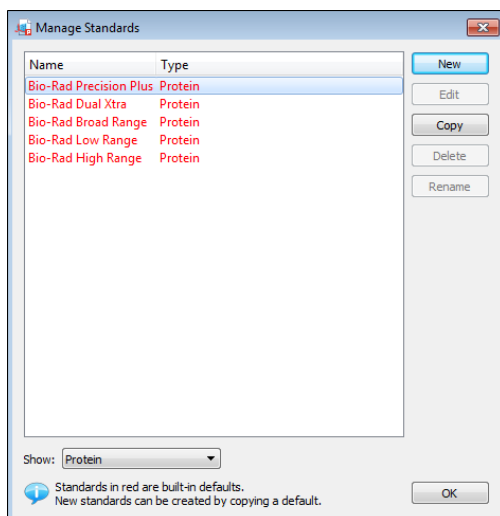
3. Select the standard and click OK.

   If the standard you are using is not on the list, do one of the following:

   ■ Use the Show dropdown list, select All Standards, select the standard you are using, and click OK.

   ■ Add a standard, select it, and click OK. See To add a standard to the list of predefined standards on page 58.

   ■ Copy a standard for modification. See To copy a standard and add it to the predefined list of standards on page 58.

4. Choose the lane(s) that contain your standards by typing lane numbers or the words *First* and *Last* in the Standard Lanes box. The format is xx, xx, xx, and so on, where xx is the lane number. For example, if you run an 18-well gel and want your standards in lanes 1, 10, and 18, enter *First*, *10*, *Last*.

   Note: Lane detection works best when standards are placed in the first and last lanes. For nucleic acid samples, use this step to determine the size of the bands in base pairs.

   For information about manually estimating molecular weight after image acquisition, see Molecular Weight (MW) Analysis Tools on page 131.

5. Select the appropriate regression method:

■ Point to Point (semi-log)

■ Linear (semi-log)

■ Logistic

■ Cubic Spine

For more information, see Regression Methods on page 147.

To copy a standard and add it to the predefined list of standards

1. Click Change in the Analyze Molecular Weight protocol setup screen.

The Manage Standards dialog box appears.

2. Select a standard that closely matches the standard you are using.

3. Click Copy.

The copy is added to the list.

4. Select the copy of the standard you chose.

5. Click Edit.

The Edit Standard dialog box appears.

6. Change the name, type, units, and values to match the standard you are using.

7. When you are finished, click OK.

To add a standard to the list of predefined standards

1. Click Change to display the Manage Standards dialog box.

2. Click New.

The Edit Standard dialog box appears.
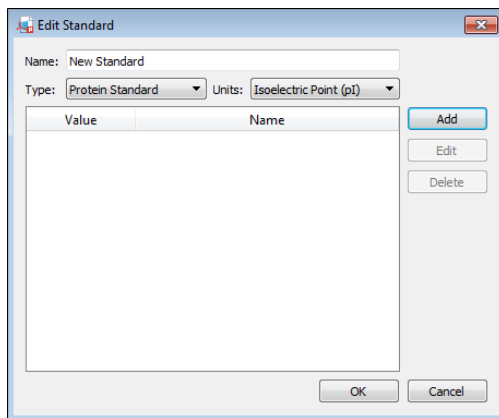
3. Type the name of the standard you are using.

4. In the Type dropdown list, select Protein Standard or Nucleic Acid Standard.

5. In the Units dropdown list, select the unit of measure for the molecular weight size markers.

6. Click Add.

   The Edit dialog box appears.
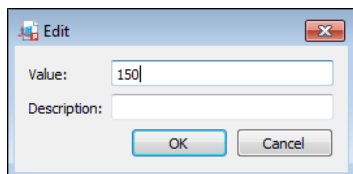
7. Type a value for each molecular weight size markers and click OK. Adding a description is optional.

   Note: You must enter at least two molecular weight size marker values.
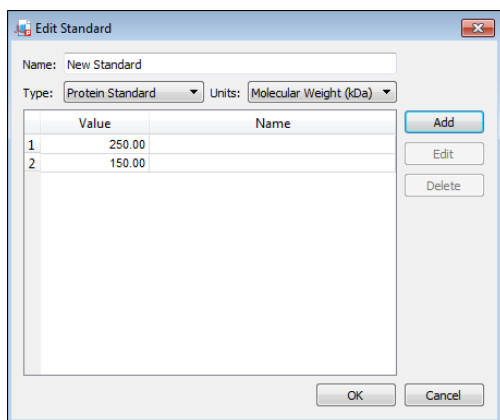
8. When you are finished entering values, click OK.

Specify Output

To specify protocol output

1. Select Specify Output in the left pane of the Protocol Setup window to display output options.
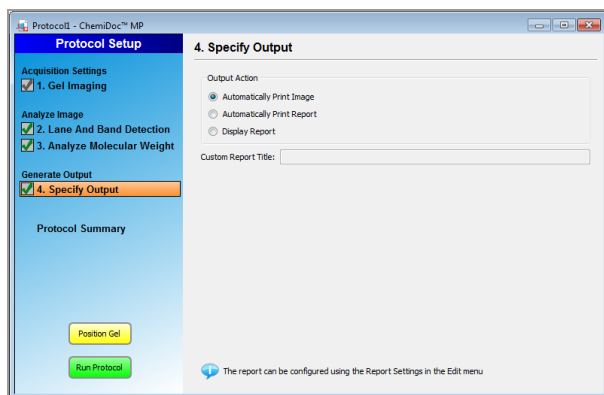
2. In the right pane, choose an output option:

   ■ Automatically print the image

   ■ Automatically print a report

   ■ Display the report

   Image Lab prints to the default printer unless you specify otherwise. For information about customizing reporting options, see Report Options on page 149.

Setting Up a Custom Application

When you create a custom application you can combine acquisition settings based on the following variables.
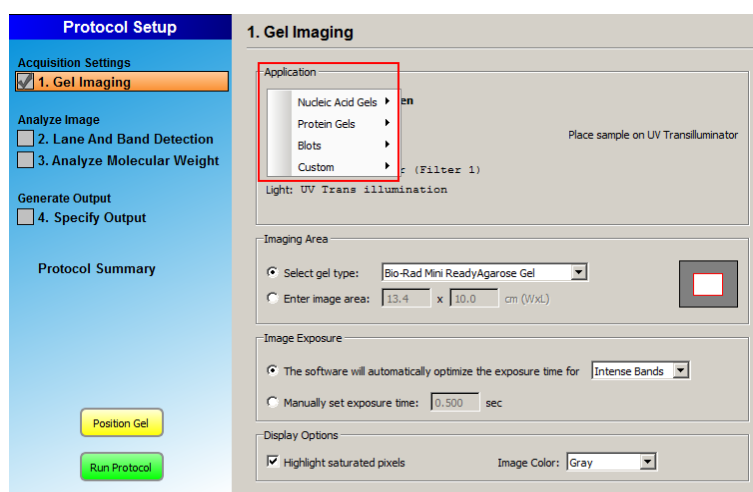
Note: The list of variables varies depending on the imaging system.

- Light source

- Filter

- Tray

- Image or display color

- Binning

- Scan mode

To learn more about creating a custom application, see the chapter Acquiring Images in the instrument guide for your imaging system.

Review Protocol Settings

Click Protocol Summary in the left pane of the Protocol Setup window to display a summary of all protocol settings:

Editing a Saved Protocol

You can open a protocol, change settings, disable a protocol step, and save the protocol.

Running a Protocol

Detailed procedures for running a protocol appear in the chapter Acquiring Images in the instrument guide for your specific imaging system.

Note: (GelDoc EZ only) Running a protocol in this imaging system requires different steps. See the detailed procedure in your instrument guide.

To run a protocol

1. Position the gel in the imager.

2. On the Protocol Setup screen, click Run Protocol.
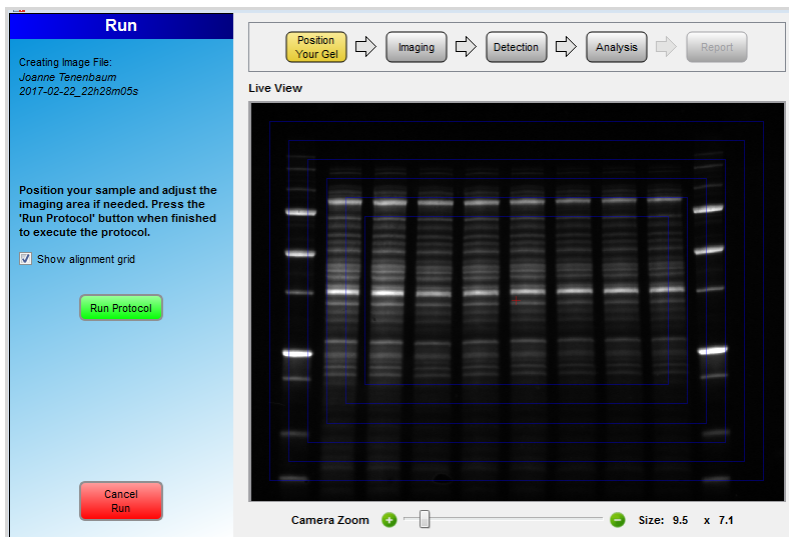
Image Lab runs the protocol in imaging mode.

After acquiring the image, Image Lab continues with the steps selected during protocol setup, such as detection, analysis, and output. The protocol steps appear in boxes across the top of the screen. Image Lab highlights each step as it is completed.

When the protocol ends, the image appears in the workspace. You can edit and save the image or analyze it further.

To cancel the protocol in progress

▶   Click Cancel Run.

Regression Methods

A regression method is used to calculate the molecular weight of unknown bands. Image Lab uses the relative front and molecular weight values of the standard bands to calculate the standard curve. The standard curve is then used to calculate the values of the unknown bands. The shape of the standard curve is based on the selected regression method.

During Protocol Setup step 3. Analyze Molecular Weight, you must choose one of the following regression methods.

Regression Methods

Regression Method	Minimum number of standard bands
Linear (semi-log)	2
Point-to-point (semi-log)	2
Logistic	5
Cubic spline	5

The molecular weight of each band appears in the Mol. Wt./Base Pair column in the analysis table. If you do not have enough data points for the method you select, the molecular weight of the unknown bands is not calculated.

You can check how well each regression method fits the data in the standard curve window. For more information, see Standard Curve on page 99. The linear (semi-log) regression method provides a measurement that describes how well the standard curve fits the data R^2 value. The closer the R^2 value is to 1.0, the better the data fit the standard curve.

To display the standard curve

1. Click Standard Curve on the toolbar.

2. In the Standard Curve dialog box, select the Volume Standard Curve tab.

Note: When the Force Through Origin checkbox is selected, the standard curve graph starts at 0,0, regardless of the best curve fit.

See Appendix A, Regression Calculation Methods, to learn how each regression method is calculated.

See Molecular Weight (MW) Analysis Tools on page 131 for more information about molecular weight.

Chapter 5 Viewing Images and Image Data

After a gel is imaged, the image appears in the Image Lab software workspace.

The following image shows a gel image with band and lane detection and annotations. The labels are overlays that you can display or hide.

You can view data as an analysis table, a standard curve, and a report.
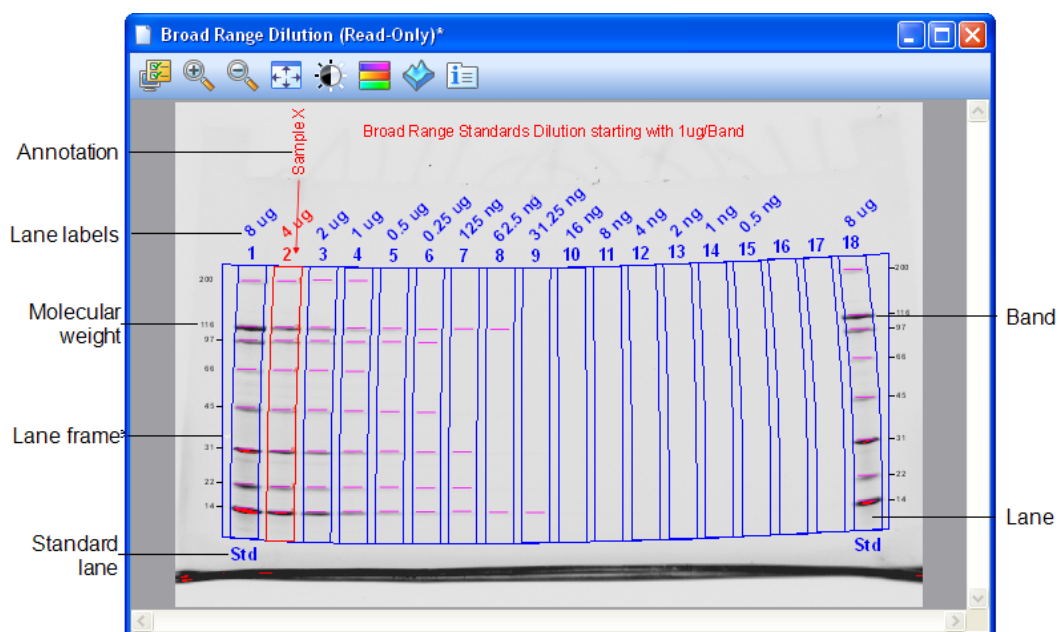
Displaying Gel Images

The Display Toolbox appears above the gel image. Each tool is described in the following sections.
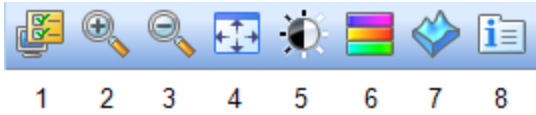
LEGEND

1	Display Gel Options	5	Image transform
2	Zoom in	6	Change image colors
3	Zoom out	7	Switch to 3D view
4	Fit in window	8	Image info

Display Gel Options

To display gel options

▶ Click the Display Gel Options icon in the Display Toolbox above the image.

The Display Gel Options dialog box appears.
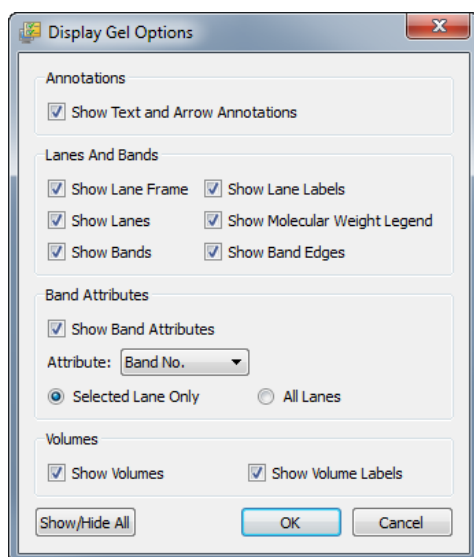
Annotations

Under Annotations, you can choose whether to show text and arrow annotations that have been drawn on the image.

Lanes and Bands

Under Lanes and Bands, you can turn on or off image overlays, such as lane frames, lanes, bands, lane labels, molecular weight legends, and band edges.

Band Attributes

Under Band Attributes, you can select one of the following attributes to display for a selected lane or for all lanes.

- Band number

- Band label

- Molecular weight

- Relative front

- Volume

- Absolute Quantity

- Relative Quantity

- Band %

- Lane %

Volumes

Under Volumes, when you draw volume boundaries on the gel, you can display the boundaries and their volume labels.

Zoom Tools

Zoom tools resize the gel image.

To enlarge the image

▶   Click the magnifying glass with the plus sign.

To reduce the image size

▶   Click the magnifying glass with the minus sign.

You can also zoom in and out using the mouse.

To zoom in and out by dragging

1.   With the pointer in the image, hold down the Alt key and drag the pointer to surround the area of interest.

   The image area you selected fills the screen.

2.   Double-click in the image to return it to full size view.

To zoom in and out by scrolling

1.   With the pointer in the image, hold down the Ctrl key (PC) or Command key (Mac) and use the mouse wheel to scroll up and down to enlarge or shrink the image.

2.   Double-click in the image to return it to full size view.

Fit in Window

After you zoom in on an area of an image, clicking Fit in Window restores the image to full size view.

Fit in Window is also useful when you display a channel image in single view. Click Fit in Window to display the selected channel image in full size view.

Image Transform

In this dialog box, you can adjust image brightness and contrast to optimize the image display so faint details can be seen.

Note: Image Transform adjustments do not change the data. They change only the way the data are displayed.

To access image transform options

▶   Click the Image Transform icon in the Display Toolbox above the image.

The Image Transform dialog box appears.
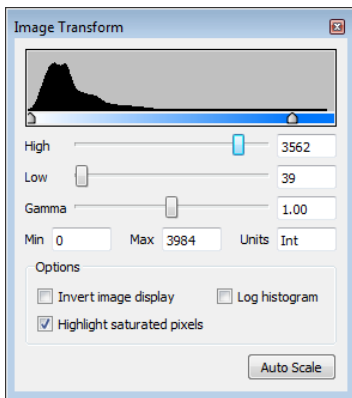
The minimum to maximum range varies depending on the light and dark values present in the image. The human eye cannot see the full range the image contains.

The frequency distribution histogram shows the total data range in the image and the amount of data at each point in the range.

Auto Scale determines an optimal setting for the image automatically. The lightest part of the image is set to the minimum intensity. The darkest is set to the maximum.

Set Intensity Values with Sliders

■ The High slider determines the intensity value used as the maximum grayscale point in the gel image.

■ The Low slider determines the intensity value used as the maximum grayscale point in the gel image.

■ The Gamma slider changes the grayscale curve. A value of 1 is linear. A value <1 redistributes a greater proportion of the grayscale to the first half of the intensity values. A value >1 redistributes a greater proportion of the grayscale to the second half of the intensity values.

Set Intensity Values by Entering Numbers

You can also type numerical values in the boxes next to the sliders.

▶ Click anywhere on the sliders to move them incrementally.

Options

■ **Invert image display** — inverts dark bands on a light background to light bands on a dark background. Light bands on a dark background are inverted to dark bands on a light background.

■ **Highlight saturated pixels** — areas of the image with saturated signal intensity (higher than a measurable range) appear highlighted in red. In multichannel images, you can highlight in red individual saturated pixels in a channel. You cannot highlight saturated pixels in a composite image.

■ **Log histogram** — changes the y-axis on the histogram to display the number of pixels at each intensity value using a linear or logarithmic scale.

Changing Settings in Multichannel Images

In the Image Transform dialog box, you can change settings for each channel in a multichannel image. To do so, select a channel at the bottom of the dialog box and change the settings for that channel. Repeat this process for each channel.

In addition to the adjustments described in Image Transform on page 72, you can change the color of each channel. Changing the channel color in the Image Transform dialog box also updates the title bar and the channel buttons on the multichannel image.
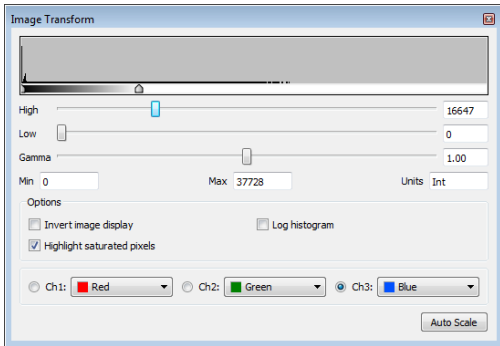
When you change the color of a selected channel in Image Transform, its color also changes in the composite image.

Image Colors

You can choose a color map for the image results file. Viewing the image with a different color scheme can make all of the elements in the image more visible.

Note: In multichannel images, you can change colors in individual channels. You cannot change colors in the composite image.

Changing the color map for an image does not change the data. It changes only the way the data are displayed.

To access image color options

▶ Click the Image Colors icon in the Display Toolbox above the image.

The Image Colors dialog box appears.
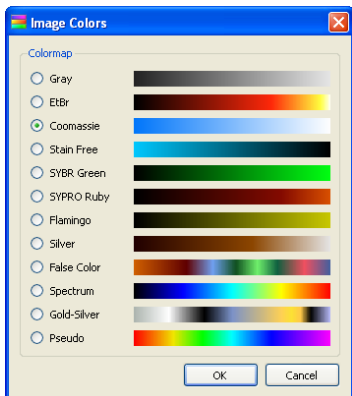
The first eight color choices imitate the colors of stained gels. The remaining choices supply enough color variation to highlight small differences in the image data. The dialog box lists each available color.

3-D Projection

The 3-D view transforms the gel image into a solid three-dimensional model spinning in space with x, y, and z dimensions. Accentuate or diminish the relative heights of data points by moving the slider at the bottom of the window right or left.

Note: In a multichannel image, you can view each channel separately in 3-D. You cannot display a composite image in 3-D.

To view the intensity of various bands

1. Click 3-D in the Display Toolbox above the image.

    The image appears in 3-D.

2. Drag the model to rotate it into your preferred view.

3. Click the image to bring the window into focus.

To evaluate the intensity of various bands

1. On your keyboard, press C to display an inverted green cone in the 3-D view.
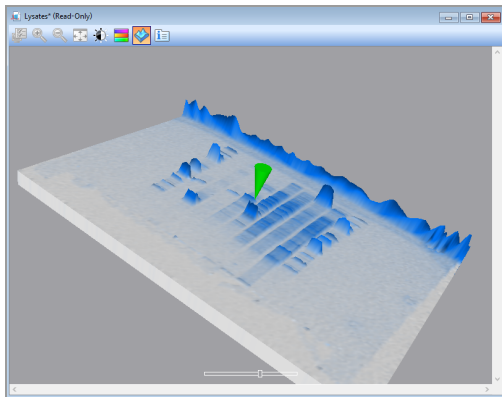
2. Drag the inverted cone around the image.

    The intensity value at the cone's location appears in the right corner of the status bar.

3. Press C again to hide the cone.

To leave 3-D view

▶   Click 3-D in the Display Toolbox.

Image Info

The Image Info dialog box reports information about the active image.

