ChemiDoc™ MP System with Image Lab™ Software

Instruction Manual

Catalog # 170-8280

BIO-RAD

CREDITS

1. Image Lab software is based in part on the work of the Qwt project (http://qwt.sf.net).
2. Image Lab software is based in part on the work of the CImg project (http://cimg.sourceforge.net/).
   See license for details at
   http://www.cecill.info/licences/Licence_CeCILL-C_V1-en.html
3. Image Lab software is based in part on the work of the Independent JPEG Group (http://www.ijg.org/)

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Safety and Regulatory Compliance

Important Safety Information

Please read these instructions before attempting to operate the ChemiDoc™ MP imaging system.

This instrument is suitable for research use only. It must be used, therefore, only by specialized personnel who know the health risks associated with the reagents that are normally used with this instrument.

Use of the ChemiDoc MP system involves UV illumination. Proper precautions must be taken to avoid eye and skin exposure to the UV radiation. This instrument is meant for use only by trained personnel who know the health risks associated with UV radiation normally used with this instrument. The acrylic shield provides some UV protection. However, it does not guarantee complete protection, and it is designed to shield only the person working in front of the system.

> **WARNING!** Use of the acrylic screen does not guarantee the user protection from UV radiation. The use of protective eyeglasses, mask, and/or gloves is strongly recommended.

Warranty

The ChemiDoc MP imaging system is warranted against defects in materials and workmanship for one year. If any defect occurs in the instrument during this warranty period, Bio-Rad Laboratories, Inc. will repair or replace the defective parts at its discretion without charge. The following defects, however, are specifically excluded:

- Defects caused by improper operation
- Repair or modification done by anyone other than Bio-Rad Laboratories, Inc. or the company's authorized agent
- Use of spare parts supplied by anyone other than Bio-Rad Laboratories, Inc.
- Damage caused by accident or misuse
- Damage caused by disaster
- Corrosion caused by improper solvents or samples

General Precautions

- Please read the instruction manual carefully.
- The instrument must be used only for the intended purpose of gel documentation in research laboratories.
- The instrument must be connected to a grounded power source line and protected by a circuit breaker.
- Do not pour liquids directly on or inside the instrument.
- Switch off all the lights immediately after use.
- Clean the transilluminator platen after use.

Regulatory Notices

The ChemiDoc MP imaging system is designed and certified to meet EN61010, the internationally accepted electrical safety standards, EMC regulations, and TUV requirements. Certified products are safe to use when operated in accordance with this system instruction manual. This instrument should not be modified or altered in any way. Modification or alteration of this instrument will:

- Void the manufacturer's warranty
- Void the regulatory certifications
- Create a potential safety hazard

WARNING! Bio-Rad Laboratories, Inc. is not responsible for any injury or damage caused by use of this instrument for purposes other than those for which it is intended or by modifications of the instrument not performed by Bio-Rad Laboratories, Inc., or an authorized agent.

Power Safety Information

Voltage Setting Information

The universal hood of the ChemiDoc MP imaging system has a power supply that automatically chooses the correct voltage for your country or region.

FUSES

The universal hood of the ChemiDoc MP imaging system has two user-serviceable fuses, F1 and F2, which are located on the bottom rear panel and are a part of the power entry module. Refer to the Maintenance chapter for fuse replacement instructions.

Contents

1 Introduction

The ChemiDoc™ MP imaging system offers exceptional application flexibility, high performance, and ease of use. The system contains a charge-coupled device (CCD) camera to capture images in real time and enable you to accurately position your sample and generate optimized image data.

The ChemiDoc MP system uses a new generation lighttight enclosure (the universal hood III), which contains built-in UV and white light illumination as well as available red, green, and blue Epi LED light sources. The system features dynamic flat fielding technology for superior image uniformity and accurate quantification.

Bio-Rad Image Lab™ software controls image capture and optimization for your selected applications, analyzes results, and produces reports based on your specified output, all in a single workflow.

ChemiDoc MP Imaging System

The ChemiDoc MP system is a high-resolution gel documentation system that allows fast, easy quantification of gels and blots. Position your sample inside the imager and follow the onscreen steps to run a protocol with only one click. You can customize your applications within an existing protocol or create a new protocol using the many options presented in Image Lab software.

The ChemiDoc MP imaging system also offers sensitive chemiluminescent detection. The system includes a supersensitive 16-bit CCD camera that is deeply cooled for faint-sample detection and for accurate quantification of image data.

Features include:

- Smart, application-based protocol setup using Image Lab software, which assists by presenting appropriate filter and illumination sources for imaging applications that require excellent sensitivity
- Exceptional sensitivity and a dynamic range greater than four orders of magnitude
- Flexibility to image chemiluminescent, fluorescent, and colorimetric samples with dynamic flat fielding specific to each application

System Components

CCD Camera and Lenses

The ChemiDoc MP camera is placed on top of a lighttight enclosure (the universal hood) for capturing images. The camera comes with a motorized zoom lens (MZL) that allows remote adjustment of the lens control functions (zoom, focus, and iris).

A patent-pending software algorithm controls the MZL, giving the user automatic image focus once an initial calibration is performed during system installation. See the Technical Specifications table for complete specifications of each system.

A +1 diopter lens is factory installed to allow the entire sample stage to be visible. This lens should always remain on the MZL assembly.

Universal Hood III

The universal hood III is designed to capture fluorescent and chemiluminescent images without using a photographic darkroom. The enclosure has built-in white light epi-illumination and UV transillumination. For easy sample loading, the UV transilluminator is located in the drawer of the universal hood and can be accessed from the front of the enclosure. When not imaging, the lights in the darkroom enclosure turn off automatically.

The universal hood III has touchpad buttons to perform various functions; however, Image Lab software controls all of these functions remotely, removing any requirement for manual control of the lens and lights. Running a protocol overrides touchpad input.

Image Lab Software

The system ships with a full version of Image Lab software. In addition to controlling the imager system, image capture, and optimization, Image Lab software can be used to annotate and document images, analyze molecular weights (or base pairs, when imaging nucleic acid gels), and determine accurate quantification and purity of samples.

You can print all or a subset of your data in a report. Alternatively, you can export your data to other software, such as Microsoft Office programs, for further analysis or presentation options. See Chapters 5 to 9 for detailed instructions on the software.

Emission Filters

The universal hood III can hold up to six different emission filters for fluorescent applications. No filter is required to image chemiluminescent samples.

A standard filter is used for colorimetric (white light) applications and is included in the installation kit.

Optional Accessories

Bio-Rad Laboratories, Inc. offers a selection of optional filters and illumination sources. See Chapter C, Accessories on page 135 for ordering information and a complete listing of accessory filters, UV light sources, optional parts, and replacement parts.

Printer

For your convenience, Bio-Rad offers an optional USB printer for use with the ChemiDoc MP system: the Mitsubishi thermal printer (catalog #170-8089).

Conversion Screens

White Light Conversion Screen

The white light conversion screen is a phosphor screen that produces white light transillumination when placed on top of the UV transilluminator.

XcitaBlue Conversion Screen

The optional XcitaBlue™ screen kit (catalog # 170-8182) converts UV to blue light, which enables you to visualize DNA samples while protecting them from UV damage.

Optional Light Sources

Red LED Module

The optional red LED module kit contains the emission filter and excitation source for fluorescent applications. Instructions are also included.

Green LED Module

The optional green LED module kit contains the emission filter and excitation source for fluorescent applications. Instructions are also included.

Blue LED Module

The optional blue LED module kit contains the emission filter and excitation source for fluorescent applications. Instructions are also included.

System Requirements

Image Lab software runs under Windows XP Professional, Windows 7, and Mac OS X. Images scanned at high resolution can be quite large. The amount of memory required for using the program is determined mainly by the size of the images you scan and analyze.

For this reason, we recommend that you archive images on a network file server or on removable storage media. Bio-Rad can also provide an appropriate computer to use with this system. Contact your local Bio-Rad representative for more details.

Computer Specifications

Specifications	Minimum	Recommended
Operating system	Windows XP SP3 Windows 7, 32- and 64-bit Mac OS X 10.6	Windows XP SP3 Professional Windows 7 Professional, 64-bit Mac OS X 10.6
Processor	Pentium 4 or equivalent (Windows) at 2.0 GHz	Intel Core 2 Duo 2.0 GHz or higher
Hard disk space	20 GB	>100 GB
Memory (RAM)	1,024 MB	>1,024 MB
Ports for connecting instrument	1 free USB 2.0 port	1 free USB 2.0 port
Other software (optional)	Microsoft Excel 2000 or later (Windows)	Microsoft Excel 2000 or later (Windows)
	Office 2008 or iWork software (Mac)	Office 2008 or iWork software (Mac)

ChemiDoc MP Applications

The ChemiDoc MP system is capable of running protocols to image all the detection reagents from a variety of samples, either single-plex or multiplex applications, plus the others added to this list on the next page. Those listed are examples of the detection reagents that may be used to image your samples and not a complete list. Contact Bio-Rad technical support to determine whether your application can be imaged on this instrument.