To access image info

1. Click the Image Info icon in the Display Toolbox above the image.

    The Image Info dialog box appears.
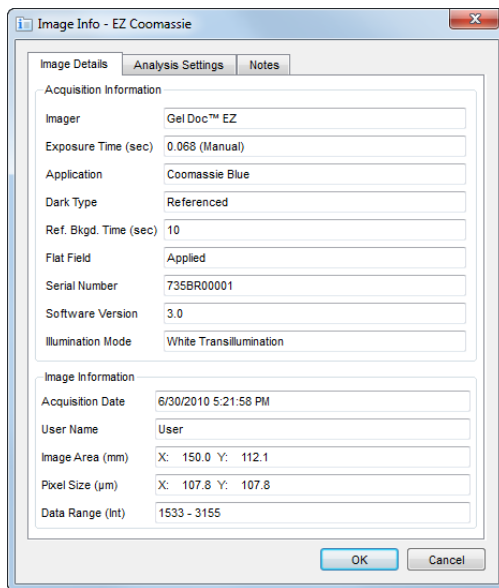
2. (Multichannel images only) Select a channel.
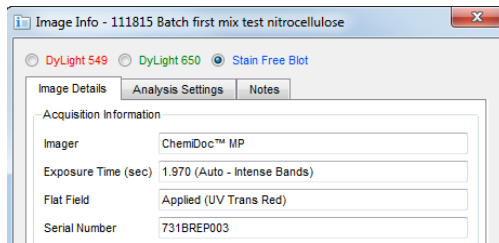
Detailed information about the selected channel appears.

Three tabs display the following kinds of image information:

- **Image Details** — acquisition and image information.

- **Analysis Settings** — analysis settings used to image the gel. For example, if Band Detection and Molecular Weight Analysis were performed, their data appear here.

- **Notes** — Enter any information you like to describe the image, such as the types of samples used, and add other information about the results. Under Lane Information, you can specify custom labels for lanes. In a multichannel image, custom labels apply to lanes in all channels.

Displaying Multichannel Images

The multichannel view displays the composite of all channels in the image file as well as an image of each channel. The name Multichannel appears in the title bar of the composite. An application name appears in the title bar of each channel image. A yellow border surrounds the active image.
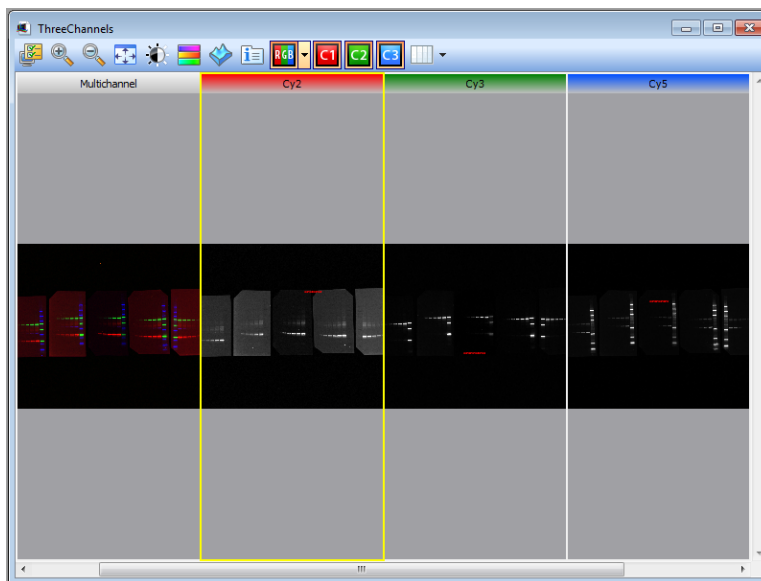
Multichannel View Settings

In multichannel view the Display Toolbox includes multichannel settings. These settings enable you to view the multichannel image in color or grayscale, show or hide channels in the multichannel view or in the composite image, and change the layout of the display.

Most multichannel view settings offer two or more alternatives (for example, the Change Layout icon). Click a setting to select its alternative.
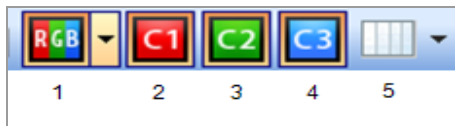
Legend

1	Show RGB/Grayscale Composite
2	Show/Hide Channel 1
3	Show/Hide Channel 2
4	Show/Hide Channel 3
5	Change Layout

To display or hide the composite image

▶   Click RGB to display or hide the composite image in the multichannel display.

To display a multichannel image in RGB or Grayscale

▶ In the RGB dropdown list, do one of the following:

■ Click Show Grayscale Composite to display the composite image in grayscale.

■ Click RGB Composite to display the selected channels in color.
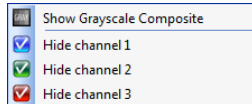
In the Display Toolbox RGB changes to Gray or vice-versa to match the setting you choose.

Note: The setting you select also applies to reports, printouts, and exports for display.

To display or hide a channel in the multichannel display

■ In the Display Toolbox, click a numbered channel (C1, C2, C3).

To show or hide a channel in the composite image

■ In the RGB dropdown list, click Hide or Show a channel in the composite image.

To change the multichannel layout

▶ In the change channel layout dropdown list, choose a layout.
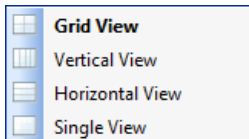
Change Layout

You can view multichannel images in four different views.

To select a display view

1. Click the Change Layout icon in the Display Toolbox above the image.

    A list of layout views appears.

	Grid View
	Vertical View
	Horizontal View
	Single View
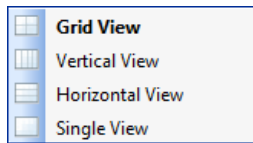
2. Click the view you want.

Grid View

By default, multichannel images appear in grid view.
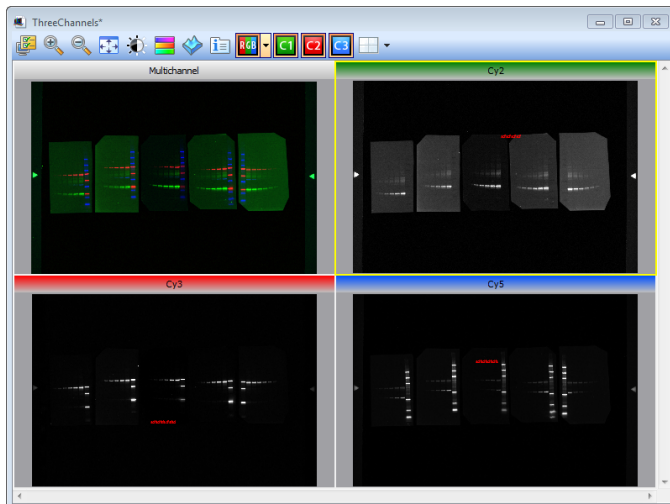
Vertical View — Images Side by Side
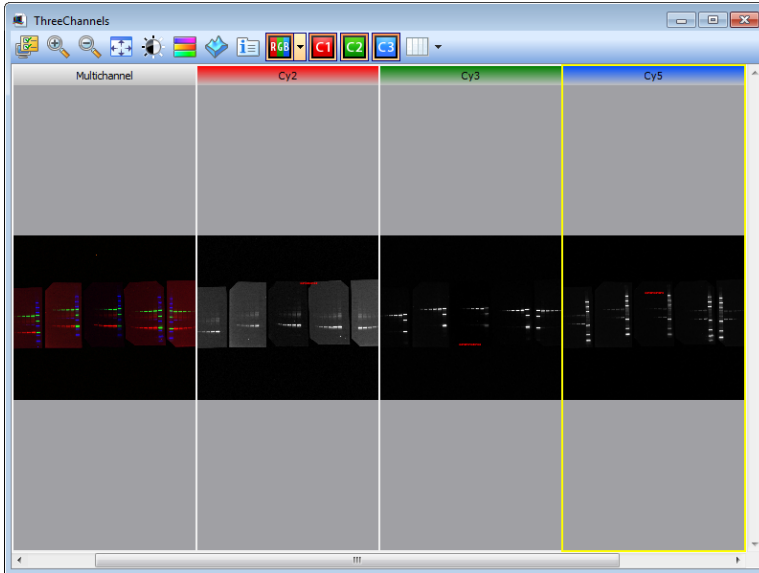
Horizontal View — Images Stacked

Single View — Selected Channel
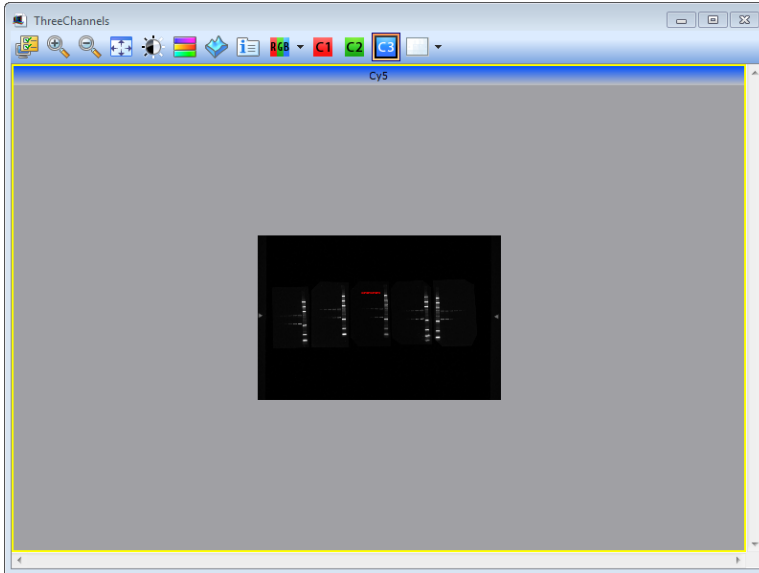
Tip: Click Fit in Window in the Display Toolbox to fill the screen with the image.

Splitting Multichannel Images

You can split a multichannel image into individual image files. When you split a multichannel image, Image Lab creates a new file for each channel except the RGB channel. Each new file has the same name as the multichannel image plus the application name in parentheses. All acquisition settings and overlays are copied to the new files.

To split a multichannel image into separate files

1. Open a multichannel image.
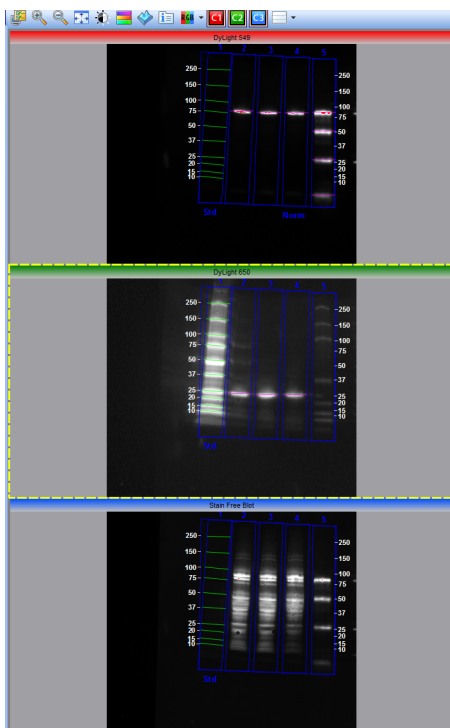
2. In the File menu, click Split Multichannel Image.

   Each channel, except the RGB channel, appears in a separate window. The first channel image appears in the workspace.
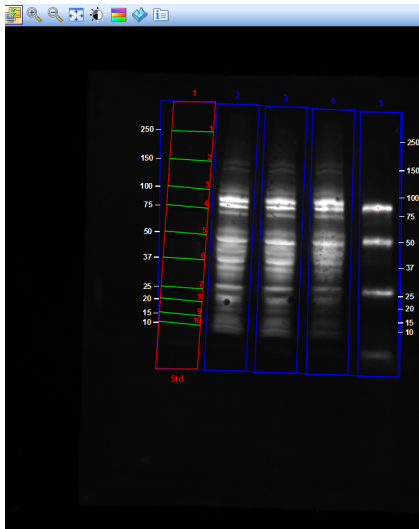
3. Save the channel image as a new file.

4. To view another channel image, select it in the Windows menu.

5. Save each channel image in a separate file.

Creating a Multichannel Image from Acquired Single Images

You can create an unlinked multichannel image from acquired single images or from single channels in a multichannel image that are open in Image Lab software. Only images with the same aspect ratio can be combined in a multichannel image. Because the images are not linked, a change you make to one image has no affect on the others.

Note: The type of multichannel image described in this section is different from a multichannel image that is created during acquisition with a multichannel-capable imager. Any imager can acquire single images that you can put together using the Create a Multichannel Image option in Image Lab.

To create a multichannel image from single images

1. Open the single images from which you want to create a multichannel image.

2. In the File menu, click Create Multichannel Image. The list of open images appears in the Create Multichannel Image dialog box.

3. Drag each image into a channel box in the right pane. Once you select the first file, the Available Open Images list displays only files with the same aspect ratio.

4. (Optional) Specify a color for each channel in the accompanying dropdown lists. The resulting multichannel image appears in the Image Preview.

5. Click OK to save the multichannel image.

To replace a channel in a multichannel image

1. Open the multichannel image and the new image you want to use.

2. In the File menu, select Create Multichannel Image. The open image files appear in the Compatible Open Images list.

3. Drag the images you want to keep from the Available Open Images list (left pane) into the channels in the New Multichannel Image pane (right pane).

4. Drag the new image you want to use into one of the available channel boxes.

5. Click OK to save the new multichannel image.

Displaying Data
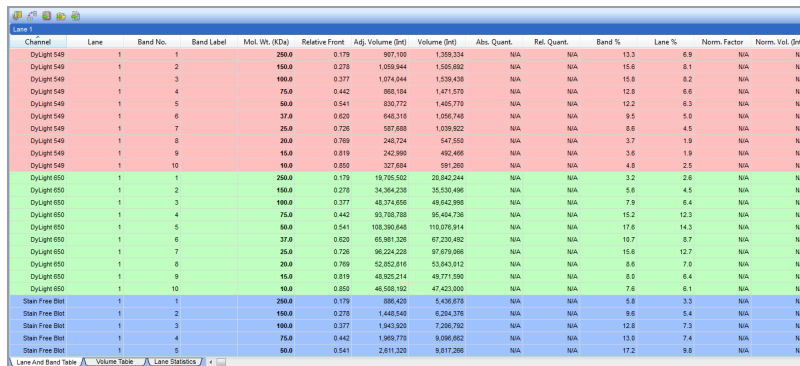
You can view results from analyzed data associated with a gel image in an analysis table, a lane profile, a standard curve, or a report. Main toolbar buttons turn these views on or off. You can view data in all views simultaneously.

Analysis Table

The Analysis Table tool appears in the Results Data section of the main toolbar. The analysis table displays image data for the selected image in results tables that appear in three tabs.

Lane and Band Table

This table displays lane and band volumes in order by lane number. For a multichannel image, the table displays data for each channel in its channel color.

Lane and Band Measurement Columns

Channel — the channel that contains the volume.

Lane — the number of the lane that contains the band.

Band No. — The number of the band in the lane.

Band Label — to assign a custom label to each band, click the Band Label box in the row for the band and type a name.

Molecular Weight — the molecular weight of the band is calculated based on the standard and regression method you define. Italic values indicate extrapolated values. For nucleic acid gels, the band size band appears in base pairs.

Relative Front — values between 0–1 indicate the relative movement of the band from top to bottom.

Adj. Volume — The volume with background subtracted.

Volume — the sum of all the intensities in the volume.

Abs. Quant. — absolute quantification of the band.

Rel. Quant. — relative quantification of the band compared to the reference band.

Band % — percentage of the band's volume compared to all band volumes in the lane.

Lane % — percentage of the band's volume compared to the entire volume of the lane.

Norm. Factor —the correction factor for the lane that contains the band.

Norm. Vol. (Int) — the adjusted volume corrected by the normalization factor.

Volume Table

This table displays volume analysis values obtained arbitrarily, that is, in a slightly more manual way than in automatic analysis. A greater variety of shapes can be selected. Volume analysis also includes two ways to control background that are different from those applied in lanes and bands analysis.

Channel	No.	Label	Type	Volume (Int)	Adj. Vol. (Int)	Mean Bkgd. (Int)	Rel. Quant.	# of Pixels	Min. Value (Int)	Max. Value (Int)	Mean Value (Int)	Std. Dev.	Area (mm2)
DyLight 549	1	U1	Unknown	761,600	761,600	N/A	N/A	2945	24	532	258.6	99.0	13.7
DyLight 650	1	U1	Unknown	33,380,892	33,380,892	N/A	N/A	2945	544	39,328	11,334.8	11,079.2	13.7
Stain Free Blot	1	U1	Unknown	36,081,980	36,081,980	N/A	N/A	2945	2,172	35,904	12,251.9	8,111.8	13.7

Volume Measurement Columns

Volume Number — a unique number assigned to each volume.

Volume Label — software-generated labels for different types of volumes
(U – unknown, B – background, S – standard). You can change labels can in Volume Properties.

Volume — the sum of all the intensities within the band boundaries.

Adjusted Volume — the background-adjusted volume.

Mean Background — the mean intensity of the background of the band.

Absolute Quantity Volume — the quantity of the volume based on the standard volumes and the regression method.

Relative Quantity Volume — the ratio of the band to the reference volume.

 # Pixels — number of pixels in the volume.

Minimum Value — the minimum pixel value in the volume.

Maximum Value — the maximum value of the pixels in the volume.

Mean Value — mean value of all pixel values in the volume.

Standard Deviation — standard deviation of all pixel values in the volume.

Area — the area of the volume in square millimeters.

Lane Statistics Table

This table is associated with the lanes and bands analysis. Molecular weight is based on lanes and bands found. All lane statistics apply to the whole lane and include the normalization factor associated with normalized lanes.

Channel	Lane No.	Adj. Total Band Vol. (Int)	Total Band Vol. (Int)	Adj. Total Lane Vol. (Int)	Total Lane Vol. (Int)	Bkgd. Vol. (Int)	Norm. Factor
DyLight 549	1	6,795,448	11,009,750	13,089,500	21,280,660	8,191,160	N/A
DyLight 549	2	26,127,018	26,491,738	46,557,918	55,187,870	8,629,952	1.000
DyLight 549	3	27,225,408	27,585,616	48,586,156	56,845,184	8,259,028	0.972
DyLight 549	4	25,422,676	25,802,060	48,786,940	57,665,616	8,878,676	1.079
DyLight 549	5	237,989,294	242,182,540	293,543,388	309,475,260	15,931,872	2.117
DyLight 650	1	615,035,608	627,444,548	759,329,556	782,985,032	23,655,476	N/A
DyLight 650	2	33,018,064	33,545,216	52,019,506	63,327,236	11,307,730	1.000
DyLight 650	3	33,457,608	33,979,214	49,785,596	59,689,154	9,903,558	0.972
DyLight 650	4	27,270,340	27,890,646	37,882,564	50,823,356	12,940,792	1.079
DyLight 650	5	N/A	N/A	24,510,688	33,331,554	8,820,866	2.117
Stain Free Blot	1	15,159,568	68,259,980	26,638,472	127,130,864	100,492,392	N/A
Stain Free Blot	2	N/A	N/A	451,833,560	555,425,884	103,592,324	1.000
Stain Free Blot	3	N/A	N/A	464,858,106	564,783,866	99,925,760	0.972
Stain Free Blot	4	N/A	N/A	418,658,328	518,984,058	100,325,730	1.079
Stain Free Blot	5	N/A	N/A	213,392,314	314,626,742	101,234,428	2.117

Note: You cannot change the orientation of the Lane Statistics table.

Display Data Options

Display Data options appear in the top left corner of the analysis table toolbar.

To display data options

▶   Click the Display Data Options icon in the analysis table toolbar.

The Display Data dialog box appears.
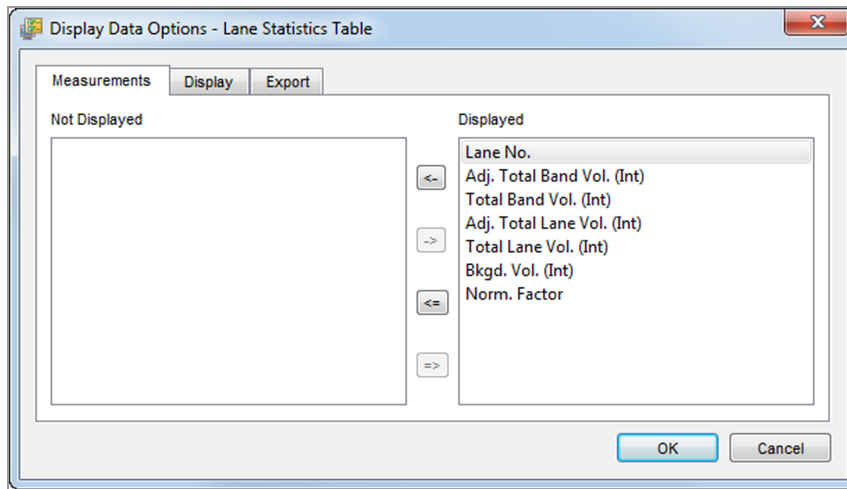
The Display Data Options dialog box displays three tabs.

Measurements — choose the measurements to display in the table.

▶ Click the arrows to move columns between Not Displayed pane and the Displayed panes. By default, all measurements appear in the analysis table.

> **Note:** For a description of each Lane and Band measurement type, see Lane and Band Table Measurements on page 91. For a description of each Volume measurement type, see Volume Measurements on page 92.

Display — specify display settings for the analysis table.

■ **Default display settings** — By default the Move selected lane to top checkbox is selected by default. When you click a lane on the image, the analysis table scrolls to display the selected lane first in the vertical or horizontal table view.

■ **Per Measurement Precision** — set the precision (decimal places) for measurements in the Lane and Band and Volume tables.

■ **Example** — display an example of how measurements look with the selected measurement and precision settings.

Export — choose how to export analysis data. The following settings appear on the Export tab:

■ **Export formatting** — select checkboxes to include lane headers (Lane and Band table tab only) or column headers, or both, in the exported file.

■ **Export delimiter** — select a delimiter for the exported file.

 □ Comma delimited

☐ Tab delimited

☐ Use other delimiter (user defined)

Change Analysis Table Orientation

The Change analysis table orientation icon appears in the analysis table toolbar.

▶ Click this icon to toggle between horizontal and vertical table orientations.

Note: You cannot change the orientation of the Lane Statistics table, which appears when you click the Lane Statistics tab in the analysis table.

Horizontal — displays lanes and volume data rows beside each other. You can scroll through the table from left to right.

Vertical — displays the lanes and volumes data rows vertically. You can scroll through the table from top to bottom.

Copy Analysis Table to the Clipboard

The Copy analysis table to the clipboard icon appears in the analysis table toolbar.

▶ Click this icon to copy the analysis table to the clipboard.

You can then paste the analysis table into a word processing or presentation application.

Note: Copying the table in vertical orientation to an 8.5 x 11-inch page best accommodates the table columns.

Export Analysis Table to a File

The Export analysis table to a file icon appears in the analysis table toolbar.

▶ Click this icon to export the table data as a CSV file that you can open in a database application.

Export Analysis Table to Excel

The Export analysis table to Excel icon appears in the analysis table toolbar.

▶ Click this icon to export the table data to a spreadsheet.

You can use the sorting and formula functions to manipulate the data. If Excel (PC or Mac) or Numbers (Mac) installed is installed on your computer, the software opens and displays the spreadsheet.

Lane and Band Table Measurements

The following measurements appear in the Lane and Band tab of the Analysis table.

▶   Click the Display data options button in the analysis table toolbar to choose columns.

Lane — each lane has a unique number.

Channel — (for multichannel images) lists the application used.

Band Number — each band in a lane has a unique number, sorted from top to bottom.

Band Label — you can assign a custom label to each band by clicking the Band Label box in the Lane and Band table.

Molecular Weight — the molecular weight of the band is calculated based on the standard and regression method you define. Italic values indicate extrapolated values. For nucleic acid gels, the size of the band is displayed in base pairs.

Relative Front — values between 0–1 indicate the relative movement of the band from top to bottom.

Volume — the sum of all intensities within the band boundaries.

Adj Volume — lists the adjusted volume of a band after background subtraction.

Abs. Quant. — absolute quantification of the band.

Rel. Quant. — relative quantification of the band compared to the reference band.

Band % — percentage of the band's volume compared to all band volumes in the lane.

Lane % — percentage of the band's volume compared to the entire volume of the lane.

Norm. Factor —(multichannel images only) the correction factor for the lane that contains the band.

Norm. Vol. (Int) — (multichannel images only) the adjusted volume corrected by the normalization factor.

Volume Measurements

The following measurements appear in the Volume tab of the Analysis table.

▶ Click the Display data options button in the analysis table toolbar to choose the columns to display.

Volume Number — a unique number assigned to each volume.

Volume Type — U – unknown, B – background, S – standard

Volume Label — software-generated labels for different types of volumes (you can change the label in Volume Properties)

Volume — the sum of all the intensities within the band boundaries.

Adjusted Volume — the background-adjusted volume.

Mean Background — the mean value of the background.

Absolute Quantity Volume — the quantity of the volume based on the standard volumes and the regression method.

Relative Quantity Volume — the ratio of the adjusted volume and the adjusted volume of the reference volume.

 # Pixels — number of pixels inside the volume boundary.

Minimum Value — intensity of the pixel with the minimum intensity inside the volume.

Maximum Value — intensity of the pixel with the maximum intensity inside the volume.

Mean Value — mean value of all pixels inside the volume boundary.

Standard Deviation — standard deviation of all pixels inside the volume boundary.

Area — area of the volume in mm^2.

Lane Profile

The Lane Profile icon appears in the Results Data section of the main toolbar.

▶ Click Lane Profile to display a cross-section view of a single lane rotated 90°.
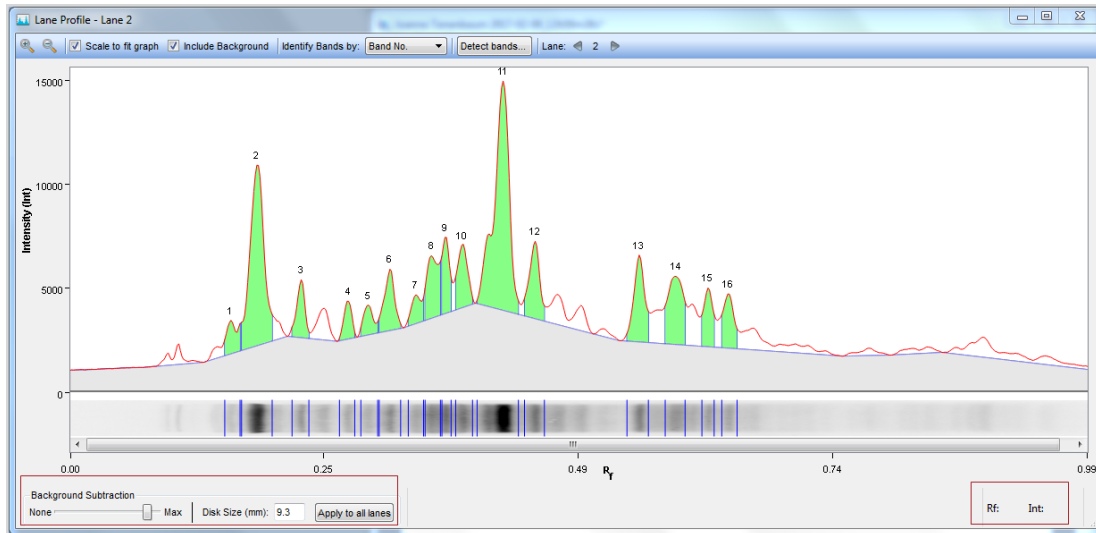
A Background Subtraction bar appears in the lower left corner of the Lane Profile window. When you move the pointer over a lane profile, the current relative front (Rf) value and the average value at the Rf value appear in the lower right corner of the window.

An image of the selected lane appears below the graph of lane intensities. The transform and color map are applied to the grayscale image.

The composite channel of a multichannel image always displays a grayscale image of the lane with the default transform applied to that lane. For single channel images and individual channels of a multichannel image, the transform and color map are applied to the grayscale image.

The title bar identifies the lane profile in view (Lane 1, Lane 2, and so on). Lane profile titles of multichannel images include the channel name, for example, Lane 2 (DyLight650).

The following settings appear in the Lane Profile toolbar from left to right:

■ Zoom in and out

■ Scale to fit graph

■ Include Background

■ Identify Bands by

■ Detect bands

■ Lane (select lane number with Previous and Next arrows)

To view profiles of other lanes

Do one of the following:

■ In the toolbar, click the Previous and Next arrows next to Lane to view profiles of other lanes in the image.

■ In the window, click a lane to display its profile.

To view the lane profile of each channel in a multichannel image

1. Click a channel.

   By default, the Lane Profile window displays the lane profile for Lane 1 in the selected channel.

2. Click the Previous and Next arrows to view the profiles of other lanes in the channel.

3. Repeat steps 1–2 to view lane profiles in other channels.

Scale to Fit Graph

1. Select this checkbox to define the range of the lane profile graph by its highest vertical point.

    This setting fits the entire graph into the window.

2. Clear the Scale to Fit Graph checkbox to display the entire range of possible intensity values in the graph.

    Doing so allows valid comparisons between different lanes.

Include Background

The area used for band quantification appears in green between the red and blue lines in the lane profile graph.

1. Select the Include Background checkbox to display the subtracted background in gray, just below the blue line.
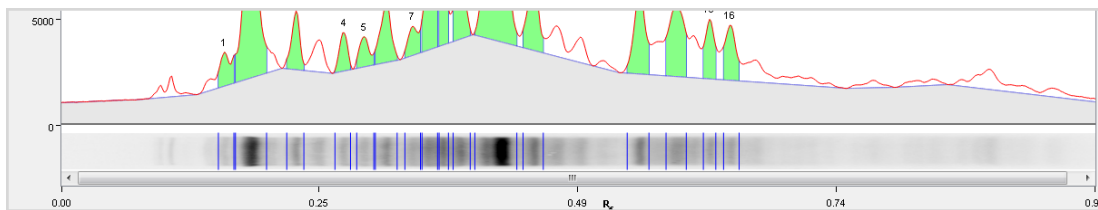
2. Clear the checkbox to remove the background.
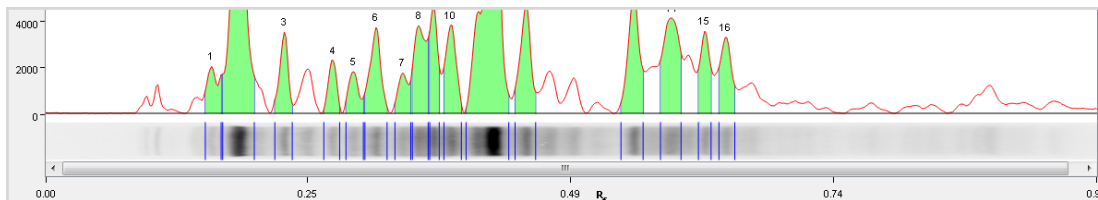
Identify Bands by

By default, bands are labeled by band number.

To change how bands are labeled in the lane profile, select a label type from the dropdown list:

- Band Number (Band No.)

- Band Label

- Molecular Weight (Mol. Wt.)

- Relative Front

- Volume

- Adjusted Volume (Adj. Volume)

- Absolute Quantity (Abs. Quant.)

- Relative Quantity (Rel. Quant.)

- Band %

- Lane %

- Normalization Factor (multichannel images only)

- Normalization Volume (multichannel images only)

Adjusting Band Boundaries

The lane profile graph displays bands in a lane as green areas. Below the lane profile graph, a lane image strip displays each band delimited by a pair of vertical lines.
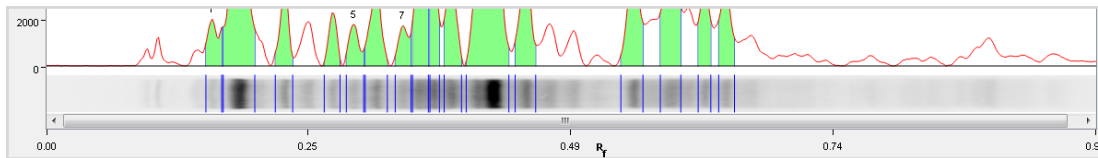
You can move the vertical lines or the green graphic areas to change band boundaries.

Note: Boundary lines cannot overlap. You cannot move a boundary line beyond the next boundary line.

To adjust band boundaries

1. Point to a boundary line in the graph or strip.

    The cursor changes to a double-arrow and a statistics box appears briefly.

2. Drag the boundary to a new position.

Adding and Deleting Bands — Lane Profile

You can add and delete bands in the Lane Profile window if you have the right to modify the image.

When you attempt to modify an image, the cursor changes to a plus sign. If you are unable to modify the image, the cursor does not change and you cannot add or delete bands from the image.

Note: You can also edit bands in the Bands window. For information, seeEditing Bands on page 121.

To add a band in the graph

1. Position the pointer where you want to add the band.

2. Press Ctrl-click (PC) or Command-click (Mac).

    The cursor changes to a plus sign.

3. Image Lab adds the band and renumbers the bands in the lane profile.

To delete a band in graph

1. Position the pointer on the band you want to delete.

2. Hold down the Ctrl key (PC) or Command key (Mac).

    The cursor changes to an X.

3. Hold down the Ctrl/Command key and click inside the band.

    Image Lab deletes the band and renumbers the bands in the lane profile.

Standard Curve
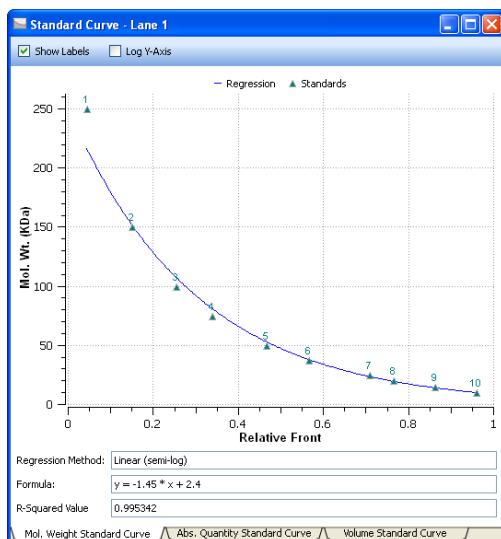
The Standard Curve dialog box displays the best curve fit for the defined standards and the bands relative to this curve for the lane selected in the image. The tabs at the bottom of the dialog box display the standard curves for three different analyses.

Standards appear in green. Unknown bands appear in red. You can toggle the molecular weight display on the y-axis between linear and log scale by clicking the Log y-axis box at the upper left. The regression method you chose in Molecular Weight Analysis Tools appears, as well as the formula (if applicable) and the R^2 value of the regression method.

Tabs in this window enable you to view the molecular weight standard curve, the absolute quantity standard curve, or the volume standard curve.

Report

See See Generating Reports for information about reports.

Chapter 6 Analyzing Images

Analysis Tool Box tools are active when an image file is open and in focus.

- In the Windows environment, an active or in-focus Image Lab window displays a slightly darker title bar.

- On a Mac computer, active window control icons appear brighter than others.

Image Types

Image Lab creates the following image types:

- Single-channel images

- Linked multichannel images

- Unlinked multichannel images

Linked Multichannel Images

The multichannel view of a multichannel image is a composite of single channels collected by a multichannel protocol. A multichannel image can consist of up to three images. A linked multichannel image is acquired using the multichannel protocol. When you detect lanes in one channel of the image, Image Lab automatically detects lanes in the other channels.

Unlinked Multichannel Images

Unlinked multichannel images are generated by combining several images using the Create Multichannel Image feature. Detecting lanes in one channel has no effect on the other channels. You must apply lane detection to each channel separately.

Auto Analysis

Auto analysis detects lanes and bands. It can also calculate the molecular weight of the bands in an image. Auto Analysis does not find bands in the normalization channel because these bands are not usually of interest.

▶ Click Auto-Analysis in the Analysis Tool Box to do the following:

- Analyze images obtained with protocols without steps for auto detection and analysis.

- Change analysis parameters to reanalyze images.

Note: When you change settings for an analyzed gel, Image Lab overwrites the initial analysis. To preserve both analyses, save each image file with a different name. To recover an overwritten analysis, click Undo in the main toolbar.

(Multichannel images only) Band Detection Sensitivity and Molecular Weight Analysis settings apply to all channels in a multichannel image. You can select different sensitivity levels to detect bands in each channel using Lane and Bands settings.

In unlinked multichannel images with no normalization channel, lane and band detection are performed independently for each channel. For unlinked multichannel images with a normalization channel, lane finding is performed in the normalization channel and copied to the other channels. Band finding is performed only in the sample channels.

Auto Detection Settings
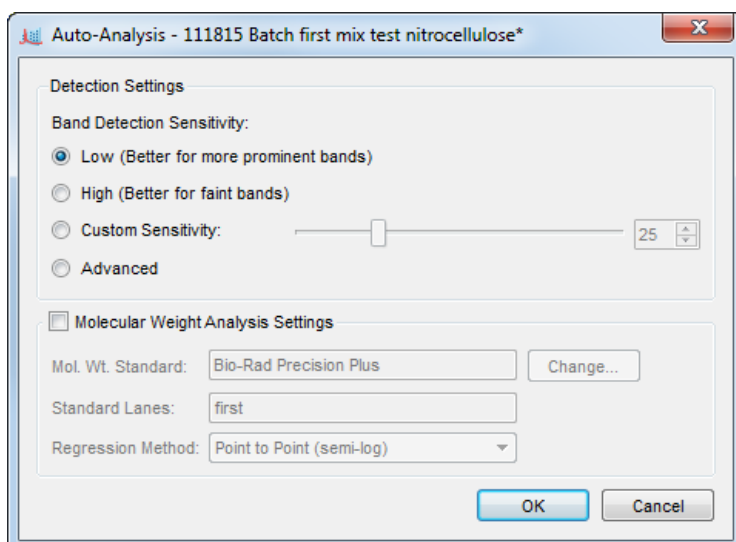
Low Band Detection Sensitivity — sets detection at a low level for images with prominent bands. Faint bands are not detected with this setting.

High Band Detection Sensitivity — sets detection at a higher level for images that are faint. Extraneous bands can be removed using the Band Tools in the Analysis Tool Box. See Lane and Bands Settings on page 109

Custom — enables you to set a value between 1 and 100 to select the best detection sensitivity for your sample. You can also drag the sliding bar left or right to set the value.

When Low Band Detection Sensitivity is selected, the numerical value is set at 25; when High Band Detection Sensitivity is selected, the value is set at 75.

Molecular Weight Analysis Settings

Molecular Weight Standard — choose any of the many Bio-Rad standards or a standard you added to the standards list.

Standard Lanes — choose or change the lanes in which the standards are placed.

Regression Method — four regression methods are available. For more information, see Regression Calculation Methods on page 179

Analysis Tool Box Tools

Analysis Tool Box tools customize the analyzed data in image files. These tools are available only when an image file is open in the workspace.

Note: Some tools delete the existing analysis. Screen messages notify you before you complete actions that delete the analysis.

To access a tool

▶   Click a toolbox button.

To return to the Analysis Tool Box

▶   Click the Back arrow to the left of the tool name.

To hide the toolbox pane

▶   Click the double arrow to the right of the tool name.

See Analysis Tool Box on page 36 for more information about these tools.

Image Tools

Image tools enable you to manipulate images.

To display the image tools menu

▶ Click Image Tools in the Analysis Tool Box.
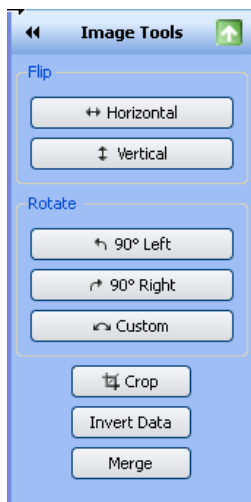
Flip — flip the gel image horizontally or vertically.

Rotate — rotate the gel image 90° using the Left or Right buttons. You can also set a custom rotation using the Custom button.

Crop — trim the outer edges of your image to any shape or area.

Invert Data — toggle the image data from positive to negative.

Merge — merge any two single channel images of the same size, for example, a chemiluminescent blot image, with a colorimetric image of the same blot

Correcting a Slanted Gel

To correct a slanted gel

1. In the Rotate section under Image Tools, click Custom.

    Red arrows appear on the gel image.
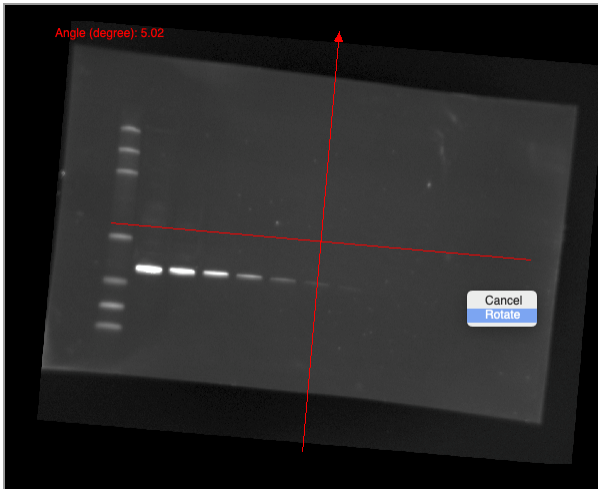
2. Drag the red arrows to rotate them any degree between 0 and 360.

3. Right-click the gel image and click Rotate to set the gel in the new position.

Cropping a Gel Image

You can save crop settings and use them to crop other images. Saved crop settings are useful when you want to crop the same area in several images.

To crop a gel image

1. Click Crop under Image Tools in the Analysis Tool Box.

    A red box outlines the image area.

2. Drag the red box to surround the image area you want to keep.

3. (Optional) Right-click the image to open the Crop menu and click Save Crop Settings.

    The Save Crop Settings dialog box appears.

4. (Optional) Type a name for the crop settings and click OK.

5. Right-click and select Crop.

6. Image Lab crops the image to the area inside the red box.

To crop an image using saved crop settings

1. Click Crop.

    A red box outlines the image area.

2. Right-click the image to open the Crop menu and click Load Crop Settings.

3. Select the saved crop settings you want to use and click Load.

    The red box resizes and the crop specifications appear on the image.

4. Right-click and select Crop.

    Image Lab crops the image to the area specified in the crop settings.

To delete crop settings

1. With an image open, click Crop.

2. Right-click the image inside the red box to open the Crop menu.

3. Click Delete Crop Settings.

4. Select the crop settings in the dialog box that appears and click Delete.

Inverting Data

Invert Data is used for negative stains and zymograms. In order to analyze the gel, the intensity values of its bands must be greater than its background.

To determine if image data must be inverted

▶ View the gel as a 3-D projection.

To invert data

▶ With the image open, click Invert Data under Image Tools in the Analysis Tool Box.

   The image data changes from positive to negative.

Merging Images

You can merge any two single channel images of the same size. For example, you can merge a chemiluminescent blot image with a colorimetric image of the same blot. Using colorimetric prestained standards for a chemiluminescent blot, you can acquire an
epi-white light image of the blot to show the standards and a chemiluminescent image to show immunodetection. Data from the two images can then be combined into a third single image with both signals.

Note: Merging images can adversely affect quantification. If accurate quantification is required, perform analysis on the original, separate images.

To merge two same-size images

▶ With both images open in the workspace, click Merge under Image Tools in the Analysis Tool Box.

Lane and Bands Settings

Lane and Bands settings enable you to identify the lanes and bands in an image.

To access Lane and Bands settings

▶ With an image open in the workspace, click Lane and Bands in the left pane.