See Chapter 4, Acquiring Images, for detailed instructions on designing protocols.

Nucleic Acid Gels

- Ethidium bromide
- SYBR® Green
- SYBR® Safe
- SYBR® Gold
- PicoGreen
- GelGreen
- GelRed
- Fluorescein
- OliGreen
- GelStar

Protein Gels

- Stain-Free Gel
- Oriole™ fluorescent gel stain
- Flamingo™ fluorescent gel stain
- SYPRO Ruby
- Krypton
- Coomassie Fluor Orange
- Pro-Q Diamond
- Pro-Q Emerald 300
- Pro-Q Emerald 488

Blots

- Chemi
- Chemi Hi Sensitivity
- Chemi Hi Resolution
- Stain-Free Blot
- Colorimetric
- Cy2
- Cy3
- Cy5
- Cy5.5
- Alexa 488
- Alexa 546
- Alexa 647

- Alexa 680
- Dylight 488
- Dylight 549
- Dylight 649
- Dylight 680
- IRDye 680
- Rhodamine
- Fluorescein
- Qdots 525
- Qdots 605
- Qdots 625
- Qdots 705

ChemiDoc MP Technical Specifications

Applications	
Chemiluminescence	Yes
Fluorescence*	Yes
Colorimetry/densitometry	Yes
Gel documentation	Yes
Hardware Specifications	
Maximum sample size (L x W in cm)	28 x 36
Maximum image area (L x W in cm)	26 x 35
Maximum image area for standard UV-excited gels (L x W in cm)	25 x 26
Excitation source	Trans-UV and Epi-white are standard (302 nm included, with 254 and 365 nm available as options). Optional trans-white conversion screen. Optional XcitaBlue™ UV/blue conversion screen. Blue, green, and red Epis.
Detector	Supercooled CCD
Pixel size (H x V in microns)	6.45 x 6.45
Cooling system	Peltier cooled
Camera cooling temperature	–30°C controlled
Filter selector	6-position filter wheel, 1 without filter for chemiluminescence
Emission filters	1 included (standard), 3 optional (530, 605, 695)
Dynamic range	>4.0 orders of magnitude
Pixel density (gray levels)	65,535
Dynamic flat fielding	Application-specific, for all applications
Instrument size (cm)	36 (L) x 60 (W) x 96 (H)
Instrument weight (kg)	32
Operating Ranges	
Operating voltage	110/115/230 V AC nominal
Operating temperature	10–28°C (21°C recommended)
Operating humidity	<70% noncondensing
Automation Capabilities	
Workflow automated selection	Application driven, user selected or recalled by a protocol
Workflow automated execution	Controlled by a protocol via application-specific setup for image area, illumination source, filter, analysis, focus, and reporting
Workflow reproducibility	100% repeatability via recallable protocols; from image capture to quantitative analysis and reports
Autofocus (patent pending)	Precalibrated focus for any zoom setting

Image flat fielding (patent pending)	Dynamic; precalibrated and optimized per application
Autoexposure	2 user-defined modes (intense or faint bands)

*Using the optional XcitaBlue kit (catalog # 170-8182) is highly recommended if performing preparative DNA applications with blue excitable stains. The UV to blue conversion screen allows you to visualize DNA samples while protecting against UV damage.

ChemiDoc MP Imager Workflow

Following are the basic steps to acquiring, analyzing, and archiving an image using the ChemiDoc MP system and Image Lab software:

1. Select an existing protocol or customize a new one.
2. Position the sample to be imaged.
3. Run your selected protocol.
4. View the displayed results.
5. Optimize the analysis.
6. Generate a report.
7. Save or export the results.

When running chemiluminescent blots, you can use a signal accumulation mode, which takes a series of exposures within a period of time. This enables you to choose an image with optimal signal. Refer to Chapter 4, Acquiring Images, for instructions.

For More Information

Refer to the ChemiDoc MP Installation Guide found in your ChemiDoc MP installation kit for instructions on installing Image Lab software and assembling and calibrating the ChemiDoc MP system.

To recalibrate your system because you have acquired new accessories, refer to Chapter 2, System Calibration.

2 System Calibration

When your system is installed, system calibration is performed with a calibration wizard. See the Installation Guide in your installation kit for detailed instructions.

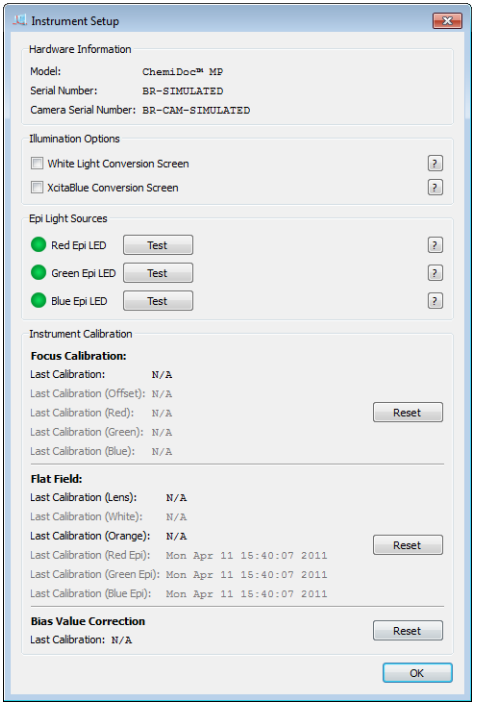
The instrument calibration wizard performs several procedures required to automate the system and prevent focus problems. Each of these calibrations affects your system as follows:

- **Focus Calibration** — this calibration allows automated focus settings at any zoom point, using a (patent pending) software algorithm. Therefore, your focus remains correct whether you view an entire sample or an area of interest.
- **Focus Calibration with Height Offset** — this calibration takes the tallest of the available conversion screens into account, and extrapolates values for the others, so that focus remains optimal, whichever screen is used.
- **Dark Reference Image** (ChemiDoc™ MP only) — this calibration determines and corrects any background signal present in your imager.
- **UV Flat Field Calibration** — this calibration generates the flat field correction profiles needed for the UV light source. Because of this calibration, your images have backgrounds of even intensity.
- **Lens Flat Field Calibration** — this calibration corrects for the intensity roll-off inherent in any lens.
- **White Conversion Screen Calibration** — this calibration generates a flat field correction profile needed for the white light conversion screen.

The Instrument Setup dialog box available from the Edit menu displays these calibration results for reference. The calibration wizard needs to be run only once, when your system is installed. The calibrations remain valid over the life of the instrument.

The calibration need not be changed unless you add equipment, such as a new light source.

When you add light sources or filters to a ChemiDoc MP system, recalibrate your system using the dialog box found at Edit > Instrument Setup.



1. If you have a new illumination source, select the appropriate box in the Illumination Options field.
2. If you are adding new filters, use the dropdown list to match what is installed in your instrument.
3. The software prompts you to reset the calibrations needed for the new illumination sources.

4. Click OK to exit the dialog box. Your settings persist until you make further changes.

3 Image Lab™ Software Overview

Image Lab image acquisition and analysis software works with the ChemiDoc™ MP imaging system to create a reproducible, automated, and time-saving workflow for imaging and analyzing gels.

In Image Lab software, a protocol is any combination of imaging, analysis, and report settings that has been saved to run as a single workflow. Researchers can run one protocol repeatedly or easily design a wide range of protocols.

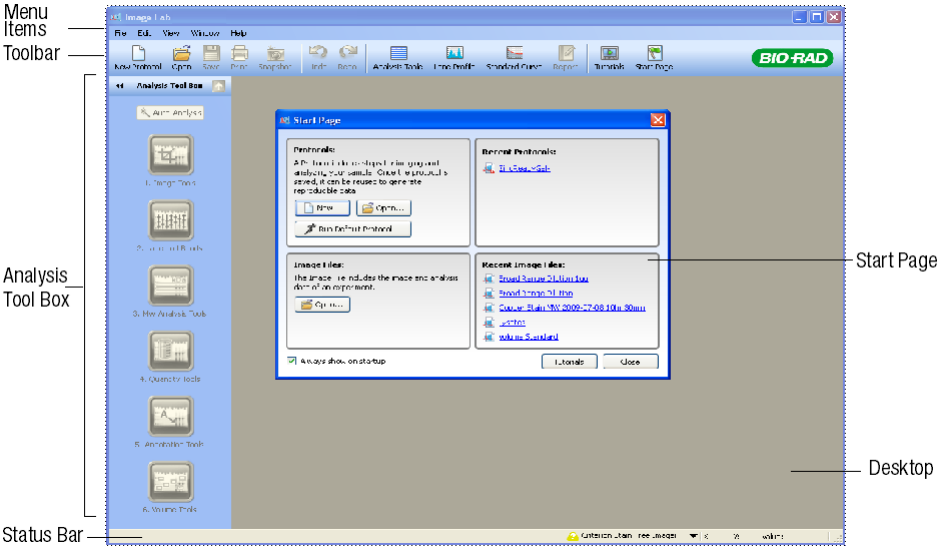
With Image Lab software you can view analyzed data, edit the analysis, and produce customized reports that show precisely the settings applied in order to ensure repeatable results.

Two types of files are generated by Image Lab software:

- Image files, which contain the imaged gel, annotations, and analysis performed on the gel.
- Image files are saved with an .scn extension. An imaged gel, run according to a protocol file, generates an image file. Multichannel image files are saved with an .mscn extension.

Interface Overview

The following illustration shows the Image Lab software main window. The paragraphs below describe the main software elements.



Main Window

Image Lab software displays a single main window. All image and protocol dialog boxes that present choices open in the workspace, which is the gray area of the main window.

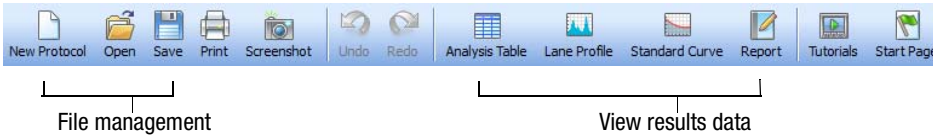
If many screens are open in the workspace, you can make one visible by clicking the title bar at the top of the selected screen. A list of open protocols and image files also appears in the Window menu; you can select one to bring it to the top.

You can view the complete analysis for an image or protocol at one time or compare image results by arranging the screens in the workspace.

Toolbar

Many Image Lab software tools can be selected by clicking toolbar icons. The Screenshot tool enables you to send a screen capture of your image to the clipboard or to save it as a file. You can view demonstrations of various functions by clicking Tutorials. The unlimited Undo and Redo buttons enable you to correct missteps easily.

Additional tools for viewing protocols and results data presented in various forms are labeled below. These tools are described in Chapter 5, Viewing Images.



Results Data

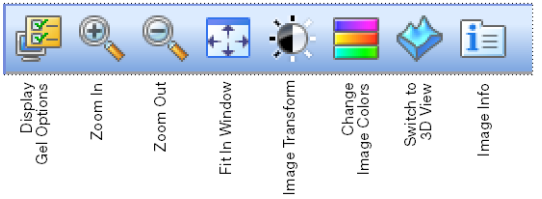
Results data associated with gel images can be viewed as an analysis table, a lane profile, a standard curve, or in a report. These views always display the analysis for the selected image.

Buttons to toggle these views on and off are located in the main toolbar, as shown. All of them can be viewed at once.

See Displaying Data on page 67 for details.

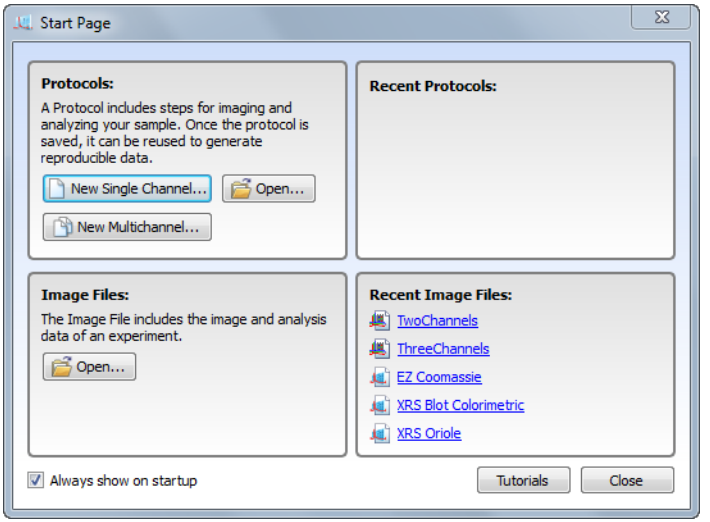
Display Toolbox

The display toolbox near the top of every image enables you to display images in the most useful ways. See Chapter 5,Viewing Images for a description of each option.

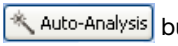


Start Page

The Start Page guides you through creating, opening, and viewing protocols and images.



Analysis Tool Box

The Auto Analysis 

button quickly analyzes images. The remaining tools customize the *analyzed* data.

> **Note:** An image file must be selected (on a Windows computer, the title bar is dark blue) to make analysis tools available.

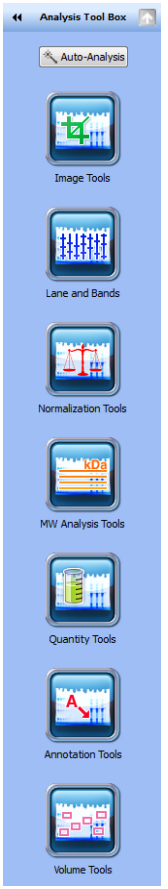


Image Tools enable you to flip, rotate, and crop images and to transform the image files.

Lane and Band Tools control the detection function, enabling you to resize, adjust, and bend lanes and to detect, adjust, add, or delete bands.

Normalization Tools enable you to normalize volume data in multichannel images, allowing you to correct for sampling loading errors in your gels.

MW (molecular weight) (or base pairs, if using nucleic acid gels) analysis calculation enables you to choose standard samples, assign standard lanes, and choose a regression method.

Quantity Tools enable you to automatically quantify bands, using either relative or absolute values.

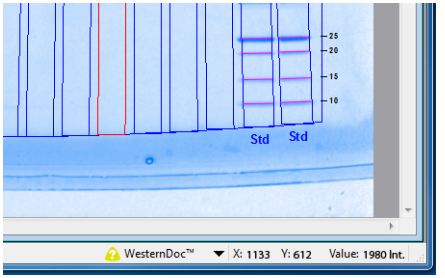
Annotation Tools are useful for drawing attention to any area of a gel.

Volume Tools enable you to manually quantify an object inside a boundary that you define.

These tools are described on page 77.

Status Bar

The status bar in the lower right-hand corner of the main window shows the imager in use and the X and Y values for the cursor position on an image file.



The Int (intensity) values show a numerical value for the image intensity at the mouse position. The maximum data range using a ChemiDoc MP is 0 to 65,535, but the range varies depending on the values contained within each image.

> **Note:** For multichannel images, hovering over a multichannel pane displays color coded intensities for all channels.

Menu Commands

The following 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

New Protocol enables you to create a new protocol that contains the necessary steps and choices. See Acquiring Images on page 25 for detailed instructions. Protocols can also be altered and stored for reuse.

Open browses the file system to retrieve a previously saved protocol file or image file.

Recent Images gives you the choice of opening a recent image file.

Recent Protocols gives you the choice of opening a recent protocol.

Save enables you to save a protocol or image file once the protocol or image is named.

Save As enables you to name and store a protocol or image. Protocols are stored with a .ptl file extension. Image files are stored with an .scn extension.

Multiplex protocols are stored with an .mptl file extension. Multiplex image files are stored with an .mscn extension.

Create Multichannel Image enables you to create a multichannel image from single-channel images and from single channels in other multichannel images.

Split Multichannel Image enables you to split the multichannel image into individual image files. Each file has the same name as the multichannel image; the application name is appended in parentheses.

Close closes the active screen.

Close All closes all the screens.

Export enables you to export gel images or analysis tables with the following options:

- **Export for Publication** — exports a displayed image to a file. You can select from .bmp, .png, .jpg, and TIFF formats. The gel displays with any lanes, bands, and annotations that appear on the screen. See Exporting Gel Images for Publication on page 120 for more information.
- **Export for Analysis** — creates a TIFF formatted file that retains all gel image data. Analysis data are not included. Use this option to analyze the image in other software such as Quantity One®,

FPQuest™, or InfoQuest™FP. See Exporting Gel Images for Analysis on page 122 for more information.