Lane and Bands detection settings appear in the right pane.
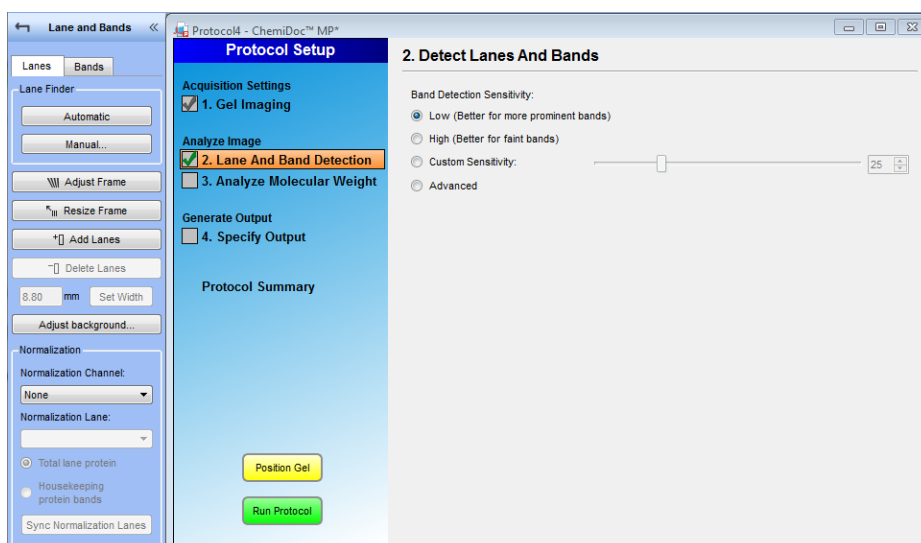
Normalization settings also appear on the Lanes tab.

Detecting Lanes

To detect lanes in an image

▶ Do one of the following:

- If the imaged gel is a standard size, click Automatic.

- If automatic lane detection does not find all lanes, click Manual.

- To detect a specific number of lanes, click Manual and enter the number of lanes you want to find.

Detecting Lanes in a Multichannel Image

Automatic lane detection works differently in multichannel images depending on whether the channels are linked or unlinked. In a linked multichannel image, lane detection is based on the selected channel, and lanes are detected in all channels. In an unlinked multichannel image, lane detection is based only on the selected channel. You must select and apply automatic lane detection to each lane separately. The lanes in an unlinked multichannel image can fall in slightly different places on each channel.

To detect lanes automatically in a multichannel image

▶ Select a channel and click Automatic in the Lanes tab.

To correct the position of detected lanes in an unlinked multichannel image

1. In the Lanes tab, click Automatic or Manual to detect the lanes in one channel.

2. Do one of the following:

- Repeat step 1 for each channel separately.

- Copy the detected lanes into the other channels and align the lanes in each channel. See Copying Lanes on page 117 for more information.

Editing Lanes

After lanes are detected they appear on the image numbered from left to right. By default the first lane is selected. The selected lane appears outlined in red. You can edit individual lanes using mouse actions. Pausing the pointer over a lane displays helpful tooltips. Additional lane settings are available in the Lanes tab of the Analysis Tool Box.

(Multichannel images only) Edits to lanes in a linked multichannel image apply to all channels. Edits to lanes in unlinked multichannel images apply only to the channel edited.

To activate lane settings

1. Click Lanes and Bands in the Analysis Tool Box. Lane and Band settings become active. By default the Lanes tab is selected.

2. (Optional) If Adjust Frame or Resize Frame appears blue, it is active. Click the active button to inactivate it and exit Adjust Frame or Resize Frame mode so you can edit lanes.

To select lanes

▶ Do one of the following:

- To select a lane, click it.

- To select multiple, contiguous lanes, hold down the Shift key and click the first and last lanes you want.

- To select multiple lanes that are not contiguous, Ctrl-click (PC) or Command-click (Mac) each lane you want to select.

- To select all lanes, click Ctrl-A (PC) or Command-A (Mac).

To move one or more selected lanes

▶ Place the pointer on a selected lane and drag it to a new position.

All selected lanes move at once. Image Lab renumbers the lanes automatically.

To resize a lane

1. Select a lane.

White anchor points become visible.
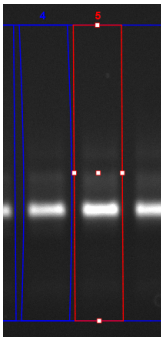
2.   Point to an anchor on the side of the lane.

     The cursor changes to a double-pointed arrow.

3.   Drag the anchor to change the lane width to your liking.

To resize lanes to a width you specify

1.   Select one or more lanes.

2.   In the Lanes tab, enter a width in millimeters and click Set Width.
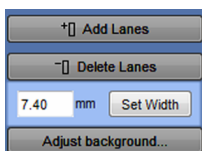
     Image Lab resizes the width of the selected lanes to your setting.

To resize multiple lanes

1.   Select one or more lanes.

2.   Point to an anchor on the side of any selected lane.

     The cursor changes to a double-pointed arrow.

3.   Drag the anchor to change the lane width of all selected lanes to your liking.

To bend a lane from the middle

1.   Select the lane you want to bend.

     Point to the anchor in the center of the lane and drag the anchor to bend the lane right or left.

To tilt a lane at top or bottom

1.   Select the lane you want to tilt.

2. Point to the anchor at the top or bottom of the lane.

3. Drag the anchor to tilt the lane right or left.

To add an anchor in a lane

▶ In the center of the lane, right-click and then click Add Anchor.

Tip: You can adjust a so-called smiling gel using an additional anchor. To do so, see the procedure, To adjust the lane frame shape, in Editing the Lane Frame on page 114.

To remove an anchor in a lane

1. Right-click the anchor you want to remove.

2. Click Remove Anchor.

Note: Top and bottom anchors cannot be removed.

Editing the Lane Frame

Using Lanes commands in the Analysis Tool Box, you can adjust the shape of the lane frame and all lanes it contains. You can also move, resize, or delete the frame. When selected, the frame appears in red.

To access Lanes and Bands settings

▶   Click Lanes and Bands in the Analysis toolbox.

Lanes and Bands settings appear in the toolbox. The Lanes tab is selected by default.

To adjust the lane frame shape

1.   Click Adjust Frame in the Lanes tab to select the frame.

2.   Drag a corner of the lane frame and reshape or reorient the frame to your liking.

3.   (Optional) If the gel is smiling, add or remove anchors to correct the shape.

4.   Click Adjust Frame again to stop adjusting the frame shape.

To resize the frame proportionally

1.   Click Resize Frame in the Lanes tab to select the frame.

2.   Point to a corner anchor.

The cursor changes to a double-pointed arrow.

3.   Drag a corner of the lane frame to resize frame height and width at the same time.

4.   Click Resize Frame again to stop resizing the frame.

To resize frame height

1.   Click Resize Frame in the Lanes tab to select the frame.

2.   Point to the top or bottom border of the frame.

The cursor changes to a double-pointed arrow.

3.   Drag the border to resize the height of the frame to your liking.

4.   Click Resize Frame again to stop resizing the frame height.

To resize frame width

1.   Click Resize Frame in the Lanes tab to select the frame.

2.   Point to the right or left border of the frame.

The cursor changes to a double-pointed arrow.

3. Drag the border to resize the width of the frame to your liking.

4. Click Resize Frame again to stop resizing the frame width.

To move the frame

1. Select the frame.

2. Place the pointer inside the frame.

    The cursor changes to a four-directions arrow.

3. Drag the frame to a new location.

Adding and Deleting Lanes

Using Lanes and Bands settings you can add or delete one or more lanes in an image.

Adding Lanes

By default, Image Lab adds lanes to an image using the width of Lane 1.

To add one or more lanes to an image
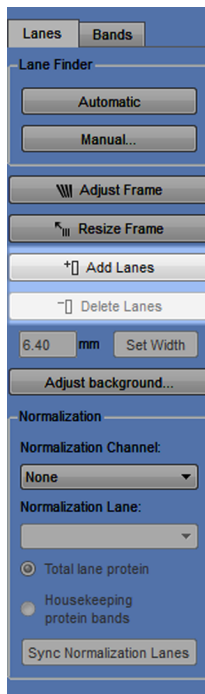
1. Click Add Lanes in the Lanes tab.

2. Click the location in the image frame where you want to add a lane.

3. (Optional) To add more lanes, click multiple places in the frame.

    Image Lab adds lanes to the image in the locations you selected.

4. Click Add Lanes again to stop adding lanes.

5. Reposition the added lanes.

6. (Optional) If lanes overlap, move the added lanes to the left or right end of the frame. The frame expands to accommodate the lanes, and the lanes are renumbered automatically.

Deleting Lanes

To delete one or more lanes from an image
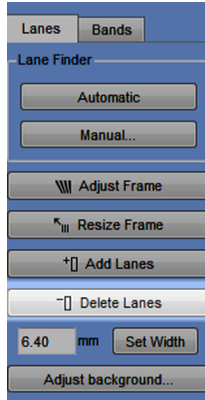
1. Click a lane you want to delete.

2. (Optional) Ctrl-click each additional lane you want to delete.

3. Click Delete Lanes in the Lanes tab.

4. To confirm the deletion, click Yes in the dialog box that appears.

   Image Lab deletes the lanes you selected.

5. Click Delete Lanes again to stop deleting lanes.

6. (Optional) Reposition the remaining lanes and resize the frame..

Copying Lanes

You can copy lanes from an image into any other image. Doing so is useful when several images have been taken of the same sample with different stains. You can create the lane frame in the image with a total protein stain and copy it to the other images. Copying lanes is especially useful when normalizing unlinked multichannel images.

Note: Individual lanes cannot be copied.

A channel can contain only one lane frame at a time. When you paste lanes into a channel that already contains a lane frame, Image Lab deletes the existing lanes and replaces them with the copied lanes.

Note: When you copy lanes into a channel of a linked multichannel image, the lanes are copied into all image channels including the composite image.

To copy lanes between channels

1. Click Lane and Bands and the Analysis Tool Box.

2. Select the channel or image that contains the lanes you want to copy.

3. In the Edit menu, click Copy.

4. Select the channel into which you want to paste the lanes.

5. In the Edit menu, click Paste.

   A screen message notifies you that existing lanes in the selected channel will be deleted.

6. Click Yes to continue.

7. Adjust the position of each copied lane in the frame individually.

Detecting Bands

Image Lab software detects bands in individual images.

To detect bands in an image

1. Click Detect Bands.
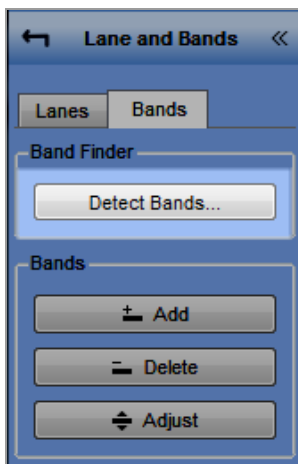
2. The band Detection dialog box appears.
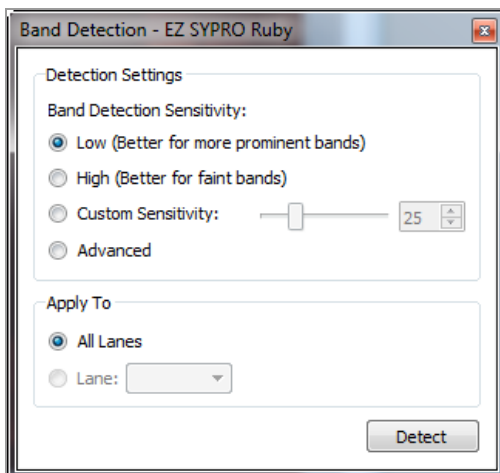
3. Select band detection sensitivity and the lanes to which it applies.

You can also detect bands in the Lane Profile window. See Adding and Deleting Bands — Lane Profile on page 98.

Detecting Bands in a Multichannel Image

In a multichannel image, you must detect bands for each channel separately whether the multichannel image is linked or unlinked. Multichannel view is a composite of the other channels. Bands cannot be detected on a composite image. Detecting bands in multichannel view is less useful because quantifying overlapping bands causes combined values from multiple channels.

Tip: When selecting bands in a multichannel image, leave the Band Detection dialog box open and select and detect bands in each channel one at a time.

Setting Advanced Band Detection Options

You can also set specific parameters for band detection under Advanced in the Band Detection dialog box.
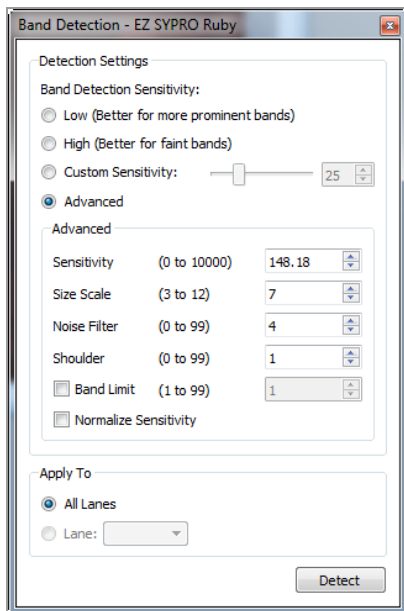
Advanced values are set in relation to the band detection sensitivity you select under Detection Settings. You can apply the specific parameters you set for the sensitivity level to all lanes or to a specific lane.

If you later change the band detection sensitivity in Detection Settings, the advanced values change in relation to the new sensitivity levels. In this case, you might have to reset advanced parameters to the original values.

Advanced Detection Options

■ **Sensitivity** — determines the minimum optical density to be defined as a band. The higher the sensitivity value, the lower the minimum signal intensity and the more bands will be detected.

If the sensitivity is set too high, background staining might be detected as bands. If the setting is too low, bands of interest might not be detected.

The default sensitivity setting is 10.0. If the gel has faint bands (that is, if the optical density is less than 0.05, and counts are less than 2,000), consider increasing this value to 20.0.

■ **Size Scale** — distinguishes between trends in signal intensity and random intensity fluctuations. Size scale is the number of pixels in a vertical column taken together to determine whether a band is present.

The Size Scale parameter uses the size of objects in the image to determine the nature of those objects. If a gel image has high levels of background noise, a larger size scale is appropriate. At low noise levels, a smaller value is preferable. You can also increase the size scale if the gel has only a small number of thick bands scanned at high resolution.

■ **Noise Filter** — minimizes the number of small fluctuations (or noise) in the image that are called bands while still recognizing larger features (the real bands). This filter becomes especially important at higher sensitivity levels.

The noise filter value refers to the size of the filter in pixels (for example, a value of 2.50 equals a filter size of 2.50 x 2.50 pixels). Features smaller than the filter size are not recognized as bands. Entering a noise filter size of zero turns the filter off completely.

If band detection identifies doublets as single bands, decrease the noise filter setting or increase the sensitivity level, or do both.

Tip: You can decrease the Size Scale parameter instead of the noise filter to improve the detection of closely spaced bands. However, if you decrease both the noise filter and the size scale, the fuzziness around bands might be mistakenly detected as separate bands.

■ **Shoulder** — band detection tries to distinguish shoulders as separate bands. When looking at a lane trace, these bands appear as flat or gently sloping abutments to darker, better-defined bands (that is, there is no dip on the trace between the two bands). Increasing the shoulder sensitivity results in more shoulders being detected as bands. Changing this setting to zero results in no shoulders being recognized as separate bands.

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

■ **Normalize Sensitivity** — compensates for differences in intensity between lanes.

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

Note: This setting does not normalize for band quantitation.

■ **Band Limit** — enables you to limit the number of bands that will be detected in each lane, reducing the need for later editing.

Editing Bands

You can optimize the bands in an image using the settings in the Bands tab:

Add — manually add a band to a lane.

▶ Click Add, and then click inside a lane. Image Lab locates a faint band close by.

Tip: To view faint bands more easily, darken the entire image. Click Image Transform in the image toolbar and adjust the image with the sliders in the box that appears.

Delete — delete a band from a lane. Useful for removing bands not relevant to the analysis. Click Delete, and then click the band you want to remove.

Adjust — adjust the height of a band.

▶ Click Adjust to display boundary lines above and below each band.

■ Move the pointer over a boundary line until the cursor changes to a double-pointed arrow.

■ Move the boundary line to adjust the band height. Image Lab repositions the band and recalculates its center.

Note: You can also edit bands in the Lane Profile window. For information, see Lane Profile on page 93.

Normalizing Volume Data

(Multichannel images only) Normalizing the volume data in a multichannel image is most useful in the following cases:

■ The lanes are loaded with the same volume, but you do not know the total protein in each lane.

■ Pipetting errors result in variations in the lane volumes.

■ Differences occur in the transfer of protein from a gel to a membrane.

You can correct for these differences when normalizing the volume data.

▶ Select one of the following settings under Normalization in the Lanes tab:

■ **Total Lane Protein** – calculates the normalization factor based on one lane in the normalization channel.

■ **Housekeeping Protein Bands** – calculates the normalization factor based on a single band of housekeeping protein. To obtain accurate results, the housekeeping protein must be stable and impervious to the treatments in the experiment. The housekeeping protein must also be the same in pretreatment and post-treatment.

Both settings use the normalization factor to calculate the normalized volumes in the lanes for all channels.

Normalization Settings

Normalization settings appear in the Lane and Bands tabs of the Analysis Tool Box under the Lanes tab.
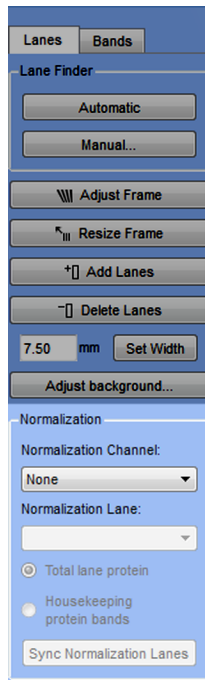
By default, the first non-standard lane is selected as the normalization lane. However, you can select any non-standard lane as the normalization lane.

Note: If a standard lane is selected that conflicts with the normalization lane, Image Lab resets the selection to the default normalization lane.

You can select one of the channels in a multichannel image, for example:
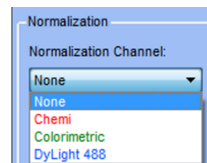
For a single channel or two-channel image, you can select Add channel in the Normalization Channel dropdown list to drag entries from other open images into the normalization data channel.
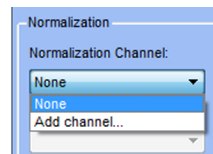
For more information, see Add a Channel to a Single Image on page 130.

Normalizing Data — General Steps

General steps outline the process of normalizing volume data. Detailed information in the following sections guides you through these general steps:

- Detecting lanes in a channel

- Adjusting the lanes

- Detecting bands

- Subtracting extraneous background

- Removing compromised data

- Viewing the data in an analysis table

Detect Lanes in Channels

(Multichannel images) You can detect the lanes in a multichannel image automatically or manually. If the multichannel image consists of unlinked images, ensure that the detected lanes are correctly mapped for each channel.

Tip: You can copy lanes in one channel to map lanes in another channel.
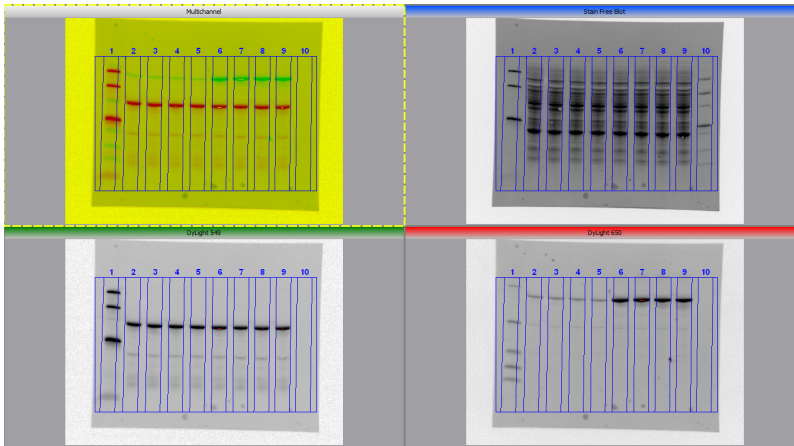
To detect lanes in channels
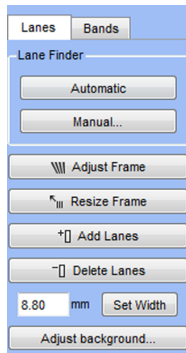
1. Under Lane Finder, click Automatic or Manual.

   Image Lab detects the lanes.

2. (Optional) If the images are unlinked, ensure that the detected lanes are correctly mapped to each channel.

Adjust the Lanes

Important: After lanes are detected, review lane borders to ensure that each lane encompasses all the protein in the lane and lanes do not overlap.

In the image shown, lanes were detected automatically. Some of the lanes exclude material that should be included.

Note: Image Lab automatically excludes the lanes you specify as standard lanes from normalization.
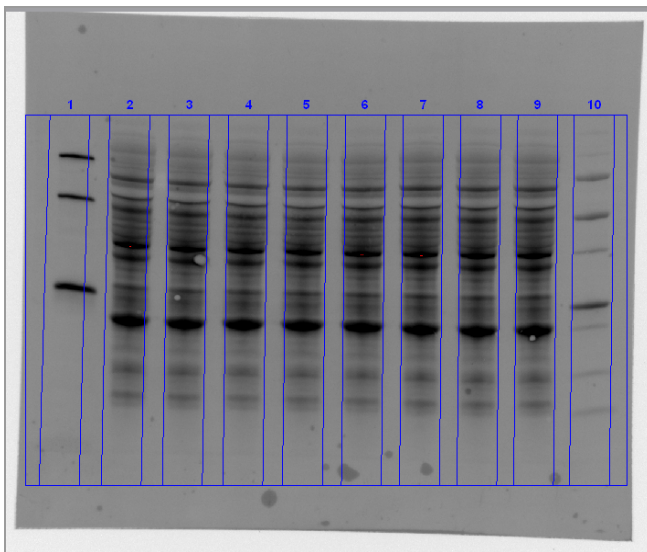
The image can be corrected by adjusting lanes using Lane and Bands settings. The lanes in the image shown were corrected by slightly widening them to include the relevant material:
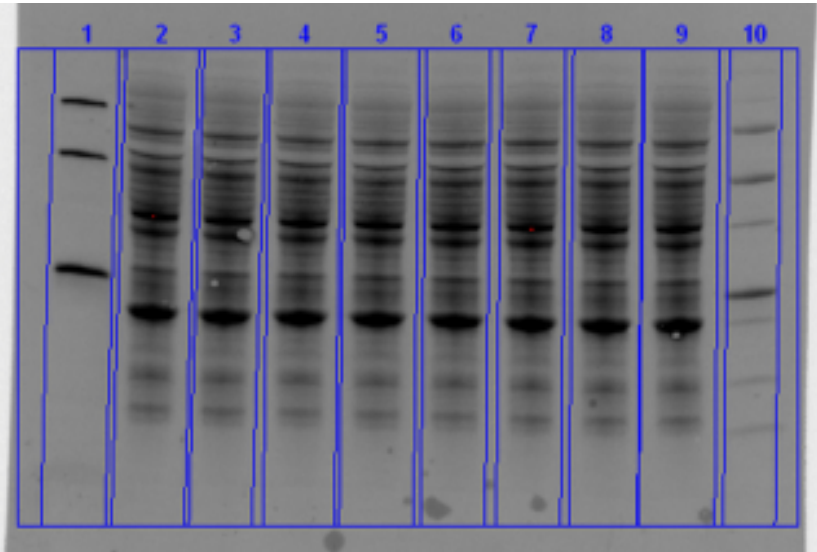
Detect the Bands

Settings in the Band tab enable you to detect the bands in image channels.