- **Export for PulseNet** — reduces the image to an 8-bit TIFF image file. Resolution is limited and file size is restricted to 300 dpi.
- **Lane and Band Table to Excel** — if Excel (or Numbers on a Mac) is installed on your computer, it will launch with your lane and band table displayed as a spreadsheet.
- **Lane and Band Table to File** — exports as a CSV (comma-separated values) file so your lane and band table can be opened in a database application.
- **Volume Table to Excel** — if Excel (or Numbers on a Mac) is installed on your computer, it will launch with your volume table displayed as a spreadsheet.
- **Volume Table to File** — exports as a CSV file so your volume table can be opened in a database application. See Exporting Results on page 119 for detailed information about exporting files.

Image Info displays information about individual gel and blot images such as acquisition date, data range, and image capture details including the exposure time and illumination source used, in three tabs (Image Details, Analysis Settings, and Notes). See Image Info on page 62 for more information. See Chapter 9 for more information about exporting files.

- **Page Setup** contains print controls such as orientation (landscape or portrait), margins, printer used, and paper size.
- **Print** displays a print preview of the gel and the header information, which includes the filename of the image, the user's name, and date and time it was printed. The usual Windows Print screen is available as well; it enables you to select a printer and the number of copies to print.
- **Exit** closes Image Lab software (after prompting you to save changes to your protocols or images).

Edit Menu Commands

Undo undoes the last action.

Redo restores the last action after an Undo.

Screenshot enables you to take a screen capture of the Lane Profile Window, the Standard Curve Window, or the default choice, Current Image View. This capture can include the name of the image, and it can be placed on the clipboard or saved in a file.

Default Imager enables users who own two or more imagers to switch between them.

Instrument Setup enables you to review the instrument serial number and how the imaging system is calibrated. If accessories are added, change the system calibration in the Instrument Setup dialog box.

Report Settings enables you to configure reports. This dialog box contains three tabs. All of the checkboxes are selected by default; clear the boxes to exclude information from reports. Your selections apply to all reports until you change them again.

- 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, or just some 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.

Preferences contains two tabs.

- The Protocol tab shows presets for naming image files. You can choose to include a designated Prefix, User Name, Date, and/or Time in the name of your image files.
- The Colors tab enables you to choose colors for the graphic elements in your gels, such as Lane Frame, Lane, Band, Band Attribute, and MW Legend. This functionality ensures that these elements are visible, whatever colors your gels are.

VIEW

Image Overview opens a small window that shows the entire 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 opens a window showing a histogram, which allows you to adjust the light and dark values of a gel image. This adjustment does not change your data; it changes only the way the data display on your monitor.

Operations History opens a window showing the sequence of actions performed by both the user and the software.

Window

The Window controls enable you to show and hide multiple open image files in your workspace. A list of all currently open images and protocols appears in this menu.

Tile aligns all open image files so all are visible at once.

Tile Horizontal places all open image files from top to bottom.

Tile Vertical places all open image files from left to right.

Cascade stacks all open image files and protocols with overlapping title bars, so each one can be easily chosen for view.

Imitate Zoom changes the zoom setting of all open images to the same zoom setting as the current image file.

Imitate Transform changes the brightness and contrast of all open images to the same transform settings as the current image file.

Next cycles through all open image files from oldest to newest.

Previous cycles through all open image files from newest to oldest.

Help

Image Lab Help displays the help system.

User Guide displays the instruction manual in .pdf form.

About displays Image Lab software version and release date.

4 Acquiring Images

Image Lab™ software runs specific applications with repeatable workflows using custom protocols that have a wide variety of settings. These protocols can be retrieved, revised, and reused.

In Image Lab, a protocol is any combination of settings for imaging, analyzing, and reporting that runs as a single workflow.

Image Lab supports two kinds of protocols: single-channel and multichannel. A single-channel protocol enables you to choose one application for acquisition of a single image from a sample, with the exception of signal accumulation mode for chemiluminescence. Multichannel protocols enable you to choose up to three different applications, resulting in sequential imaging of a sample. These images can be combined into an RGB (red, green, blue) color-composite image.

> **Note:** The Image Lab software is designed to work with different imagers, some of which work only with single-channel images. The New Protocol menu choices change depending on whether the imager supports multichannel images or not. If you do not see the multichannel option, check to see that your imager selection is set to ChemiDoc™ MP.

To access the Protocol Setup window

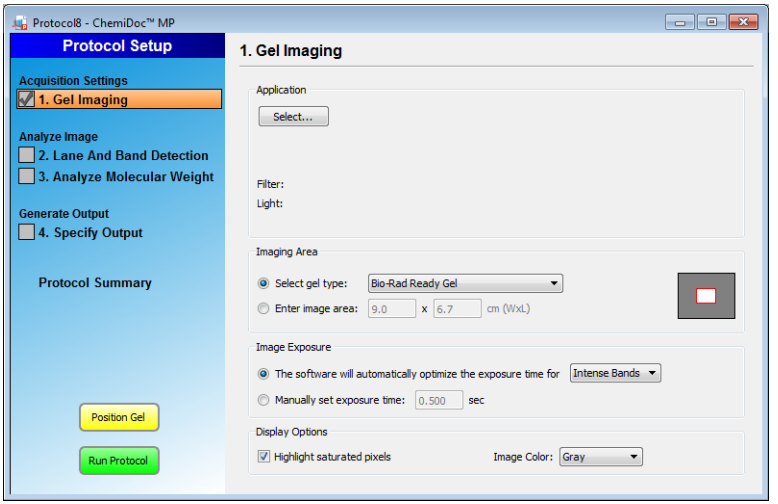
- Click the New Protocol button in the toolbar, and then select Single channel or Multichannel in the menu that appears.
- In the Protocols box on the Start Page, click New Single Channel or New Multichannel.

The appropriate Protocol Setup window appears for the type of protocol you selected.

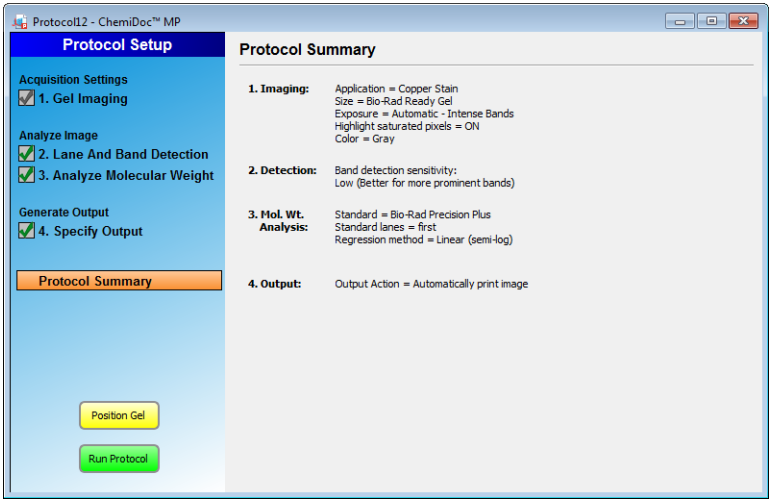
The Protocol Setup Window

Single-Channel Protocols

This is the Protocol Setup window for a single-channel protocol. The left pane displays headings. Under the headings are numbered protocol steps. You can enable or disable a step by selecting or clearing its checkbox. When you select a step, the right pane of the window displays the detailed settings for that step.

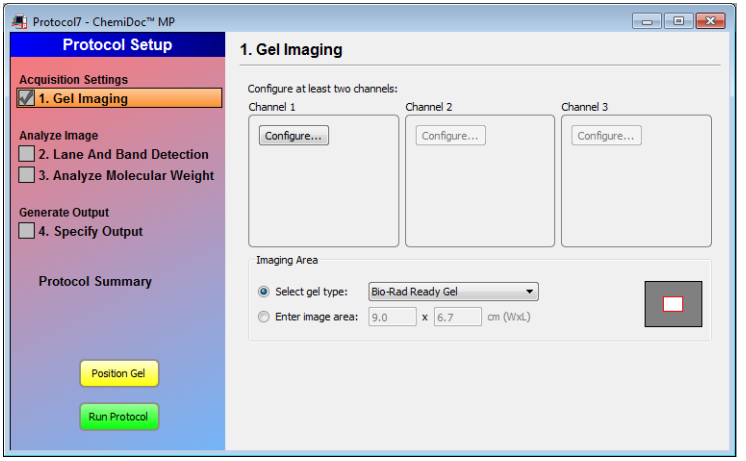


You can review protocol settings by selecting Protocol Summary, which lists the settings for each step in the right pane of the Protocol Setup window.


Protocol12 - ChemiDoc™ MP
Protocol Setup
Acquisition Settings
1. Gel Imaging
Analyze Image
2. Lane And Band Detection
3. Analyze Molecular Weight
Generate Output
4. Specify Output
Protocol Summary
Position Gel
Run Protocol
Protocol Summary
1. Imaging:
Application = Copper Stain
Size = Bio-Rad Ready Gel
Exposure = Automatic - Intense Bands
Highlight saturated pixels = ON
Color = Gray
2. Detection:
Band detection sensitivity:
Low (Better for more prominent bands)
3. Mol. Wt. Analysis:
Standard = Bio-Rad Precision Plus
Standard lanes = first
Regression method = Linear (semi-log)
4. Output:
Output Action = Automatically print image

Multichannel Protocols

The Protocol Setup window for multichannels has some settings that differ from the single-channel Protocol Setup window, but you work with it the same way as the single-channel Protocol Setup window.



Select a step in the left pane and configure the settings for that step in the right pane.

Click Protocol Summary in the left pane to view all your protocol settings in the right pane.

Creating a Protocol

There are three categories of settings when setting up a protocol:

- **Acquisition Settings** – settings to acquire the image
- **Analyze Image** – lane and band analysis and molecular weight analysis settings
- **Generate Output** – settings to generate the output

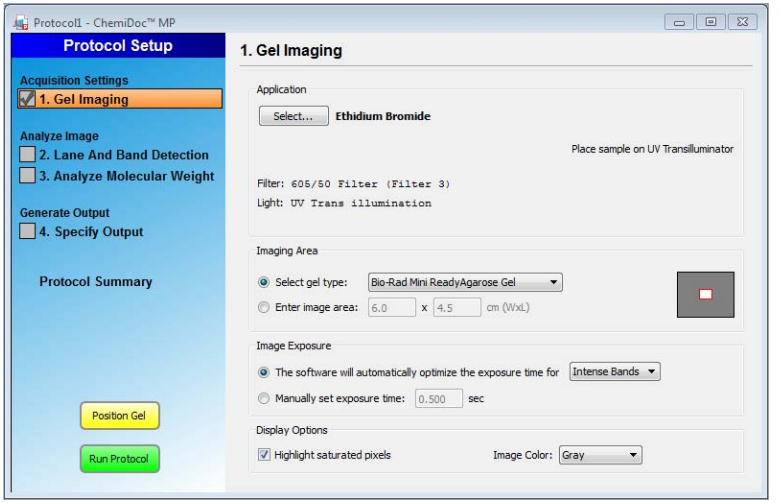
The three processes are listed in the left pane of the Protocol Setup window. Numbered steps in each process appear under these headings. To select an option under a protocol step, select the accompanying checkbox. Options for that step appear in the right pane of the window. To disable any step, clear its checkbox.

There are some differences in the gel imaging settings of single-channel protocols and multichannel protocols. (These settings are selected in "Step 1. Gel Imaging," in the Protocol Setup window.) The remaining steps in the protocol setup are virtually identical.

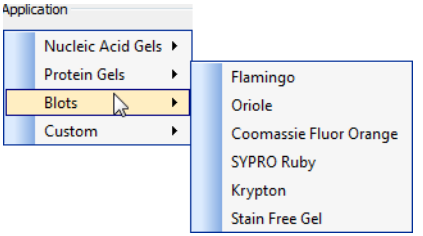
To start creating your protocol

- To create a single-channel protocol, start with "Step 1. Gel Imaging for Single-Channel Protocols" on page 30.
- To create a multichannel protocol, start with "Step 1. Gel Imaging for Multichannel Protocols" on page 34.

Step 1. Gel Imaging for Single-Channel Protocols



1. Click Select and choose an application from the menu. The applications appear in submenus under each application type. When you choose an application, any required filter or illumination source displays in the Protocol Setup window.

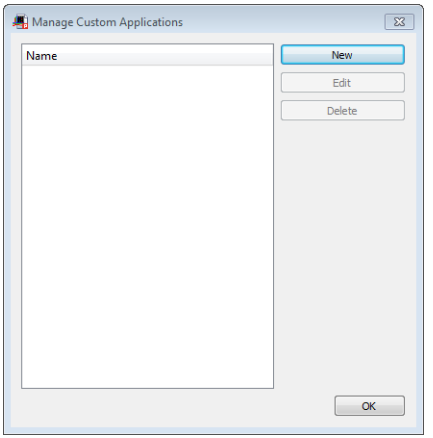


Note: For a list of applications with all required detection reagents, light sources, and any conversion screens or filters noted, see Application Tables on page 53.

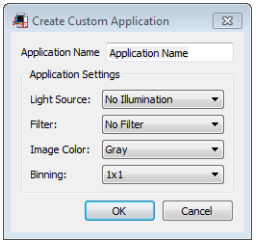
Note: If you select the Stain Free application you have the option of selecting the gel activation time. See Using the Criterion Stain Free™ System, for more information.

Choose the Custom application to run an existing application with a new name or an application unlike existing applications. Selecting Custom on the Application menu displays the Manage Custom Applications dialog box. If you previously saved custom applications, they display here.

To create a new custom application, click New in the Manage Custom Applications dialog box.

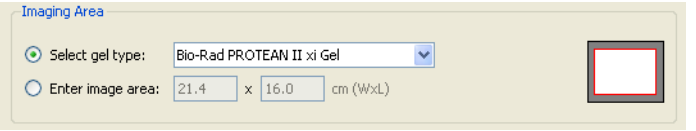


The Create Custom Application dialog box appears.

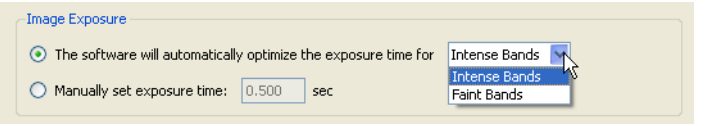


2. Choose a unique application name.
3. Select a light source, filter, and image color from the lists.
4. Select a binning setting. Choosing a higher binning setting combines pixels to increase the amount of signal without increasing noise. While a higher setting provides optimal sensitivity for low-light applications such as chemiluminescence, it also reduces image resolution.
5. Click OK to create your custom application.

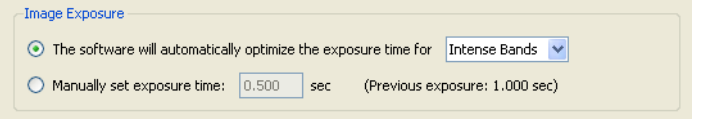
6. In Imaging Area, select from the list of Bio-Rad gels or enter image area dimensions. The red line represents the imaging area for the selected gel, and the gray rectangle represents the imager sample stage.



7. In Image Exposure, select from one of the following options:
   - **Auto Exposure** — this setting estimates an optimal exposure time and ensures the best use of the dynamic range. Intense Bands optimizes for all bands and Faint Bands makes faint bands more visible but may overexpose more prominent bands.
   - **Manual Exposure** — use this setting to manually override automated imaging. Exposure time can range from 0.001 to 7200 seconds with the ChemiDoc MP system.

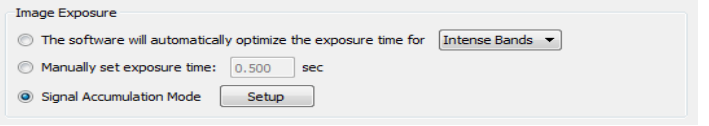


After imaging a gel with automatic exposure optimization, the display provides a reference point (see below) from which to set your manual exposure time.



Note: You can also view the exposure time of the image later, in the Image Info window (see Image Info on page 62).

Signal Accumulation Mode — if you are running a chemiluminescence application on the ChemiDoc MP system, you can also use signal accumulation mode (SAM).



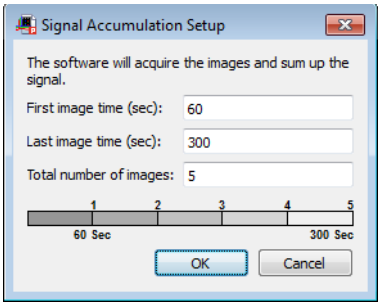
SAM allows you to get a cumulative sequence of exposures from a chemiluminescent sample to increase the likelihood that you will get the correct image.

Rather than manually acquiring a series of independent images with different imaging times, SAM presents a series of cumulative images with progressively greater signal in each image. SAM is a partially automated method for image acquisition because it requires that you estimate the shortest and longest times expected to generate an image with the appropriate signal intensity. You then decide how many total images to acquire in this time frame.