Note: Detecting bands on the normalization channel is not recommended when total lane protein normalization is selected. Detecting bands using this normalization method generates large amounts of superfluous data in the analysis table.

To use a housekeeping protein to calculate the normalization factor, you must isolate this protein in the image.

▶ Remove all bands other than the housekeeping protein from the image.

The normalization channel should have only one detected band in each lane.
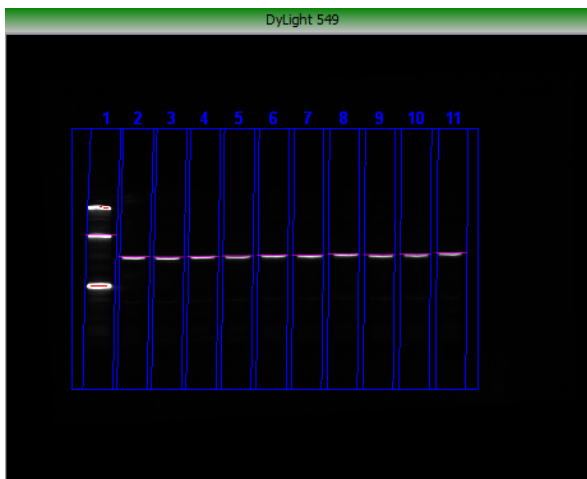
To exclude all other bands from the calculation

▶ Click Delete Bands in the Bands tab. Delete each band you do not want.

Subtract Extraneous Background

The lane volume data is background subtracted.

1. Review the background subtraction applied to each lane in the Lane Profile window.

2. Verify that an equivalent background profile is being subtracted from each lane in the normalization channel so the normalization factor is accurate.

For more information, see Volume Background Subtraction on page 146.

Remove Compromised Data

▶   Exclude from the analysis lanes that have any of the following characteristics:

 ■ **Empty lanes** — the first nonstandard lane in the selected channel is used as the normalization factor against which all other lanes in all channels are compared. If the first nonstandard lane is invalid (for example, an empty lane), delete it from the channel or select a different lane as the normalization lane.

 ■ **Lanes contain saturated pixels** — check the images for saturated pixels, indicated in red. These points can neither be read nor used in quantification.

 ■ **Poor transfer quality** — check the quality of the transfer. If the transfer is poor with splotchy or blurred areas, delete the lanes.
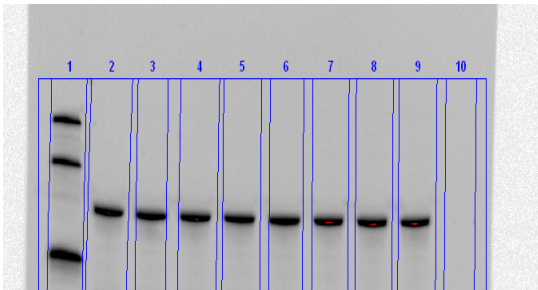
View the Data in the Analysis Table

The first nonstandard lane in the selected channel is used as the normalization factor against which all other lanes in all the channels are compared. Normalization values are calculated based on the total background-corrected signal in the selected channel.

The analysis table displays the uncorrected and normalized volumes, as well as the normalization factor used to calculate the normalized volume.

The Lane Statistics tab displays detailed lane data:

Channel	Lane No.	Adj. Total Band Vol. (Int)	Total Band Vol. (Int)	Adj. Total Lane Vol. (Int)	Total Lane Vol. (Int)	Bkgd. Vol. (Int)	Norm. Factor
Colorimetric	**1**	**55,629,000**	**78,044,925**	**69,035,250**	**143,317,800**	**74,282,550**	**1.000**
Colorimetric	2	115,071,975	148,996,050	141,824,025	206,041,650	64,217,625	0.487
Colorimetric	3	113,952,825	145,636,425	143,851,350	207,537,525	63,686,175	0.480
Colorimetric	4	78,335,250	106,958,100	102,738,900	164,657,775	61,918,875	0.672
Colorimetric	5	81,327,300	110,054,775	106,342,350	169,253,325	62,910,975	0.649
Colorimetric	6	80,115,075	108,952,500	104,782,125	167,759,700	62,977,575	0.659
Colorimetric	7	60,177,525	86,039,625	81,262,350	143,757,600	62,495,250	0.850
Colorimetric	8	59,203,575	84,586,425	80,746,725	142,268,100	61,521,375	0.855
Colorimetric	9	58,277,850	83,812,650	78,707,775	139,701,150	60,993,375	0.877
Colorimetric	10	56,073,825	75,223,350	73,884,975	137,772,525	63,887,550	0.934

Lane And Band Table \ Volume Table \ Lane Statistics /

Tip: You can hide the normalization channel from the analysis table. (See Multichannel View Settings on page 78.)

Add a Channel to a Single Image

Normalization settings can be used only for multichannel images, but you can add a channel to a single image using a normalization setting. Doing so results in a multichannel image. Then you can normalize the data in the usual way. Images in the multichannel image must have the same aspect ratio. Images created using different imaging systems or cropped images might not have the same aspect ratio as the first image. Image Lab checks the aspect ratio of each image. Images with aspect ratios different from that of the first image do not appear in the list of Compatible Open Images.

To add a channel to a single image

1. Click Lane and Bands in the Analysis Tool Box.

2. In the Lanes tab, under Normalization Channel, select Add channel from the dropdown list.

   The Add Normalization Channel dialog box appears. Images with compatible aspect ratios are listed in the left pane.

   The image on screen when you clicked Add Channel appears in the dialog box under Channel to Normalize. This image is the first channel in the multichannel image. Its name appears in the Sample data box.

3. (Optional) To add a second image, drag an image in the Compatible Open Images list into the Normalization Data box or click Browse to find the image you want.

   The image you added appears in the Channel to Normalize box.

4. Click OK.

   The Auto-Analysis dialog box appears.

5. Select a sensitivity level for bands and click OK.

   A multichannel image of the two channels appears in the workspace. The Normalization channel is labeled Norm.

Molecular Weight (MW) Analysis Tools

Molecular Weight Analysis tools enable you to determine molecular weight (or base pairs, when using nucleic acid gels) by comparing a test sample with known standards.

Note: Before you can use the Molecular Weight Analysis tools, you must detect the lanes and bands in the image.

You can view each band's molecular weight in the molecular weight column of the Lane and Band tab in the Analysis Table.

| Lane 1 - Precision Plus | | | | | | | | |
Band No.	Band Label	Mol. Wt. (KDa)	Relative Front	Volume (Int)	Abs. Quant.	Rel. Quant.	Band %	Lane %
1		250.0	0.096	177,345	N/A	N/A	6.1	5.3
2		150.0	0.195	155,799	N/A	N/A	5.4	4.6
3		100.0	0.290	149,184	N/A	N/A	5.2	4.4
4		75.0	0.367	543,501	N/A	N/A	18.8	16.1
5		50.0	0.480	593,271	N/A	N/A	20.5	17.6
6		37.0	0.567	215,145	N/A	N/A	7.4	6.4
7		25.0	0.694	556,416	N/A	N/A	19.2	16.5
8		20.0	0.743	173,250	N/A	N/A	6.0	5.1
9		15.0	0.831	148,239	N/A	N/A	5.1	4.4
10		10.0	0.913	181,062	N/A	N/A	6.3	5.4

Lane And Band Table / Volume Table

You can also display the molecular weight of the bands on the gel image by opening the Display Gel Options dialog box and selecting Mol. Wt. in the Attribute dropdown list under Band Attributes. (See Displaying Gel Images on page 68 for information about displaying band attributes.)

Changing Molecular Weight Standards

You can change the standards to standards more relevant to your samples.

To change standards

1. Click Change to open the Manage Standards box.
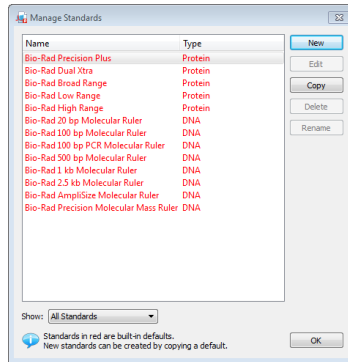
2. Choose ar standard or add one.

Standard Lanes

By default, standard samples are placed in the first and last lanes. You can specify other standard lanes by selecting the box below each lane or by entering the standard lane numbers separated by commas in the MW Analysis Tools dialog box. In the Lane and Bands view, the notation *Std* below the lanes identifies them as standard lanes. In the Molecular Weight Analysis view, a check mark appears below each standard lane.

Molecular Weight Analysis in Single-Channel Images

Three standard lanes appear in the image shown (lanes 1, 16, and 17). A check mark appears below each standard lane. The molecular weights of the bands in the standard lanes appear on both sides of the lane frame. The red lines running across the lane frame identify the location of the bands in the standard lanes. You can use these lines to determine where the bands in the other lanes fall relative to the bands in the standard lanes.

Names of the standard lanes appear as labels above them.
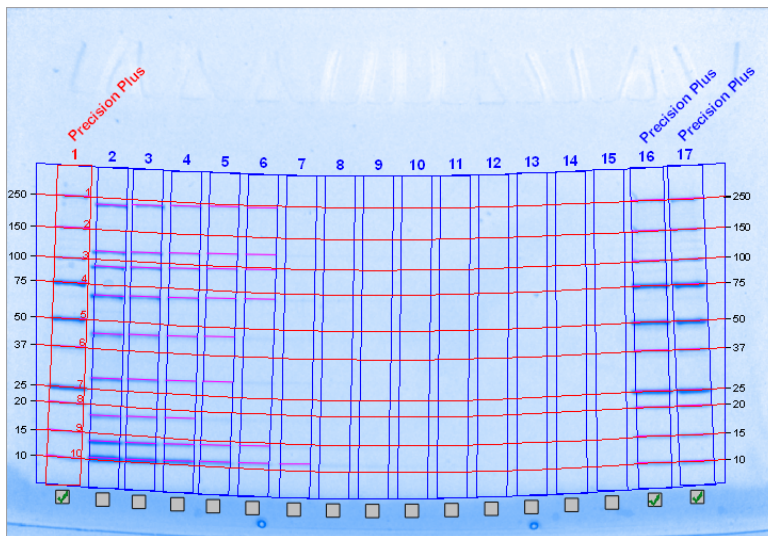
Values for the molecular weight in a standard lane appear in bold. In the table shown, the values for the first lane appear in bold. (Lanes 16 and 17 are not included in the image; their values would also appear in bold.) The molecular weight of the bands in the remaining lanes is calculated relative to these standards.

Lane 1 - Precision Plus		Lane 2		Lane 3		Lane 4	
Band No.	Mol. Wt. (KDa)	Band No.	Mol. Wt. (KDa)	Band No.	Mol. Wt. (KDa)	Band No.	Mol. Wt. (KDa)
1	**250.0**	1	203.8	1	207.9	1	210.9
2	**150.0**	2	118.0	2	119.7	2	121.6
3	**100.0**	3	99.0	3	100.4	3	102.0
4	**75.0**	4	70.6	4	71.6	4	72.4
5	**50.0**	5	45.1	5	45.8	5	46.3
6	**37.0**	6	26.8	6	27.2	6	27.5
7	**25.0**	7	17.4	7	17.6	7	17.8
8	**20.0**	8	12.9	8	13.0	8	13.2
9	**15.0**	9	10.6	9	10.7	9	10.8
10	**10.0**						

Molecular Weight Analysis in Multichannel Images

Molecular weight analysis is performed differently in multichannel images depending on whether the channels of the image are linked or unlinked. If the channels are linked, the standard lanes are synchronized across all channels. If the channels are unlinked, each channel has its own standard.

When you select the standard lane in one channel of a linked multichannel image, the same lane is selected as the standard in all other channels. The combined data in the multichannel image are used to detect the bands in this standard lane. These bands are synchronized across all channels. All the bands in all the channels are calculated using the same standard lane. If you deselect this lane as the standard, the bands in the standard lane are deleted from all channels, including the multichannel image.

In unlinked multichannel images, each channel is treated like a single channel image. You select a standard lane for each channel and the molecular weight is calculated only for the bands in that channel.

In the next image, lane 1 in the linked multichannel image is selected as the standard lane for all channels. Because the images are linked, the same molecular weights for the standard lane and the same red lines showing the location of the bands in the standard lane appear in all channels.
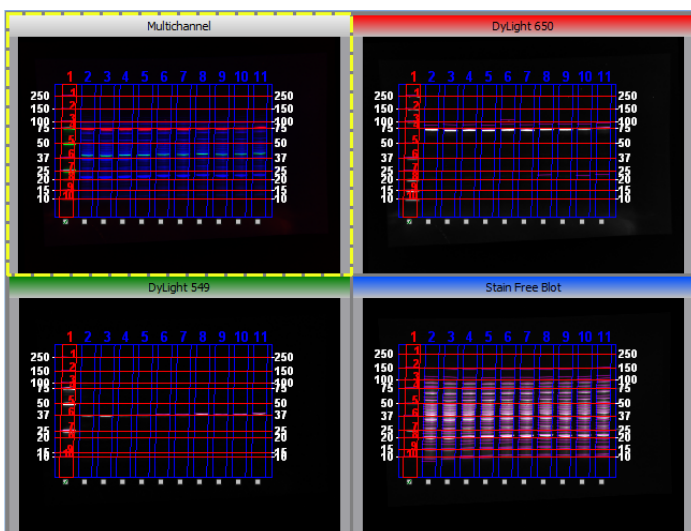
The molecular weights of the bands in the three channels are calculated based on standards established in lane 1 and appear in the analysis table.

Regression Methods

Three regression methods are available to generate the volume quantification curve used for absolute quantity:

- Linear (semi-log)

- Point-to-point (semi-log)

- Cubic spline

The data for volume standards appear in the Absolute Quantity column of the Volume Table.

To display the standard curve

1. Click Standard Curve on the toolbar.

2. In the Standard Curve dialog box, select the Volume Standard Curve tab.

Note: When the Force Through Origin checkbox is selected, the standard curve graph starts at 0,0, regardless of the best curve fit. See Appendix A, Regression Calculation Methods, to learn how each regression method is calculated.

Quantity Tools

You can quantify bands in test samples automatically in the Relative or Absolute tab under Quantity Tools in the Analysis Tool Box.

Relative Quantity Tab

To compare the relative quantities of bands

1. Select the Relative tab under Quantity Tools.
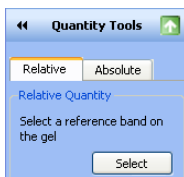
2. Click Select.

3. Click the band you want to use as a reference. A small R appears near the band you selected.

Band No.	Band Label	Mol. Wt. (KDa)	Relative Front	Volume (INT)	Abs. Quant.	Rel. Quant.	Band %	Lane %
1		298.4	0.053	18,232	N/A	0.20	1.7	1.5
2		164.5	0.224	167,427	N/A	1.85	15.5	13.7
3		138.0	0.274	75,684	N/A	0.83	7.0	6.2
4		95.1	0.381	26,235	N/A	0.29	2.4	2.1
5		57.6	0.525	90,683	N/A	1.00	8.4	7.4
6		34.7	0.671	188,839	N/A	2.08	17.5	15.4
7		22.1	0.802	131,864	N/A	1.45	12.2	10.8
8		16.3	0.889	380,593	N/A	4.20	35.3	31.1
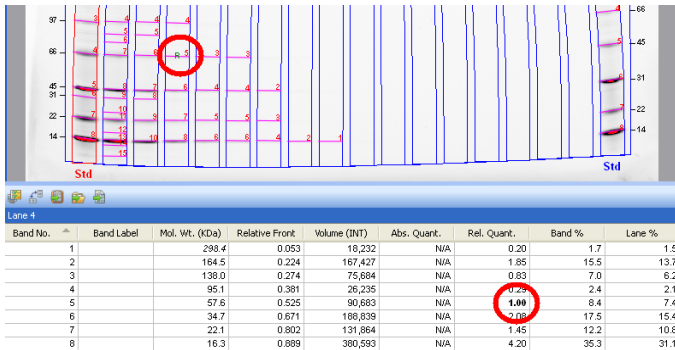
To review the relative quantities of bands

▶ Go to the Rel. Quant. column of the Analysis table (Lane and Band tab). The relative quantity is the ratio of the band volume divided by the reference band volume.

All other bands now display numerical values that are relative to the reference band. If the reference band value is 1.00, values higher than 1.00 indicate that the band quantity is greater than the reference band. Values lower than 1.00 indicate the band quantity is less than that of the reference band.

Absolute Quantity Tab

Absolute quantification is used to quantify bands based on known standard bands using a calibration curve.

To calculate the absolute quantities of the bands

1. Select the Absolute tab under Quantity Tools.

2. Click Select.

3. Select at least two standard (known) bands and assign quantity values. A small appears, circled in red, near each band you selected.
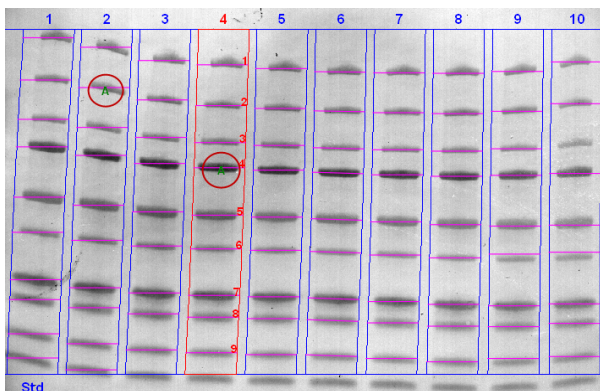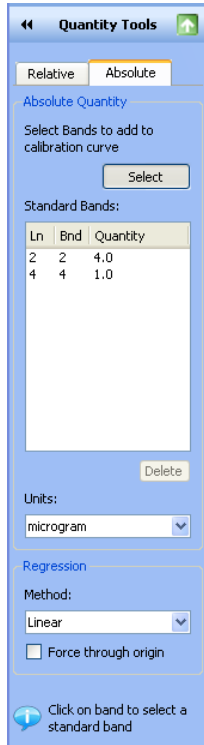
The values appear in the Standard Bands table. The greater the number of known bands and the wider the range of their values, the more accurate the absolute quantity calculation of the unknown bands will be.

Note: Any standard band selection can be deleted. Select the entry in the Standard Bands box and then click Delete.

4. Select a unit of measure in the Units dropdown list.

5. Select a regression method in the dropdown list.

    Consider the following guidelines when selecting a method.

    Linear — generates a straight line that is the best fit of the values you provided and is preferred in most cases.

    Point-to-point — generates a curve in which each data point is connected directly to the next, regardless of the shape of the resulting curve.

    Cubic spline — generates a smooth curve that connects each data point. At least four standard points are required to use this method of least-squares polynomial fits.

6. Click Standard Curve on the toolbar and select the Absolute Quantity Standard Curve tab.

Regression Method	Min Number Standard Bands	Min Nr with Force Through Origim
Linear	2	1
Point-to-point	2	1
Cubic spline	5	4

The calibration curve appears. Standards are represented by green triangles. Unknown values are represented by red triangles.

Note: Selecting the Force Through Origin checkbox always starts the standard curve graph at 0,0, regardless of the best curve fit.
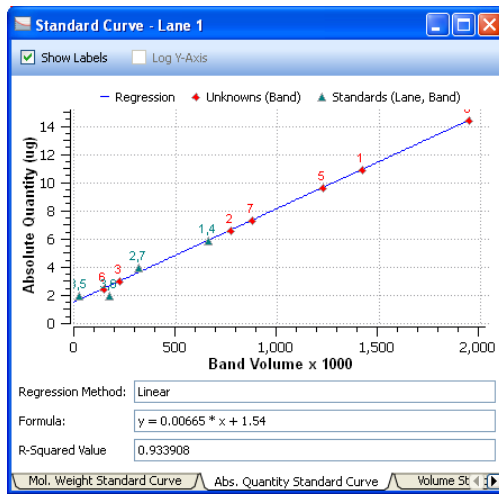
Note: Clicking the Standard Curve table generates a crosshair tool that displays the numerical values associated with the placement of the cursor in the graph.

Annotation Tools

You can annotate results with text and arrows to emphasize areas of interest.
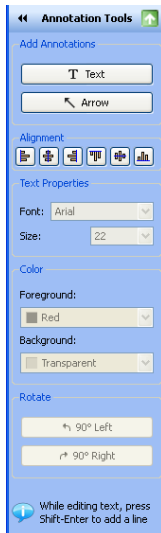
Add Annotations

Text — enables you to add text annotations to images to emphasize important details.

1. Click Text, and then click an area on the image where your comment should be inserted .A box appears with a dotted-line border.

2. Type your comment in the box.

   Tip: To add a new line to the box, place your cursor where you want the break and press Shift+Enter.

To move the box

▶ Click and drag the box to change its position.