Note: For any given exposure time, you will get more background noise with a SAM acquisition than you would with a single image taken at the specified exposure time.

For example, if the minimum time that is expected to image the sample is 1 min and the maximum is 5 min, these values are entered (in sec) in the setup window. By entering 5 in the “Total number of images” field, three images will be acquired between the first and last images.

To use SAM, click Signal Accumulation Mode and select Setup to display the Signal Accumulation Setup dialog box.



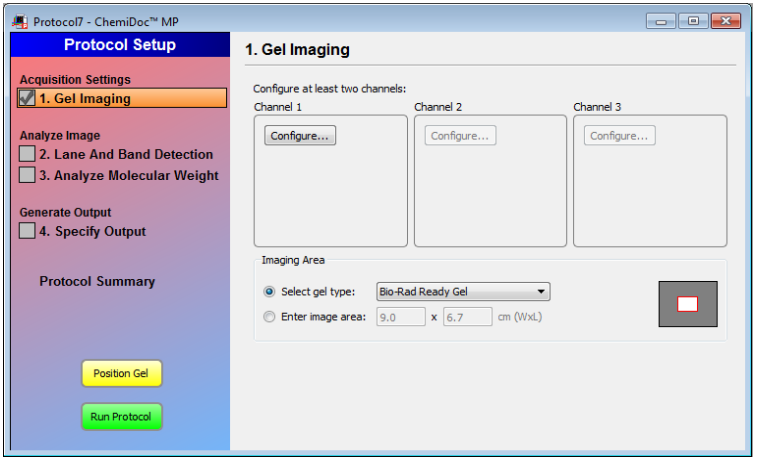
In this example, the bar in the Signal Accumulation Setup dialog box shows that images will be acquired at 1 min intervals, beginning at 1 min and ending at 5 min. The second 1 min image is added to the first 1 min image, resulting in a 2 min total integration time image. The third 1 min image is added to the previous image, and so on, until the last image is presented.

Although SAM is useful for determining the optimum imaging time for a chemiluminescent sample, it results in data that are not as accurate as data from a single image. Signal that is near the intensity of background noise becomes increasingly masked as the number of cumulative images increases. To identify extremely faint signals in an image, reacquire it as a single image, using the time the SAM tool found to be appropriate.

8. In Display Options, choose one of the following:
   - **Highlight saturated pixels** — select this checkbox to see any saturated pixels in red. This shows how much of the gel image is saturated. This option can be changed later by selecting View > Image Transform.
   - **Image color** — select color choice to display the sample image. Viewing the image with a different color scheme can make it easier to see all of the elements. See Image Colors on page 60 to view the color choices dialog box.

   Note: Continue with the next step in setting up the single-channel protocol by going to Step 2. Detect Lanes and Bands on page 39.

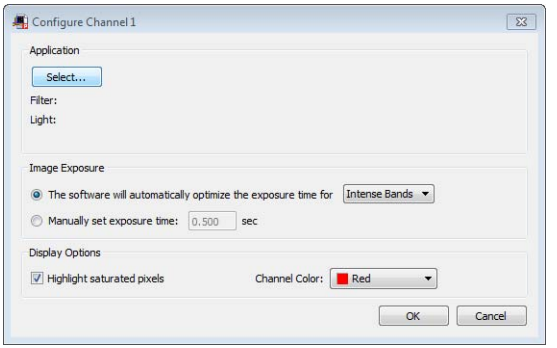
Step 1. Gel Imaging for Multichannel Protocols



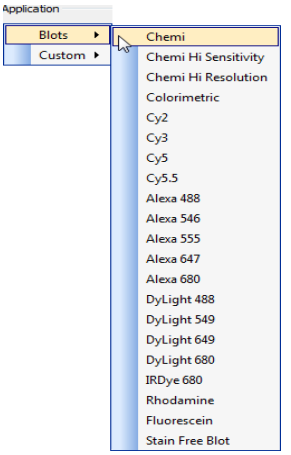
You must configure acquisition settings for at least two channels to create a valid multichannel protocol. Each channel can be assigned a color (red, green, or blue) so you can easily identify each channel. Configure each channel separately.

To configure the acquisition settings

1. In the right pane of the Protocol Setup window, click Configure in the Channel 1 box. The Configure Channel 1 dialog box appears.



2. Click Select and choose an application from the dropdown list that appears.

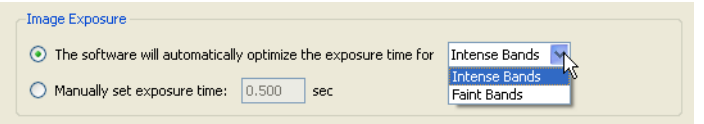


Note: Use the Custom application to run an existing application with a new name or an application unlike existing applications.

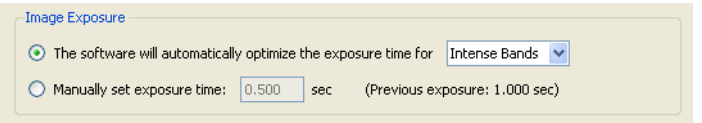
See Setting Up a Custom Application on page 52 for more information.

For a list of applications with all required detection reagents, light sources, and any conversion screens or filters noted, see Application Tables on page 53.

3. In Image Exposure, if you select the first radio button, Image Lab acquires an image estimating an optimal exposure time. This option ensures the best use of the dynamic range.
   - If you choose Intense Bands from the list, exposure is optimized for all bands.
   - If you choose Faint Bands from the list, a longer exposure makes faint bands more visible, but more prominent bands may be overexposed.
   - If you select the second radio button to manually override automated imaging, you can set the exposure time from 0.001 to 7200 seconds with the ChemiDoc MP system.

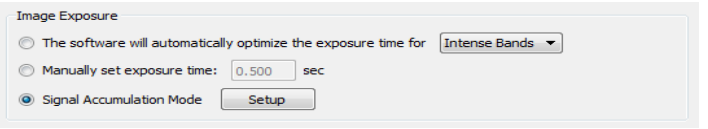


After imaging a gel with automatic exposure optimization, the exposure time displays in the protocol so you can manually adjust it if needed.



Note: You can also view the exposure time of the image later, in the Image Info window (see Image Info on page 62).

Signal Accumulation Mode — if you are running a chemiluminescence application on the ChemiDoc MP system, you can also use signal accumulation mode (SAM).

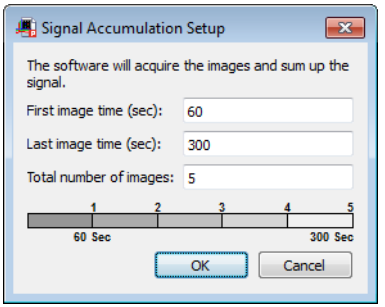


SAM is used to simplify capture of a good image from a chemiluminescent sample. This sample type often requires long integration times to obtain an image that represents the best range of signal.

Rather than manually acquiring a series of independent images with different imaging times, SAM presents a series of cumulative images with progressively greater signal in each image. SAM requires you to estimate the shortest and longest times expected to generate an image with the appropriate signal intensity. You then decide how many total images to acquire in this window of time.

For example, if the minimum time that is expected to image the sample is 1 min and the maximum is 5 min, these values are entered (in sec) in the setup window. By entering 5 in the “Total number of images” field, three images will be acquired between the first and last images.

To use SAM, click Signal Accumulation Mode and select Setup to display the Signal Accumulation Setup dialog box.



In this example, the bar in the Signal Accumulation Setup dialog box indicates that images will be acquired at 1 min intervals, starting at 1 min and ending at 5 min. The second 1 min image is added to the first 1 min image, and the image is the result of integrating these two images. The third 1 min image is added to the previous image, and so on, until the last image is presented.

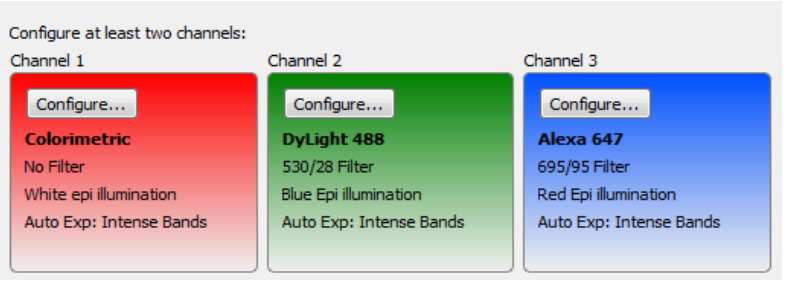
Although SAM is useful for determining the optimum imaging time for a chemiluminescent sample, it results in data that are not as accurate as data from a single image. Signal that is near the intensity of background noise becomes increasingly masked as the number of cumulative images grows. To identify extremely faint signals in an image, reacquire it as a single image, using the time the SAM tool found to be appropriate.

4. Set the following attributes in Display Options:

   Highlight saturated pixels — select the “Highlight saturated pixels” checkbox to see any saturated pixels in red. This shows how much of the gel image is saturated. This option can be changed later by selecting View > Image Transform.

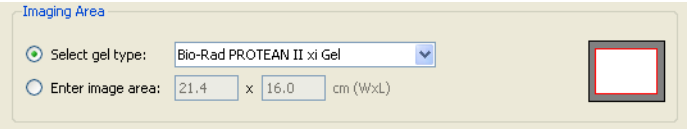
   Channel Color — select a color to display the sample image. Assigning each channel a different color makes it easy to identify each channel. Once you set up the first channel, the second channel box becomes active.

5. Repeat steps 1 through 5 to set up the second channel and the third channel, if applicable. The software determines which applications are available based on the previously selected applications. Each Configure box displays the settings for that channel.



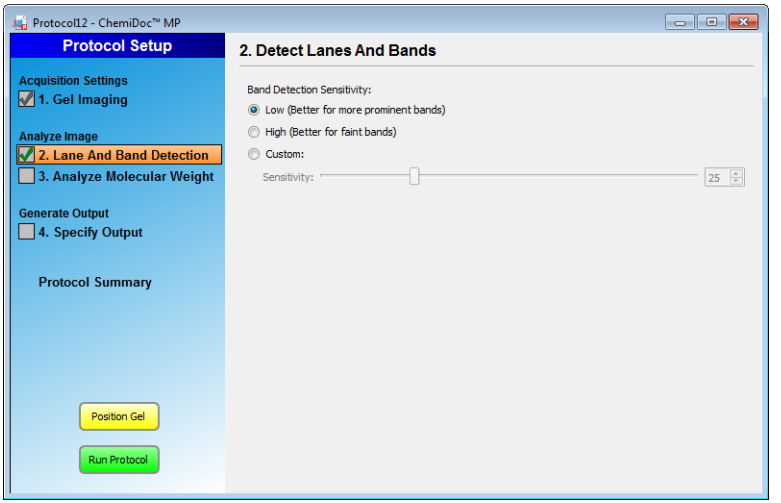
   You can reconfigure channel settings by clicking Configure in the channel box and changing the settings.

6. In Imaging Area, select from a list of Bio-Rad gels or enter the image dimensions. The red line represents the imaging area for the selected gel, and the gray rectangle represents the imager sample stage.



Step 2. Detect Lanes and Bands

The following is a screen shot of the Detect Lanes and Bands window for single-channel protocols.



To analyze the gel or blot, Image Lab must detect lanes and bands on the image. Lanes are detected automatically, and then the background is subtracted automatically. Refer to Using the All Lanes and Single Lane Tools on page 82 for details. Customize band detection with the following options.

To configure the lane and band detection settings

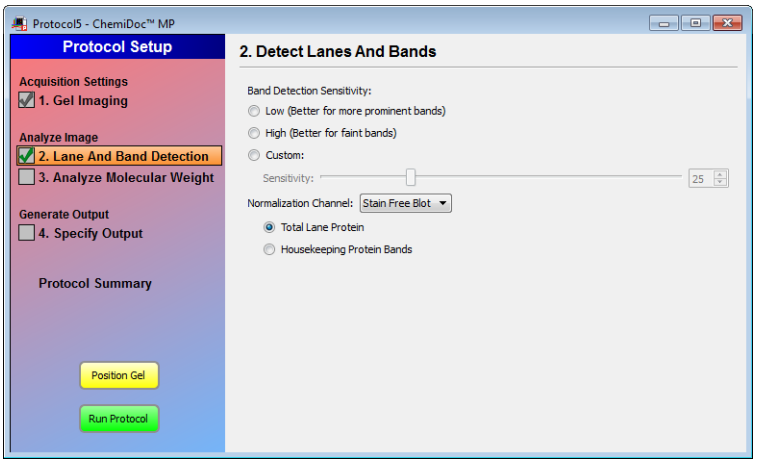
1. Select the Lane and Band Detection checkbox in the left pane of the Protocol Setup window.

2. In the right pane, select one of the following lane and band detection options:
   - **Low Band Detection Sensitivity** — this option 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** — this option sets detection at a higher level (75) for images that are more faint. Extraneous bands can be removed later, using the Band Tools in the Analysis Toolbox. See Lane and Bands Tool on page 80.

- **Custom** — select a numeric value between 1 and 100 to choose the best detection sensitivity for your sample.

Note: You cannot specify different sensitivity levels for individual channels of a multichannel image. The same sensitivity level is applied to all channels. After the image is generated, you can change the sensitivity level for individual channels using the Lane and Bands Tools.

The following is a screen shot of the Detect Lanes and Bands window for multichannel protocols.



To configure normalization settings

1. (Multichannel protocols only) Click Normalization Channel and specify which channel to use as the normalization channel.

   The selected channel is used as the normalization factor against which all other lanes in the channels are compared to normalize the volume data.

   You can defer making a selection during the protocol setup and normalize your data after the image is generated. For more information on normalizing volume data, see Normalizing Volume Data on page 87.

   Note: You must select an application for at least one channel in "Step 1. Gel Imaging" in order to see a list of choices.

2. Select the type of normalization you want to use:
   - **Total Lane Protein Normalization** – One lane in the normalization channel is used to calculate the normalization factor.

- **Housekeeping Protein Bands Normalization** – A single band of a housekeeping protein is used to calculate the normalization factor.

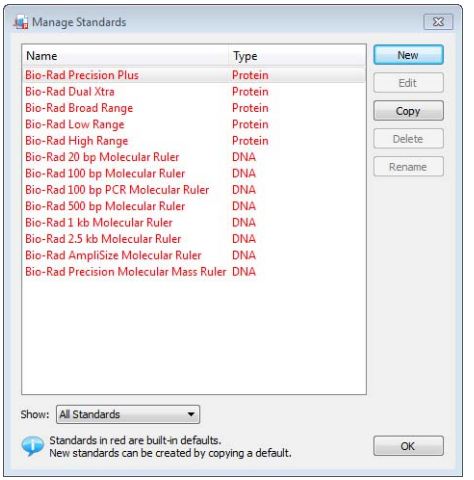
Step 3. Analyze Molecular Weight

The following is a screen shot of the Analyze Molecular Weight window for single-channel protocols.

Note: The Analyze Molecular Weight window for multichannel protocols is identical except that the background color of the left pane is pink instead of blue.

Determining molecular weight depends on selecting the proper protein standards. Many protein standards are available from Bio-Rad. Many different DNA standards are also available. See the Ordering Information on page 141 for all Bio-Rad standards and their catalog numbers.

Click Change to open the Manage Standards dialog box, where you can add third-party standard samples and edit the list.



To specify how molecular weight is analyzed

1. To analyze molecular weight automatically, select the Analyze Molecular Weight checkbox in the left pane of the Protocol Setup window. The software calculates the molecular weight for each band based on the specified standard.

2. To estimate the size of the molecules in the bands of your gel, enter the standards you are using and specify the lane(s) in which the standards are placed. For nucleic acid samples, use this step to determine the size of the bands in base pairs.

   Choose which lane contains your standards by typing lane numbers or the words *First* and *Last* in the Standard Lanes field. The format is xx, xx, xx, ... 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 more information, see Molecular Weight Analysis Tools on page 96.

Regression Methods

A regression method is used to calculate the molecular weight of the unknown bands. The software uses the relative front and molecular weight values of the standard bands to calculate the standard curve. This 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. Choose one of the four regression methods listed below.

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

If you do not have enough data points for the selected method, the molecular weight of the unknown bands is not calculated.

To set the regression method for the protocol

1. Select the appropriate method for the gel type:
   - **Gradient gels** — the linear (semilog) regression method works well for these gels because the mobility of the bands is linear to the log of their molecular weight. As an alternative, the point-to-point (semilog) method can be used if the R^2 value is not sufficient.
   - **Fixed percentage gels** — these gels have a nonlinear relationship between the mobility and the molecular weight. For these gels, choose the logistic or cubic spline regression method.

You can check how well each regression method fits the data in the standard curve window (see Standard Curve on page 74 for more information). The linear (semilog) 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.

The molecular weight of each band is displayed in the analysis table in the Mol. Wt./Base Pair column. Refer to page 123 for more information about molecular weight.