■ **Arrow** — enables you to add arrows to images to emphasize important details. Click where you want the arrow to start and drag to stretch the arrow point to the location you want to emphasize. To move the arrow on the image, click the middle of the arrow and drag it to the new position.

To change where the arrow points

▶   Click either end of the arrow. Square boxes appear. Drag a box to change the length or orientation of the arrow.

   Note: In multichannel images, you can add annotations in all the channels. Each annotation, including the merged channel, is channel specific.

Alignment

The alignment buttons enable you to align multiple annotations, such as lane numbers, which you have manually added.

To select multiple annotations

▶   Press the Ctrl key (Command key on a Mac) and click each item or drag a selection box around them.

Note: In multichannel images, you can also copy annotations from one channel to another using the same method.

Text Properties

You can change the type size and font of your text annotations.

Font

1. Click the box you want to change.

2. Open the dropdown Font menu to show all fonts installed on your system.

3. Select a new font for the text annotation.

Size

1. Click the box whose font you want to resize.

2. Open the dropdown Size list to increase or decrease the size of the text.

3. Set the font size from 6 to 72 points using the dropdown list.

Color

You can change the color of text annotations to make them visible with any color scheme, and emphasize them further by adding a color to the annotation's background, which is invisible by default.

To change the color of multiple items

▶   Press the Ctrl key and click each item.

   Foreground

   ■   Click a text annotation or arrow. This activates the Foreground box, so you can select a foreground color from the dropdown list.

   Background

   ■   Click a text annotation. This also activates the Background box so you can select a background color from the dropdown list.

Rotate

To rotate text annotations 90° left or right

▶ Click the Rotate buttons under Imaging Tools in the Analysis Tools Toolbox.

Volume Tools

Volume tools enable you to manually quantify features on a sample image when automated lane and band analysis is not appropriate or possible, such as in dot blots.
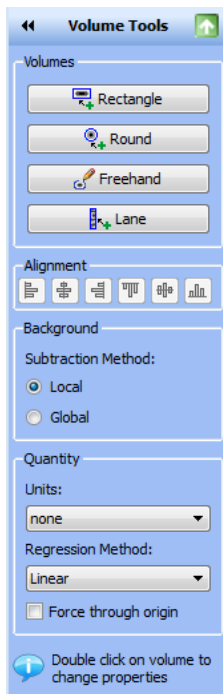
The analysis table displays the color-coded volume drawn for each channel in a multichannel image. In multichannel view, you can draw a volume on individual panes, but you cannot draw a volume on the Multichannel pane.

You can use Volume tools to quantify the signal intensity of bands, spots, arrays, and other image data. Define an area of interest by surrounding it with a shape. You can choose Rectangle, Round, Freehand, or Lane.

A default label appears inside the shape drawn. The volume label is assigned a sequential number and can be one of three types:

■ U — unknown

■ Std — standard

■ B — background
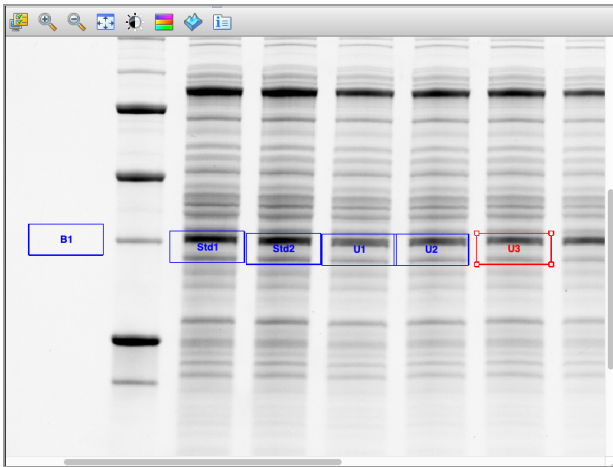
Each new volume you create initially has a red border, which indicates that the volume is selected. When you click elsewhere on the image, the border changes to blue, indicating that the volume is no longer selected.

Note: Double-click a volume area to change its properties.

To review data for the volumes

▶ Open the analysis table and select the Volume tab.

Volumes are listed based on their number and/or the associated information per volume. For information, see Volume Measurements on page 92.

Note: In multichannel images additional column bars, channel numbers, and volumes are color-coded to match their channel color.

Channel	No.	Label	Type	Volume (Int)	Adj. Vol. (Int)	Mean Bkgd. (Int)	Rel. Quant.	# of Pixels	Min. Value (Int)	Max. Value (Int)	Mean Value (Int)	Std. Dev.	Area (mm2)
Cy2	1	U1	Unknown	79,000,835	-24,648,766	465.0	N/A	222921	0	5,839	354.4	334.3	2,229.2
Cy3	1	U1	Unknown	58,288,261	22,008,480	180.0	N/A	201502	0	64,320	289.3	1,687.6	2,015.0
Cy5	1	U1	Unknown	70,533,568	1,544,826	696.5	N/A	99056	0	27,675	712.1	1,689.2	990.6

Volume Types

You can define the volume type (unknown, standard, or background), the quantity of standard volumes, or enter a custom name to replace the default label.

Unknown volumes are volumes you want to quantify.

Standard volumes are used for absolute quantities. For information, see .

Background volumes are used to remove the background from the volume calculation of non-background volumes. The result of volume background subtraction appears in the Adjusted Volume column of the analysis table (Volume Table tab).

Note: Assign this volume type only when using global background subtraction.

No.	Label	Type	Volume (Int)	Adj. Vol. (Int)	Mean Bkgd. (Int)	Abs. Quant.	Rel. Quant.
1	B1	Background	28,474,202	-44,446	1,805.7	N/A	N/A
2	Std1	Standard	44,462,671	265,608	1,909.8	100.0	1.00
3	Std2	Standard	29,249,190	695,097	2,071.2	150.0	2.62

Lane And Band Table | Volume Table

Volume Background Subtraction

When you draw a volume, some nondata background signals might be included inside the volume. These background signals usually have an intensity value that you do not want to include in your volume quantification. There are two methods of calculating this background intensity for volumes: local and global.

■ **Local** — local background subtraction calculates a separate background intensity for each unknown and standard volume you create. For each volume, the intensities of the pixels in a 1-pixel border around the volume are added together and divided by the total number of border pixels. This gives an average intensity for the background around each, which is then subtracted from the intensity of each pixel inside the volume. If the background value is greater than the pixel value inside the volume, the background-adjusted quantity of the volume can be <0. In this case, redraw the border for this volume.

■ **Global** — global background subtraction calculates a single background intensity for the entire gel. This average background intensity is then subtracted from all the volumes in the gel. The average intensity of the pixels in the background volume is calculated and subtracted from each pixel in all standard and unknown volumes. Therefore, it is not necessary for the background volume area to be the same size as your unknown.

To calculate global background subtraction

1. Use one of the Volume Tools to create a volume in a representative background region of your image (that is, a nondata region similar to the background surrounding your data).

2. Double-click the volume. The Volume Properties dialog box opens.

3. Select Background in the dialog box.

Keep the following points in mind:

■ If you select Global in the Volume Tools toolbox but do not define a background volume as described, no background subtraction is performed.

■ If you create more than one background volume, all the pixels in the background volumes are used to calculate the average background. By default, background volumes are named B1, B2, and so on based on the sequence in which they were created.

■ If the region you defined as background has a higher average intensity value than the data object, you obtain a negative value for theadjusted volume in the analysis table. If this happens, select a new background region with less intensity thanthe data object.

Relative Volume Quantity

You can choose any one volume as a reference volume by selecting the Reference Volume checkbox in the Volume Properties dialog box. The reference volume is indicated by an asterisk on the volume label, for example, U1*. Relative quantities are displayed in the Relative Quantity column in the analysis table (Volume Table tab). The relative quantity is the ratio of the background-adjusted volume divided by the background-adjusted reference volume. All other volumes now display numerical values relative to your reference volume. Values higher than 1.00 indi te that the volume is greater than the reference volume. Values lower than 1.00 indicate the volume is less than the reference volume.

Regression Methods

Three regression methods are available to generate the volume quantification curve used for absolute quantity:

■ Linear (semi-log)

■ Point-to-point (semi-log)

■ Cubic spline

The data for volume standards appear in the Absolute Quantity column of the Volume Table.

To display the standard curve

1. Click Standard Curve on the toolbar.

2. In the Standard Curve dialog box, select the Volume Standard Curve tab.

Note: When the Force Through Origin checkbox is selected, the standard curve graph starts at 0,0, regardless of the best curve fit. See Appendix A, Regression Calculation Methods, to learn how each regression method is calculated.

Absolute Volume Quantity

Note: Absolute volume quantity analysis is not available for multichannel images.

If you have drawn the volume around an object of known quantity, you can use it to calculate the quantity of unknown volumes. The quantities of unknown volumes are calculated based on the standard volumes and the selected regression method.

To classify a particular volume as a standard

1. Double-click the volume to open the Volume Properties dialog box.

2. Select the Standard option button and enter the quantity in the Quantity box.

3. Click OK to close the dialog box.

By default, standard volumes are named S1, S2, and so on, based on the sequence in which they are created.

To review the regression curve

▶ Open the Standard Curve dialog box and select the Volume Standard Curve tab.

Alignment

Align volumes by using the appropriate alignment button. To select several volumes, Ctrl-click each one, then select one of the alignment buttons. Hover over any of the six alignment buttons to display its function (Align Left, Align Right, and so forth).

Copy and paste selected volumes by pressing Ctrl+C to copy. Press Ctrl+V to paste.

When you click the Standard Curve button on the toolbar, a chart displays all unknown and standard quantities.

Chapter 7 Generating Reports

After viewing results, you can generate a report that displays the analyzed gel images, all of the settings used in the protocol, and as much information about the data as you want to include.

You can choose print settings within the Report Settings dialog box in the Edit menu or by clicking Report in the main toolbar.

Report

To produce a preview of the report

▶ Click Report on the toolbar.
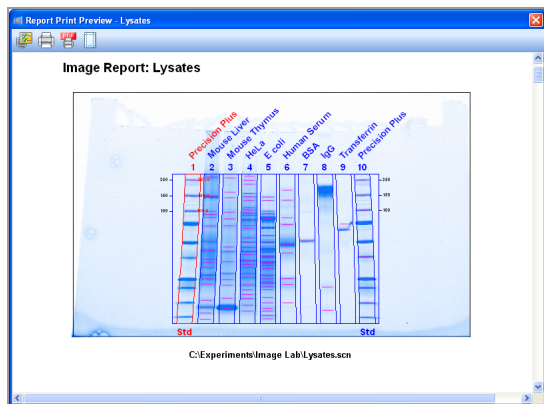
Use the following dialog boxes to customize the content in reports. Doing so does not modify the data from the analysis.

Report Options

Use this dialog box to customize the content of the report.

General Tab

The General tab includes the following options:

■ **Include Gel Image** — specify whether the image is included in the report.
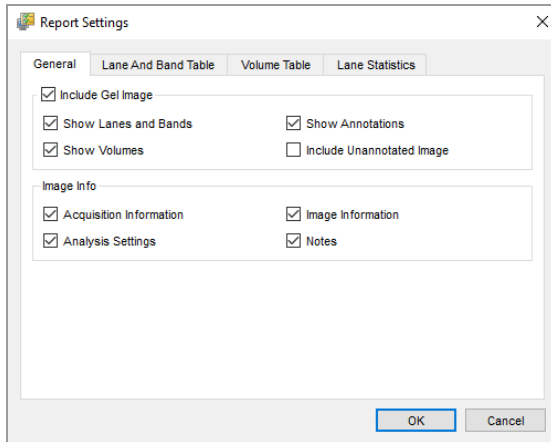
If the image is included, the following options determine which overlays are displayed on the gel image:

☐ Show Lanes and Bands

☐ Show Volumes

☐ Show Annotations

☐ Include Unannotated Image

When you select this checkbox, both the annotated image (if available) and unannotated images are printed in the report. The unannotated image precedes the annotated image in the report.

Note: If you disable the other checkboxes in the Include Gel Image group, the Include Unannotated Image checkbox is automatically disabled.

■ **Image Info** — specify what information is included in the report.

☐ Acquisition Information

☐ Analysis Settings

☐ Image Information

☐ Notes

■ **Signature History** — details on when and why a secure document was signed (applies to Security Edition only)

If a secure document has been signed, the user name, the date and time of the signature, and the reason for signing are all included in the Signature History section. If a document has not been signed, this section is omitted from the image report.

Lane and Band Table Tab
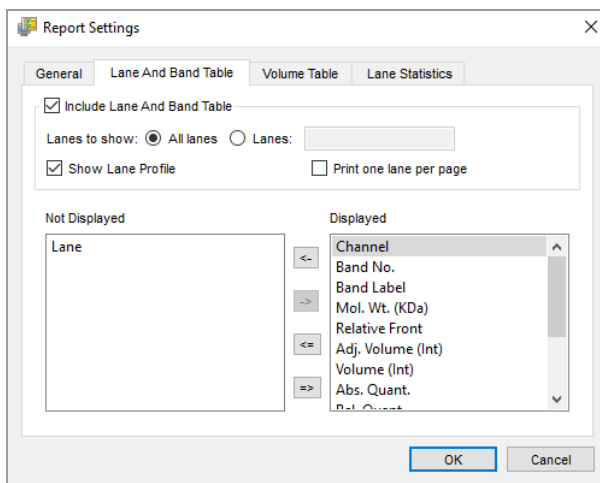
The Lane and Band Table tab includes the following settings:

■ **Include Lane and Band Table** — specify whether to include the Lane and Band table in the report.

■ **Lanes to show** — specify which lanes to display in the report.

■ **Show Lane Profile** — specify whether to display the Lane Profile view.

■ **Print one lane per page** — specify whether all lanes are printed on one page or whether each lane is printed on a separate page (adds a page break after each lane).

■ **Show Lane Profile** — include the lane profile for each lane.

■ **Not Displayed/Displayed** — remove columns that you do not want to display in the report. By default, the report displays all columns from the Lane and Bands table.

Volume Table Tab
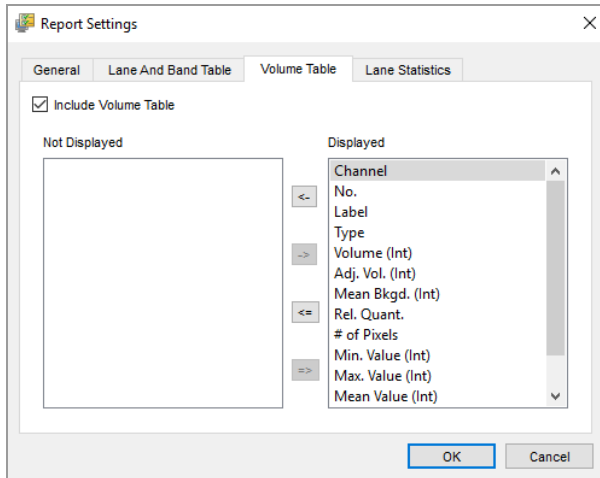
The Volume Table tab includes the following settings:

■ **Include Volume Table** — clear to exclude this information from a report.

■ **Not Displayed/Displayed** — remove columns that you do not want to display in the report. By default, the report displays all columns from the Volume table.

Lane Statistics Tab

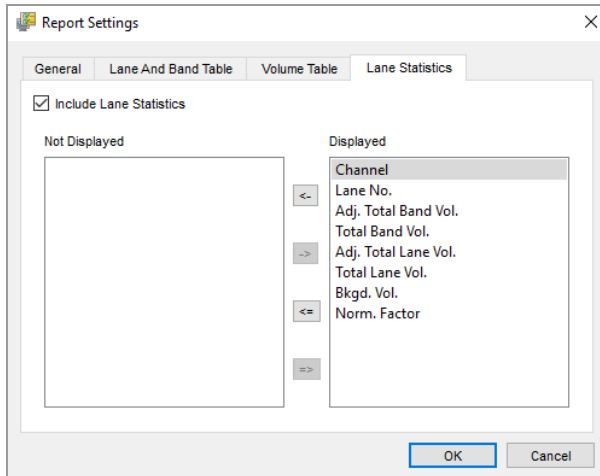In the Lane Statistics tab,you can choose to display the following information:

- Channel

- Lane number

- Adjusted total band volume

- Total band volume

- Adjust total lane volume

- Total lane volume

- Background volume

- Normalization factor

Print Report

Click the Print Report button to print the report.

Print Report to a PDF File

The Print Report to .pdf File button opens a Save dialog box so the PDF file can be saved on your system.

Adjust the Printer Settings

The Printer Settings button accesses options for paper size, orientation, and page margins.

Chapter 8 Exporting Results

The most convenient way to archive complete information about experiments is to produce reports. However, it is possible to export only gel images or analysis table data for analysis in different programs, such as Quantity One and ImageJ software. It is also possible to export only files for presentation or publication.

Exporting Gel Images

When you export gel images, you can choose an option that suits your purpose:

- Export displayed image data to a publication (choose Export for Publication).

- Export raw image data as a 16-bit .tif file (choose Export for Analysis).

- Export image data to PulseNet. Doing so reduces the image to a .tif file (8-bit), limits its resolution, and restricts its file size to 300 Kb.

- Export lane and band tables as well as volume tables to a spreadsheet program or to a file.

To select an export option

▶ On the File menu, click Export and choose an option in the context menu.
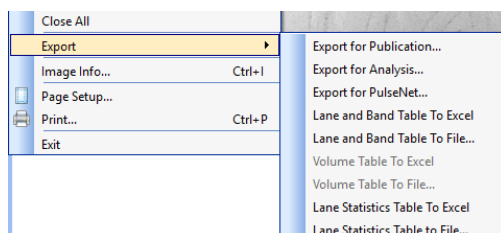
Exporting Gel Images for Publication

Choose this export option only when you want to export visual information to presentation or word processing software, such as PowerPoint or Word. You can select .bmp, .png, .jpg, or .tif format. The gel displays with the lanes, bands, and annotations that appear on screen.

(Multichannel Images) Image Lab software exports the active channel in a multichannel image. Select the channel you want to publish before you export the image.

Before You Export

You can zoom in on an area in a current view and export only that area or export the entire image.

You can exclude annotations or overlays by clicking Display Gel Options in the Display Toolbox to access the appropriate settings.
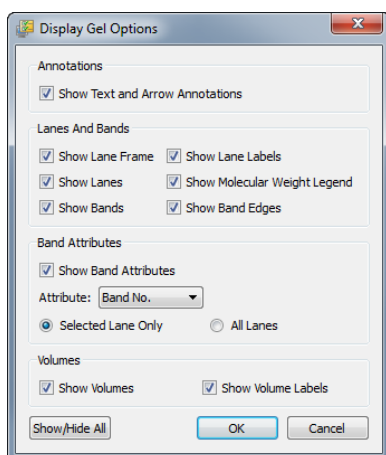
To export a displayed image to a file

1. Select File > Export > Export for Publication.

    The Export For Publication dialog box appears.
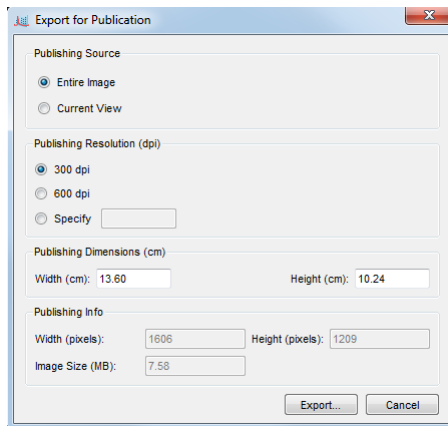
2. Do any of the following:

    ■   Select the entire image or the current view

    ■   Select the resolution or specify a custom resolution

    ■   Specify the publishing dimensions

    ■   View the resulting published image size and dimensions

3. When the image is acceptable, click Export and save the image.

Exporting Gel Images for Analysis

To export an image for analysis

▶   Select File > Export > Export for Analysis.

This procedure exports the raw data only as a 16-bit .tif file.

Note: 16-bit .tif images are not compatible with all image viewers.

The image might require contrast adjustment when it is imported into analysis software. This option creates a file that can be analyzed in other programs such as Quantity One and ImageJ.

Note: (Multichannel images) Image Lab software exports the separate channel images but not the composite image. Each exported channel image is saved with its application name appended to the filename you select.

Exporting Gel Images to PulseNet International

To export an image to PulseNet International

▶   Select File > Export > Export for PulseNet.

Image Lab reduces the image to an 8-bit .tif image file. Resolution is limited and file size is restricted to 300 Kb.

Note: Export for PulseNet is not available for multichannel images.

Exporting Lane and Band Tables to Excel

If you have Excel (or Numbers on a Mac) installed on your computer, you can export the data to your spreadsheet application.

To export the data to Excel (or Numbers)

▶ Select File > Export > Lane and Band Table to Excel.

The exported data appears in a table open in your spreadsheet program.

Tip: You can then select Save As and produce other formats.

Exporting Volume Tables to File

To export an image as a CSV file

▶ Select Export > Volume Table to File.

Image Lab exports the image as a comma-separated values (CSV) file so the data file can be opened in a database application.

Screenshot Tool Export

Use the Screenshot tool on the toolbar to capture an image open in the workspace to the clipboard or to save it to a file (.bmp, .gif, .jpg, or .png).

Chapter 9 Software Logs

Image Lab software provides the following types of logs:

- **System log** — records events related to running Image Lab, including enabling or disabling of secure mode and the users who log on to or log off of Image Lab.

- **Document log** — (Security Edition only) records events related to the creating and modifying of secure protocol and image files.

- **Instrument log** — records events related to the operation and calibration of the instrument.

Viewing the System Log

Events related to running Image Lab, including enabling or disabling secure mode and users logging on or logging off from Image Lab, are recorded in the system log.

To open the System Log Viewer

▶ In the View menu, click View System Log.
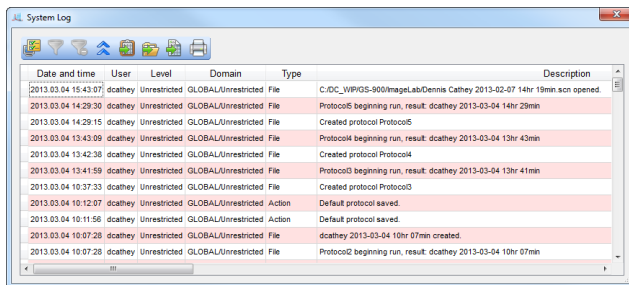
Viewing the Document Log

The View menu displays a list of logs for each open document. The document log captures information about creating and editing the Image Lab protocol and image files.

Note: Document logs are viewable only in Image Lab Security Edition, and only on Windows-based computers. Document logs are not viewable on the Mac.
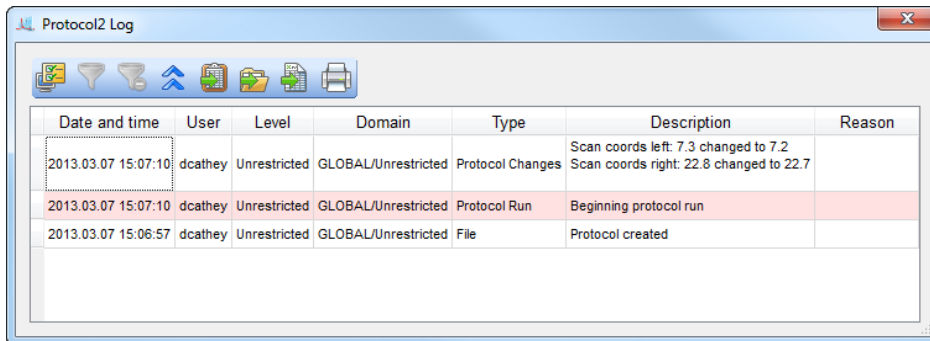
The document log captures changes from

- Image tools

- Lane and bands tools

- Normalization tools

- Molecular weight tools

- Quantity tools

- Volume tools

You can view the document log for any open file, including a previously saved file or a newly created protocol or image file open on your desktop but not yet saved.

To open the Document Log Viewer

▶   In the View menu, click the document log you want to view.

An example document log is shown next.

Viewing the Instrument Log

When your computer is connected to an instrument, an instrument log records events related to the operation and calibration of the instrument.

Displaying Log Data

The display toolbar appears above the log.

Displaying Data Columns in Logs

By default, the logs display the following columns:

■ Date and time

■ User

■ Level — the security role of the user

■ Domain — domain where the current user is logged in

■ Type — the type of event

■ Description — the event captured

■ Reason (Secure Mode only) — the reason a document was signed

You can change your view of any log by displaying or hiding data columns.

To display or hide columns

1. In the View menu, click View System Log.

2. Click the Display log viewer options icon (the leftmost icon).

3. In the Display Column Options dialog box, use the arrow keys to move columns between the Not Displayed and Displayed lists.
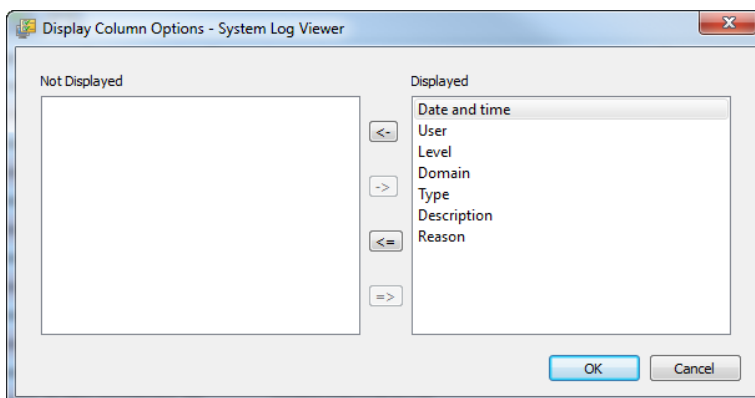
4. Click OK.

Filtering Data in Logs

For Image Lab logs, you can filter the entries in the following columns:

- Date and time

- User

- Type

For example, if you set the filter in the Type column to File, Image Lab displays only the rows in which Type is equal to File.

Setting Filters in Logs

By default, the log opens with all event types displayed. You can filter most event type columns. However, some columns cannot be filtered (for example, Description and Reason columns).
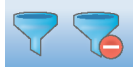
To filter column entry types

1. In the log, right-click the heading of the column that you want to filter.

   Filter options appropriate for the column appear in the Select filter values dialog box.

   Note: When you select a column that cannot be filtered, the dialog box does not appear

2. Select the filter values that you want to display in the column and click OK.

   The log displays the values you selected for the column.

Displaying or Hiding Columns

3. Click the Display Filter options icon in the toolbar.

   The Select filter values dialog box appears.
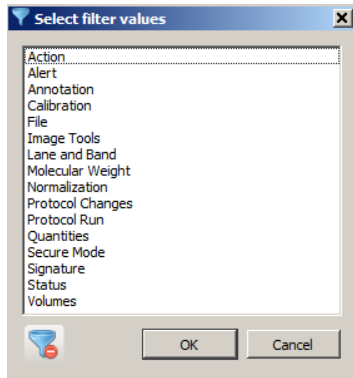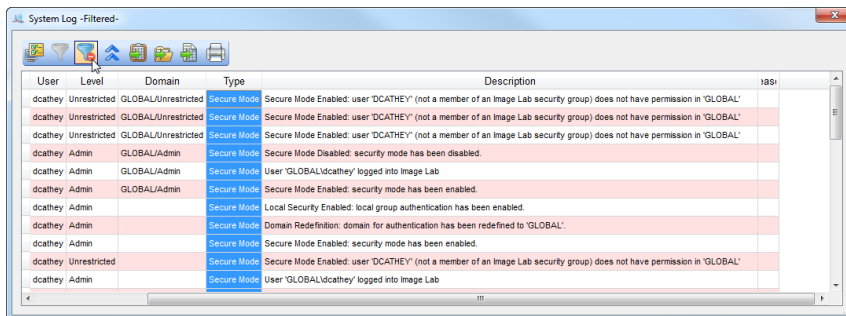
4.   Select a value and click OK.

     Tip: You can filter multiple values.

Removing filters in logs

The Remove all filters icon clears all filters on all columns.

Each filterable column also has a Remove filters icon that removes the filter for that column only.
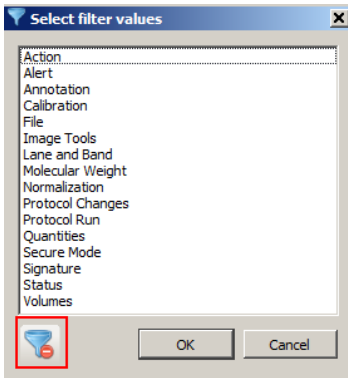
Collapsing or Expanding Data Rows

You can expand the size of rows in any log to display the full content of the row or collapse the row size to display more rows in the table. The icon toggles between collapse and expand.

To display the full content of a row

▶ Click the Collapse or expand rows icon to expand the row height. The row adjusts to fit the text of the longest entry.

This action expands all rows in the log where an entry is longer than the width of the column. Rows that do not wrap are not affected.

To display more rows in the table

▶ Click the Collapse or expand rows icon to collapse the row height to the default height setting.

This action collapses all rows in the log to display data on a single line.

Exporting Logs

In the log viewer, you can

■ **Copy log entries to the clipboard** — copies the log entries to the clipboard, enabling you to paste them into a word processing or presentation application.

■ **Export log entries to a file** — exports the log entries as a CSV file that can be opened in a database application.

■ **Export log entries to Excel** — exports the log entries to an Excel file where you can use Excel's sorting and formula functions to manipulate the data. If Excel is not installed on your computer, this feature is inactive.

To export a log file

1. In the View menu, click the log you want to export.

2. Click one of the icons to export the data in one of the supported formats.

Date and time	User	Level	Domain	Type	Description	Reason
2013.03.07 15:07:10	dcathey	Unrestricted	GLOBAL/Unrestricted	Protocol Changes	Scan coords left: 7.3 changed to 7.2 Scan coords right: 22.8 changed to 22.7	
2013.03.07 15:07:10	dcathey	Unrestricted	GLOBAL/Unrestricted	Protocol Run	Beginning protocol run	
2013.03.07 15:06:57	dcathey	Unrestricted	GLOBAL/Unrestricted	File	Protocol created	
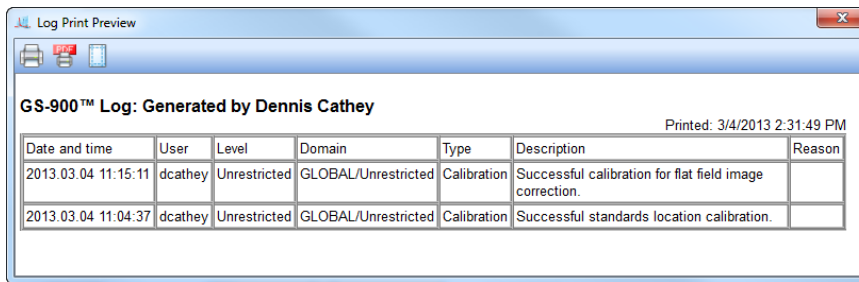
Printing Logs

Log data can be sent to a printer or saved to a PDF file.

To print a log

1. In the View menu, click the log you want to print.

2. In the log viewer, click Print log.

    The Log Print Preview window displays the contents of the log file.

In the Log Print Preview window, you can do any of the following:

◼ Click Print log to print the log to a printer.

◼ Click Print log to PDF to save the log to a PDF file.

◼ Click Adjust printer settings to prepare the file for printing.

Chapter 10 Using the Security Edition

21 CFR Part 11

Image Lab Security Edition is a module in the Bio-Rad's Image Lab software that assists users in meeting the U.S. Food and Drug Administration's regulations on good lab practices in the pharmaceutical and biotechnology industries. The Security Edition enables system administrators to ensure that Image Lab operates in compliance with Title 21 of the Code of Federal Regulations (CFR) Part 11in a closed system. A *closed system* is defined as "an environment in which system access is controlled by the persons who are responsible for the content of electronic records that are on the system" (Section 11.3 (b) (4)).

Important: Image Lab Security Edition is not supported on the Mac.

ChemiDoc Touch and 21 CFR Part 11 Compliance

The ChemiDoc Touch imaging system running Image Lab Touch Software 1.2 or earlier does not support the acquisition of images in secure mode, and any reference to the creation of secure documents does not apply.

However, Image Lab Touch, running on the ChemiDoc Touch, ChemiDoc, or ChemiDoc MP imaging systems, can export secure images but do not support signature or log viewing. Secure files exported to Image Lab software can be opened in secure mode and signed. Logs from these files can be viewed in Image Lab. For more information on this feature, see Unsecured Documents on page 175 and Signing Documents on page 176. For information about the logs that document these actions, see Document Logs on page 177.

Administering Security Controls

The security controls built into Image Lab Security Edition must be properly configured and administered by the system administrator(s) in your organization in order to be secure and in compliance with 21 CFR Part 11.

Establishing Policies and Procedures for Compliance

Bio-Rad makes no claim that Image Lab Security Edition software is CFR-compliant in and of itself, nor does the company guarantee compliance for the user. Your organization must establish policies and standard operating procedures that work in conjunction with the tools provided by Bio-Rad to ensure compliance with 21 CFR Part 11.

Standard Mode versus Secure Mode

Image Lab Security Edition can be run in either of the following modes:

■ **Standard Mode** — in this mode, there are no restrictions on controlling the instrument, operating the software, or changing the documents.

■ **Secure Mode** — in this mode, in addition to the features available in standard mode, security features are enabled, including document signing, the use of Write Once folders, and log creation.

When Image Lab is in secure mode, a padlock symbol appears in the left corner of the status bar. If no padlock is present, the software is running in standard mode.
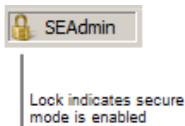
When Image Lab is installed, by default it is set to run in standard mode. It continues to run in this mode until a user with Image Lab Administrator privileges enables secure mode. This chapter assumes you are running the software in secure mode unless noted.

User Names, Groups, and Roles

To run Image Lab Security Edition in secure mode, you must log in with a user name and password. The Microsoft Windows system administrator usually creates the user names and passwords. The Image Lab administrator defines the groups with which the Security Edition roles will be associated. there are four default roles in Image Lab Security Edition. Each role is associated with one of the four default Image Lab user groups, and each user is assigned a role that gives the user access to specific features in the software.

Note: System administrators can change the user group names, if necessary, to meet their company standards. For more information about setting up groups, user names, and passwords, see

Table 7.
Image Lab Security Edition groups and roles

User Group	Image Lab Role	Description
TDS_ Administrator	Administrator	Administrators can enable or disable secure mode and view log files. They do not have access to other features.
TDS_User	Supervisor	Supervisors have full access to all application features and functions and can also sign files. They cannot enable or disable secure mode.
TDS_Tech	Clinician	Clinicians can perform instrument operations and view log files and sign files. They do not have access to other features.
TDS_Guest	Reviewer	Reviewers can view log files and can also sign files. They do not have access to other features.

Role Restrictions

Your role determines which features of the security edition you have permission to use. If you attempt to perform an action that is not permitted for a user in your role, you will see an error message. In some instances the user's role determines which Security Edition features are visible and/or enabled. Therefore, you might not see all of the features described in this chapter.

Table 8 lists the Image Lab Security Edition functions that each role has permission to perform.

Table 8. User access to features by role

Function	Administrator	Supervisor	Clinician	Reviewer
Enable/disable secure mode	X			
View log files	X	X	X	X
Enable/disable write once support	X			
Set up and recalibrate the instrument		X		
Create new protocols		X		
Open existing protocols		X	X	X

Function	Administrator	Supervisor	Clinician	Reviewer
Run protocols		X	X	
Edit protocols		X		
Create new images		X	X	
Open existing images		X	X	X
Edit images		X	X	
Analyze images		X	X	
Sign documents		X	X	X

Starting Image Lab Security Edition

When secure mode is enabled, you are prompted to log on when you start the Image Lab application.

To start Image Lab in secure mode

1. Click the Image Lab icon to start the application.

2. In the Log on to Image Lab dialog box, enter your user name and password.
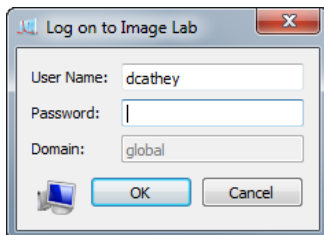
3. Click OK.

Note: If you have any questions or problems logging on, see your system administrator.

Electronic Records

Image Lab Security Edition enables you to create secure electronic records as defined by 21 CFR Part 11. In Image Lab, the following are electronic records:

■ Protocol files

■ Image files

- Document log files

- Instrument log files

- System log files

Unsecured Documents

Note: Starting with version 2.0, Image Lab Touch exports secured documents. Information in this section does not apply to imagers running Image Lab Touch 2.0 or later.

Image files created with the ChemiDoc Touch imaging system running Image Lab Touch 1.2 or earlier are unsigned, unsecure documents. Unsecure documents remain unsecure. When you open these files in secure mode, you can make changes to these files and save them without restrictions. A document log is generated for changes to these files, but the log is not viewable in standard mode. You must log in to secure mode to see the log.

You can also open unsecure documents in secure mode and sign them. You can make changes to these files and save them as secure documents. The original unsecure document remains unsigned and unsecure. The new document is saved as read-only with an incremental revision number and Image Lab generates an audit log. Image Lab will not be able to overwrite the secure file, but it can overwrite the original unsecure file.

Secure Documents

Until ChemiDoc Touch image files created using Image Lab Touch 1.2 or earlier are signed, they are saved as standard files. Once signed, they are they saved with the secure extension (.sscn).

Note: A secure document can be saved unsigned. Likewise, a signed document can be saved as a new, unsigned file. In this case, the saved file is still a secure document. All changes to the document are captured in the document log.

Documents created in secure mode can be signed at any time. When a secure document is signed, it is saved as read-only. Image Lab cannot overwrite the document. You can open signed documents and sign them again. The newly signed file is saved as a second revision, and the new signatures are captured in the document log.

Note: Image Lab can never overwrite a signed file.

You can open secure documents in standard mode of Image Lab. Selecting Save As in the File menu creates an unsecure file. The original file is preserved, and an entry is added to the original log that the file has been reverted to unsecure. Image Lab standard edition can modify the file. A log is generated for the new, unsecure document, and Image Lab no longer checks the file for unauthorized alterations.

Modifying Secure Documents

You can open and change a signed document in Image Lab. The original document is read-only and cannot be overwritten. Saving the revisions opens the Save As dialog box. The changes are saved into a new revision with an incremental revision number.

Each time a secure document is modified, you must provide a reason for *each* change before you can sign the document. The modifications are logged in the document log. The new signed document takes with it the entire history of the original document in its log.

Signing Documents

To sign a document

1. Select the protocol or results file.

2. In the Security menu, click Sign Document.

   The Signing Document dialog box appears.
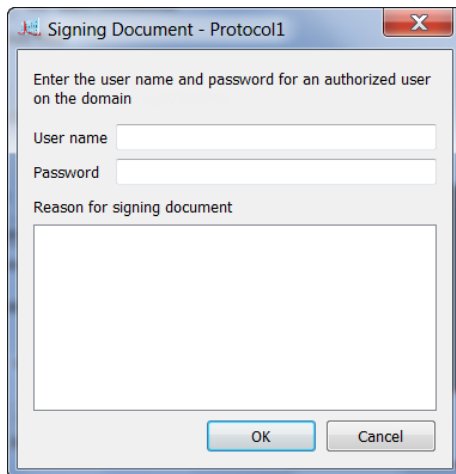
3. Enter the user name and password of a user authorized to sign documents.

   Note: The user name and password can be for a user other than the current user.

4. Enter a reason for signing the document. Typical reasons include review, approval, responsibility, or authorship.

   Note: You must provide a reason in order to sign the document.

The user name, date and time of the signature, and reason for signing are always included in the Signature History section of the image report (see .

5. Click OK.

   A Save File dialog box appears.

6. Enter a new name for the file and click Save.

   Note: Signed images are saved with an .sscn extension.

Document Logs

Changes made or actions performed on an image file generate a document log that documents each change or action. If you change a file and save it as a new file, of whether you are in secure mode or not, the document log is preserved in the new file. If you are in secure mode, the signature of the previous file is noted as part of the document log.

All major actions and changes are audited (they generate a document log). Examples of auditable actions include

■ Signing a file

■ Changing the image by cropping or rotating it, for example

■ Modifying the analysis. For example, changing the lanes and bands or adding or editing annotations to the images

Minor changes that affect only the display are not audited, such as

■ Selecting different columns in the Analysis Table

■ Changing the display options in the Lane Profile

■ Annotating or labeling the image using Annotation Tools

Each change you make to a signed image file must be documented in the Reason for Change dialog box.

Viewing the Document Log

The View menu displays a list of logs for each open document. The document log captures information about editing image files. You can view the document log for any open file. This file can be a previously saved file or a newly created image file that is open on your desktop but not yet saved.

If the document was previously saved, the log is identified with its name. If the document has not been saved, the log is identified with the time stamp that shows when the document was created.
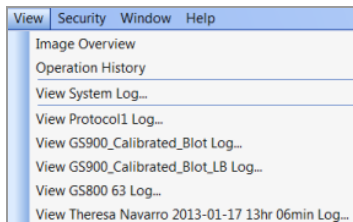
The document log includes

■   Date and time

■   User

■   Level — security role of the user

■   Type — type of event

■   Description — the actual event captured

■   Reason — reason for signing a document

For more information about logs, see Software Logs on page 161.

Appendix A Regression Calculation Methods

Each regression method calculates a standard curve. Some methods provide the formula for the standard curve. In this case, the molecular weight can be calculated by

x = relative front of the band of interest

y = molecular weight of the band of interest

Linear (semi-log): The linear equation is $y = a + bx$, where a is the intercept and b is the slope of the line.

Note: The linear equation is calculated on the **log** of the molecular weight values.

The R^2 value can be used to determine the overall quality of the linear fit. A linear regression with an R^2 value of >0.99 is considered a very good fit. The primary advantage of this method is that it is extremely simple. The primary disadvantage is that it will deliver incorrect results if the data are not very linear.

Point-to-point (semi-log): No single equation is available for the point-to-point method. The slope of each segment of the curve between data points is calculated independently.

Note: The log of the molecular weight values is used to calculate the slope for each segment of the curve.

Logistic: The logistic-4PL equation is

$$y = d + \frac{a - d}{1 + \left(\frac{x}{c}\right)^b}$$

where

x = mobility

y = molecular weight

a = estimated molecular weight at infinity

b = slope of the tangent at midpoint

c = midpoint

d = estimated molecular weight at zero mobility

Since the curve generated by the logistic-4PL regression method represents a perfectly shaped S, it might not fit the data very well in all cases.

Cubic spline: Cubic spline curves are smooth curves that go through every data point. The model is a cubic polynomial on each interval between data points. In some cases, a spline curve can work well as a standard curve for interpolation. However, because the curve is calculated individually for every pair of points, it does not correspond to any single equation.

Appendix B Setting Up Users and Groups

This appendix explains how to set up users and groups to run Image Lab Security Edition software in secure mode.

Note: This task requires System Administrator privileges on the client computers (and possibly their domain) on which Image Lab is installed.

User Accounts

To give users access to Image Lab, Security Edition, you can create new Windows user accounts, add existing user accounts to the four default user groups, or rename any of the four default user groups. Default user groups and roles are defined in User Names, Groups, and Roles on page 172.

■ A user account can have any name, but a password must be defined for the account. See Password Security on page 190 for information about setting passwords for maximum security.

■ Each user can belong to the Image Lab Administrator group and one other Image Lab user group.

For example, a user can belong to the Administrator group and the Supervisor group, but a user cannot belong to both the Clinician group and the Supervisor group.

User Authentication and Group Membership

In Image Lab authentication consists of two processes: user authentication and group membership evaluation.

User Authentication

Image Lab matches (authenticates) a user name with permissions assigned to that user name on the authentication domain. The domain can reside on your local computer (a local domain) or on a network server (a network domain). If you (or your network administrator) choose a local domain to be used for authentication, you are considered a local user. If you or your network administrator choose a network domain, you are considered a domain user.

Group Membership Evaluation

Image Lab verifies that a user is a member of one or more of the four default Image Lab user groups (TDS_Administrator, TDS_User, TDS_Tech, or TDS_Guest). The valid members for each of these groups can be specified in one of two places, as defined by the Use local groups for establishing user security levels checkbox on the Security Preferences dialog box.
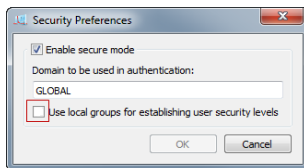
When this checkbox is selected, only users and groups that are defined on the local computer (on which Image Lab is installed) are recognized. When this checkbox is not selected, only users and groups that are defined on the network domain are recognized.
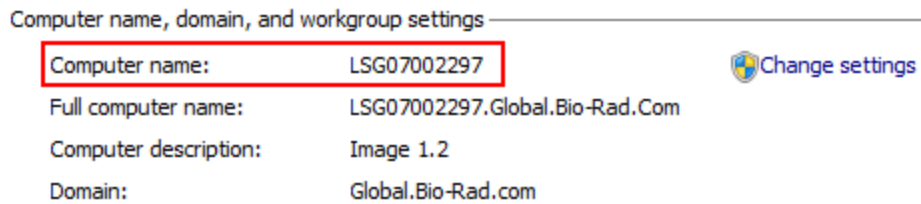
Finding Your Authentication Domain Name

Your authentication domain can be hosted on your local computer (a local domain) or on a network server (a network domain).

To find the name of your local domain

▶ In Windows, open the Control Panel and click System.

The System window appears.

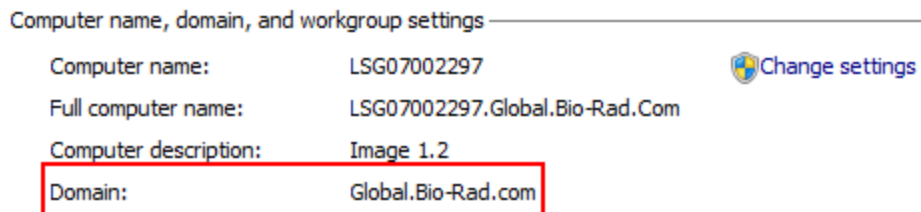Under Computer name, domain, and workgroup settings, the name of your local computer is shown as Computer name.

Computer name, domain, and workgroup settings ──────────────

Computer name:	LSG07002297	🛡Change settings
Full computer name:	LSG07002297.Global.Bio-Rad.Com	
Computer description:	Image 1.2	
Domain:	Global.Bio-Rad.com	

To find the name of your network domain

▶ In Windows, open the Control Panel and click System.

The System window appears.

Under Computer name, domain, and workgroup settings, the name of your network domain is shown as Domain.

Computer name, domain, and workgroup settings ──────────────

Computer name:	LSG07002297	🛡Change settings
Full computer name:	LSG07002297.Global.Bio-Rad.Com	
Computer description:	Image 1.2	
Domain:	Global.Bio-Rad.com	

Specifying the Domain Name

To specify the domain name

▶ In the Security Preferences dialog box, enter the exact name of the local computer or network server you want to designate as the authentication domain.
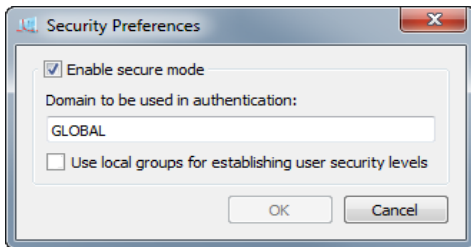
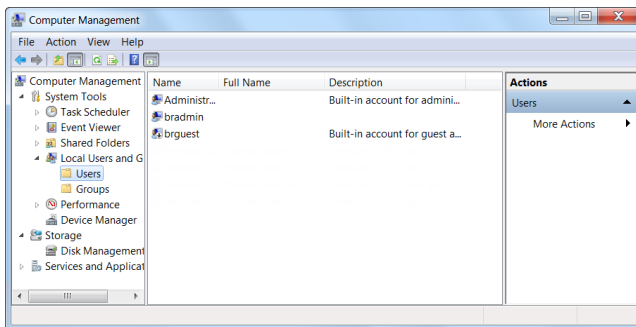## Configuring Users and Groups on a Local Computer

To access users and groups settings on a local computer

1. In the Windows Control Panel, select Administrative Tools.

2. Select Computer Management.

3. In the Computer Management window, open the System Tools folder, and then open the Local Users and Groups folder.

To create a new user on a local computer

1. Open the Users folder and click Action > New User.

    The New User dialog box appears.

2. Enter information about the user in the following boxes:

    ■ **User name** — must be unique.

    ■ **Full name** — must be unique. Bio-Rad recommends using the user's actual full name, as this name will be shown in the document log and all the log reports. This is a requirement of 21 CFR 11.50a.

    ■ **Description** — required. Bio-Rad recommends entering the user's title as the description.

    ■ **Password** — enter and confirm a password for the user.

    ■ (Optional) To prevent the Windows system administrator from knowing the user's password, you can select the User must change password at next login checkbox. In this case, the user must log on to Windows and change the password before using Image Lab Security Edition. Otherwise, Security Edition will not recognize the user.

To create a new group on a local computer

1. In the Computer Management window, open the Groups folder and select Action > New Group.

    The New Group dialog box appears.

2. In the Group name box, enter the name of one of the following default groups: TDS_Administrator, TDS_User, TDS_Tech, TDS_Guest.

3. (Optional) Enter a description in the Description box.

    The group does not need special operating-system level privileges.

4. Click Create to save the new group.
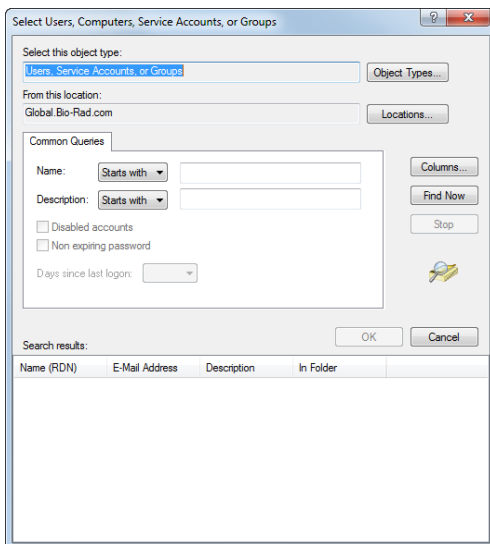
5. Repeat steps 2–4 to create the remaining groups.

To add a user to a group on a local computer

1. Do one of the following:

    ■   In the New Group dialog box, click Add.

    ■   In the Groups folder, double-click an existing group. In the Properties dialog box that appears, and click Add.
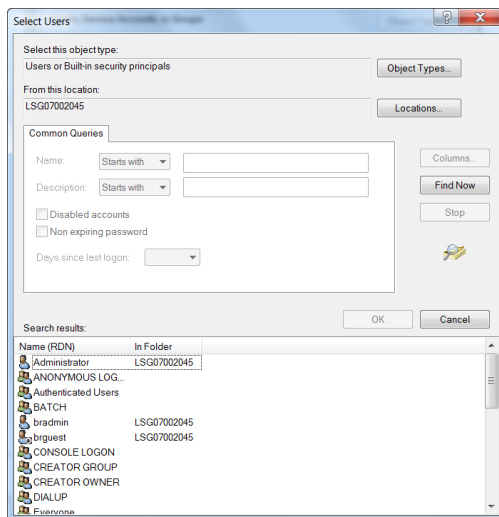
    The Select Users dialog box appears.
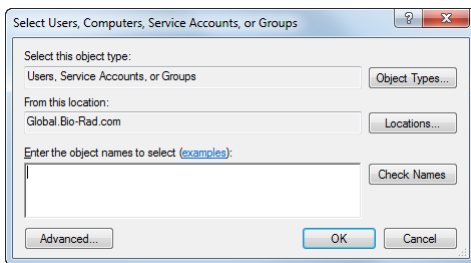
2. Click Advanced. The Search results box becomes visible.

3. Click Find Now to populate the Search results box with the names of all local computer users.

4. To add users to the group, do one of the following:

    ■ Select a user name.

    ■ Select multiple user names: hold down the Ctrl key and select the user names you want.

5. When you have selected all the users to add to the group, do one of the following:

    ■ To save the users to an existing group click OK and then click OK again to close the Select Users dialog box.

    ■ To create the new group and save the users to it, click Create to close the New Group dialog box.

Configuring Users and Groups on a Network Domain

Note: The network administrator must know how users and groups are set up using the Windows server software at your site. The following example is used to illustrate the choices.

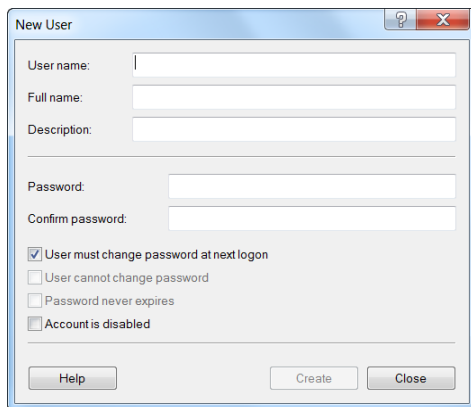**To locate the users and groups on a Windows server**

▶ Open Administrative Tools and select Active Directory.

Note that in the Active Directory window, the Users folder lists groups as well.

To create a new user on a Windows server

1. With the Users folder open, select Action > New User. Alternatively, use the right-click context menu.

   The New User dialog box appears.

2. Fill in all the boxes:

■ **User name** — must be unique.

■ **Full name** — must be unique.

   Bio-Rad recommends using the user's actual full name, as this name will be shown in the document log and all the log reports. This is a requirement of 21 CFR 11.50a.

■ **Description** — required. Bio-Rad recommends entering the user's title as the description.

■ **Password** — Enter and confirm a password for the user.

■ (Optional) To prevent the Windows system administrator from knowing the user's password, you can select the User must change password at next login checkbox. In this case, the user must log

on to Windows and change the password before using Image Lab Security Edition. Otherwise, Security Edition will not recognize the user.

To create a new group on a Windows server

1. With the Users folder open, select Action > New Group.
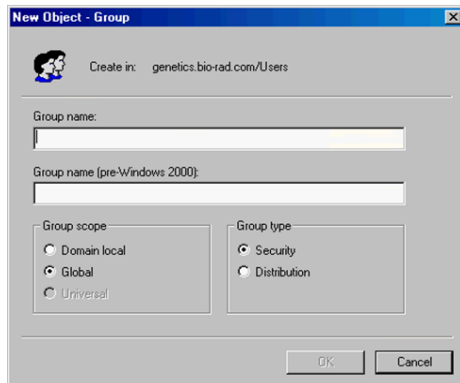
2. In the Group name box, enter one of the following group names: TDS_Administrator, TDS_User, TDS_Tech, TDS_Guest.

    Type the name exactly as specified. You can also enter a description for the group in the Description box.

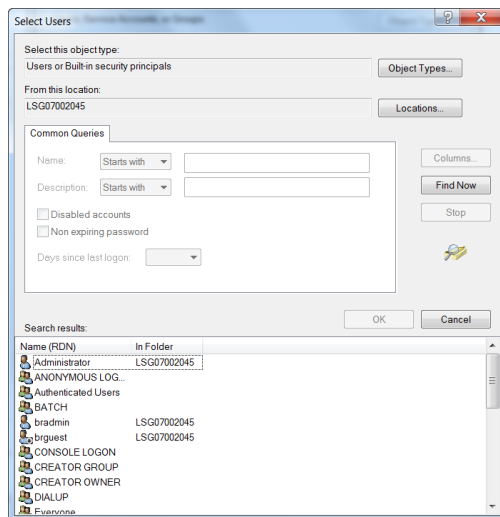   **Note:** The group does not need special operating-system level privileges.

To add a user to a group on a Windows server

1. Do one of the following:

    ■ In the New Group dialog box, click Add.

    ■ Double-click an existing group in the User Manager folder to open its Properties dialog box and click Add.

    The Select Users dialog box appears.

2. Click Advanced to expand the dialog box.

3. Click Find Now to populate Search results with all user names.

4. Click a user name in the list to select it, or press the Ctrl key and click multiple users to select them.

5. When you have selected all the users to add to the group, do one of the following:

   ■ To save the users to an existing group click OK and then click OK again to close the Select Users dialog box.

   ■ To create the new group and save the users to it, click Create to close the New Group dialog box.

Password Security

21 CFR 11.300 (b) requires that passwords be "periodically checked, recalled, or revised." Password policies are therefore recommended, although the password duration and rules are up to the system administrator and the organization. For instance, the exact duration between password changes is flexible.
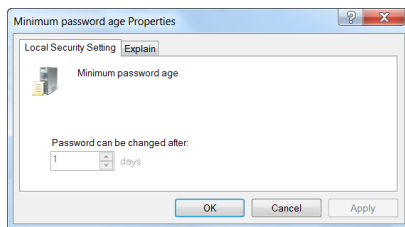
To set password policies on a local computer

1. Open the Control Panel and select Administrative Tools > Local Security Policy.

2. In the left pane of the Local Security Policy window, expand Account Policies and then select Password Policy.

   Password policies appear in the right pane.
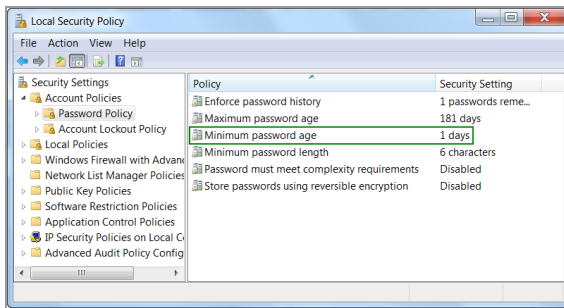
3. To change password policy settings:

   a. Right-click the policy and select Properties to open its properties dialog box.

   b. Modify the default setting to meet your company policy.

   c. Click Apply.

   d. Click OK to close the Properties dialog box.

4. Close the Local Security Policy window.

To set password policies in Active Directory

1. Open the Control Panel and select Administrative Tools > Domain Controller Security Policy.

2. In the left pane of the Domain Controller Security Policy window, expand Security Settings > Account Policies and select Password Policy.

3. To change password policy settings:

   a. Right-click the policy and select Properties to open its properties dialog box.

   b. Modify the default setting to meet your company policy.

   c. Click Apply.

   d. Click OK to close the Properties dialog box.

4. Close the Domain Controller Security Policy window.

Password Policy Setting Examples

The following examples are only suggestions. Your organization should establish its own password policy.

- Enforce password history: 12 passwords remembered

- Minimum password age: 5 days

- Maximum password age: 30 days

- Minimum password length: 8 characters

- Password must meet complexity requirements: Enabled

- Store passwords using reverse encryption: Enabled

Account Lockout Policy Setting Examples

- Account lockout duration: 0 (The account is locked out until the administrator unlocks it.)

- Account lockout threshold: 3 login attempts

- Reset account lockout counter after: 30 minutes

Auditing Windows Event Logs

Some global auditing information is stored in the Windows Event logs. It is a requirement of 21 CFR Part 11 that these logs be archived. However, by default, Windows systems automatically remove these data without warning.

Note: It is therefore critical that the event log is reconfigured to generate and preserve all necessary log data. Regular manual intervention is also required to preserve these data.

To open the Event Properties Log

1. Open Administrative Tools and click Event Viewer.

2. Right-click on each log and select Properties.

3. In the section When maximum event log size is reached: select Do not overwrite events (Clear logs manually).

4. Increase the maximum size of the event log to cover any possible messages. The smaller the maximum size of the event log, the more often the system administrator must manually view, archive, and clear the system log.

Auditing information generated by the operating system is recorded in the Security Log. Logon failures in Image Lab Security Edition are recorded in this log.

During the review process, the log should be examined for attempted breaches of security, such as a series of failed login attempts. To avoid the risk of losing data, the size should be very large and this inspection/archive process should occur daily. The Audit Policy should be set as follows:

- Audit account login events — Failure should be checked at a minimum

- Audit account management — both Success and Failure should be checked

- Audit login events — Failure should be checked at a minimum

- Audit policy change — both Success and Failure should be checked

Other Security Measures

Bio-Rad recommends that you use the built-in protections that Windows Server offers in order to protect the computer while the user is absent.

Note: Microsoft continually updates its operating systems in response to security issues. It is critical to keep all components of the Windows operating system, especially domain controllers, up to date.

Appendix B Setting Up Users and Groups

Appendix C Troubleshooting

Follow these suggestions to troubleshoot your system.

Troubleshooting tips

Problem	Possible Cause	Solution
Camera does not respond or camera not found	■ Power to the camera may be turned off. ■ The camera cables may not be seated properly. ■ The software driver for the camera is missing. ■ Computer power-saving modes may be interfering with the camera driver. ■ The cables may be defective. ■ The camera may be defective.	■ Turn on the power to the camera. ■ Make sure that all cables are connected as shown in the Installation Guide. ■ If the camera driver is not present, reload the camera driver from the Image Lab software installer. ■ Disable the power-saving modes on the computer. ■ Replace the cables. ■ Replace the camera.
Image is not visible on the monitor	■ The monitor settings are incorrect. ■ The lens cap is attached.	■ See your computer manual for the proper settings. ■ Remove the lens cap.
Printout does not look like the monitor image	■ The monitor settings are wrong. ■ The printer settings are wrong.	■ See your monitor manual for the appropriate settings. ■ See your printer manual for the appropriate settings.
Unable to focus on the sample using white light transilluminator or conversion screen	■ Focus is not calibrated for samples using this light source.	■ Select Edit > Instrument Setup to recalibrate the focus for use with this accessory.

Appendix C Troubleshooting

Appendix D Accessories

The following table lists catalog numbers and descriptions for available standards. For more information, see the Bio-Rad Life Science Research Product Catalog.

Table 9.

Catalog ID	Description
Protein Standards	
161-0363	Precision Plus Protein Unstained Standards. 1 ml
161-0373	Precision Plus Protein All Blue Standards, 500 μl
161-0374	Precision Plus Protein Dual Color Standards, 500μl
161-0375	Precision Plus Protein Kaleidoscope Standards, 500 μl
161-0385	Precision Plus Protein WesternC pack, 50 applications
161-0396	Precision Plus Protein Unstained Standards (5 pack)
161-0393	Precision Plus Protein All Blue Standards (5 pack)
161-0394	Precision Plus Protein Dual Color Standards (5 pack)
161-0377	Precision Plus Protein Dual Xtra Standards (single vial = 500 μl)
161-0397	Precision Plus Protein Dual Xtra Standards (5 pack)
161-0395	Precision Plus Protein Kaleidoscope Standards (5 pack)
161-0398	Precision Plus Protein WesternC pack (5 pack)
Nucleic Acid Standards	
170-8351	EZ Load 20 base pairs molecular ruler
170-8352	EZ Load 100 base pairs molecular ruler
170-8353	EZ Load 100 base pairs PCR molecular ruler

Catalog ID	Description
170-8354	EZ Load 500 base pairs molecular ruler
170-8355	EZ Load 1 kb molecular ruler
170-8205	2.5 kb molecular ruler
170-8200	AmpliSize® molecular ruler

Pulsed Field Standards and Markers

Catalog ID	Description
170-3624	CHEF DNA size standard, 5 kb ladder
170-3707	CHEF DNA size standard, 8 - 48 kb
170-3635	CHEF DNA size standard, lambda ladder
170-3605	CHEF DNA size marker, 0.2 - 2.2 Mb
170-3667	CHEF DNA size marker, 1 -3.1 Mb

Appendix E Mitsubishi P95 Thermal Printer

This appendix describes how to set up a thermal printer in both Windows and Mac environments.
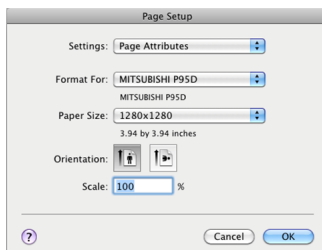
Setting up a Thermal Printer on a Mac

The printer driver can be found on the Image Lab software installation USB drive in the Misc directory.

To set up a thermal printer on a Mac system

1. Install the printer driver.

2. Connect the printer to the computer.

3. Restart the computer.

To configure the correct paper size

1. Start Image Lab software.

2. Select File > Page Setup.

3. In the Settings list, select Page Attributes.

4. In the Format For list, select the Mitsubishi printer.

5. In the Paper Size list, select 1280 x 1280.

6. In the Settings list, select Save as Default.

7. Click OK to save the settings.

For assistance with the printer driver or additional information about the printer, contact http://www.mitsubishielectric-printing.com.

Setting up a Thermal Printer on Windows

The printer driver is on the Image Lab software installation USB drive in the Misc folder.

To set up a thermal printer on a Windows system

1. Install the printer driver.

2. Open the printer section in Control Panel.

3. Click the thermal printer icon and select Printing Preferences.
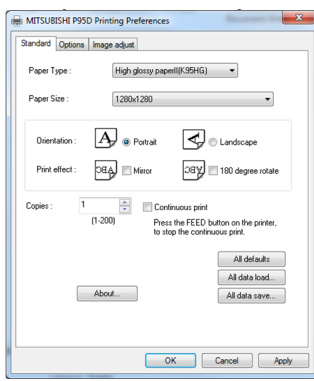
4. Configure the correct paper size. Select 1280 x 1280 from the dropdown list.

5. Click OK to apply your changes.

Glossary

Aspect ratio	The ratio of the width to the height of an image.
Color maps	Different color representations of a gel image.
Example precision	The number of decimal places chosen for displaying a measurement.
Histogram	A graphed representation of the brightness, or gray value, of an image.
Imager	The instrument without Image Lab software.
Imaging system	The instrument connected to a computer running Image Lab software.
Multichannel image	An image consisting of two or three channel images.
Native charge density	The inherent electrical charge of a protein without the addition of SDS.
Rf	Relative front value of the band. In Image Lab software, Rf has a value between 0 and 1 and indicates the relative movement of the band from top to bottom.

Glossary

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