For information about the calculations behind the regression methods, see Regression Calculation Methods.

Step 4. Specify Output

Image Lab prints to the default printer unless you select otherwise.

Creating a Multichannel Image from Single Images

You can create a multichannel image from existing single images or from single channels in other multichannel images. Only images with the same aspect ratio can be combined in a multichannel image. These images are not linked to one another; therefore, when you make a change to one image, it is not propagated to the other images.

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 left pane of the Create Multichannel Image dialog box.
3. Drag each image into one of the channel boxes in the right pane. After 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 section of the dialog box.
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 are listed in the Available Open Images list of the Create Multichannel Image dialog box.
3. Drag the images 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 channel boxes.
5. Click OK to save the new multichannel image.

Choosing a Protocol

To open a saved protocol

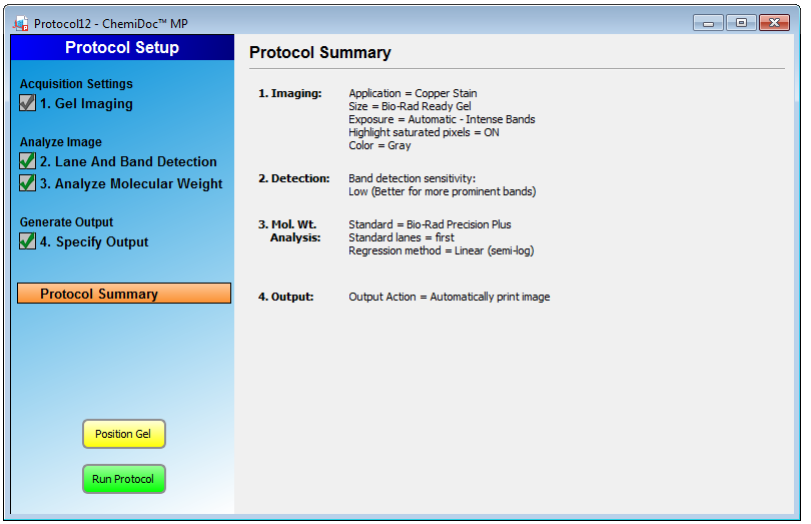
- Click Open on the Start Page or click File > Open on the menu bar.

You can also choose recently used protocols or image files from the lists on the Start Page.

Editing Protocols

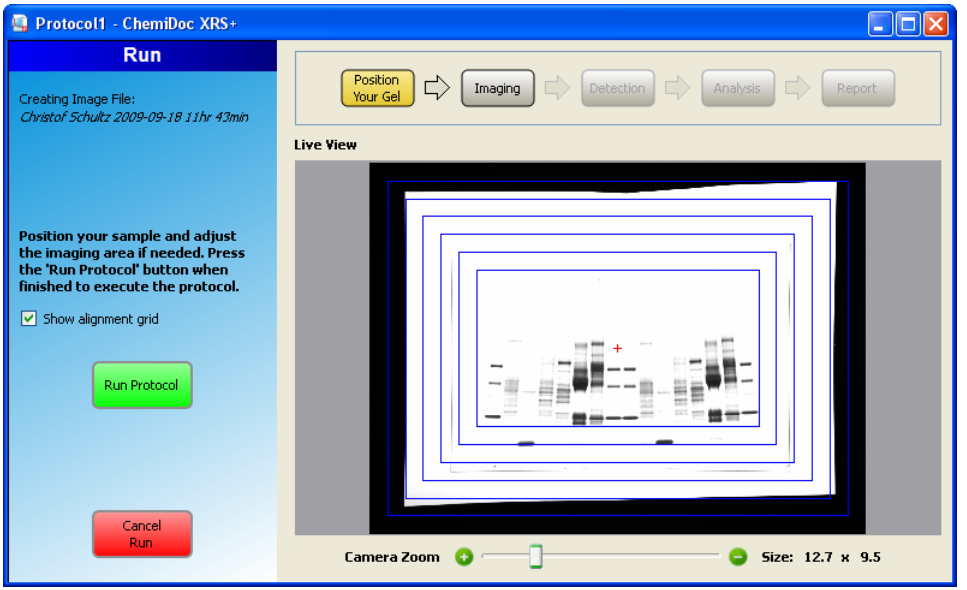
You can change the protocol settings and rename the protocol using Image Lab tools.

Open any saved protocol. You are presented with the same set of menus and choices described in Creating a Protocol on page 29.



Edit and save your changes without renaming the protocol to update an existing protocol. From the File menu, choose Save As and type a different name to create a new protocol.

Positioning the Gel



To position a gel

1. Click the yellow Position Gel button in the Protocol Setup window shown on page 47.
2. Place a gel on the imager stage and view the gel in Image Lab.
3. Use the slider below the image to zoom the image into place. You can also move the gel manually until it is centered properly on the stage.

   Note: The Bio-Rad gel alignment template kit supports four sizes of standard agarose gels to be centered quickly and easily. See Appendix C, Accessories, for more information.

Running a Protocol

To execute a protocol

- Click Run Protocol in the left pane.

The software runs through the steps in imaging mode, imaging each channel. A message at the bottom of the screen indicates the channel being imaged and a progress indicator tracks the process.

> **Note:** If you run a chemiluminescence application using auto exposure, you may see the following error message: The sample is too faint to use the auto exposure setting. Change the Image Exposure setting to use manual exposure or Signal Accumulation Mode (SAM), then run the protocol again. This error occurs if Image Lab, using the initial autoexposure algorithm, determines that it would take too long (approximately 30 minutes or more) to acquire an image. You can always use the manual Image Exposure setting and specify a longer exposure time.

After the images are taken, Image Lab continues with the detection, analysis, and reporting steps if these steps were selected in the Protocol Setup pane. When Image Lab has completed running the protocol, it displays the images that were acquired. You can then edit and save these images or do further analysis on them.

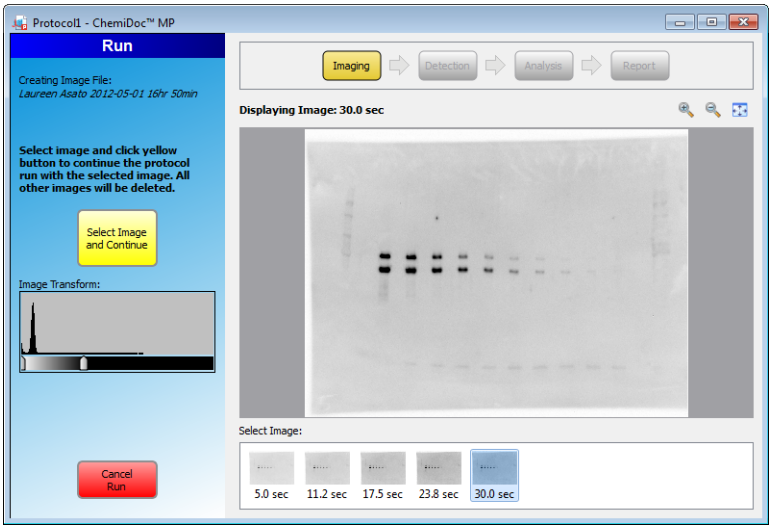
To end the protocol

- Click Cancel Run.

Running Signal Accumulation Mode (SAM) Protocols

You can interrupt the acquisition of images for a SAM-enabled protocol at any time by clicking the “Stop Acquire and Continue with Selected” button. The acquisition process stops and continues the protocol with the selected image. Any other images that were acquired are discarded.

At the end of the acquisition process, a thumbnail of each image appears at the bottom of the window. The last image acquired appears, by default, in the main window. You can view any of the SAM images in the main window by clicking on the thumbnail image.

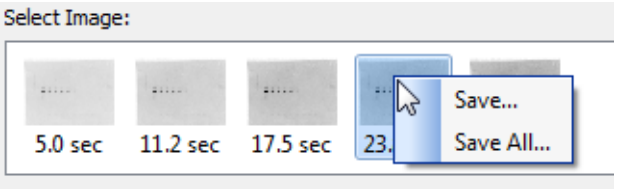


Review the images, select the image you want to use in your analysis, and click the Select Image and Continue button. Image Lab continues to the next step in the protocol using the selected image.

> **Note:** Once you click the Select Image and Continue button, only the selected image is retained. All other images are deleted. Therefore, save any other images you want to keep before continuing with the protocol. See Saving Signal Accumulation Mode (SAM) Images on page 51 for more information.

Saving Signal Accumulation Mode (SAM) Images

You can save any individual image or you can save all images once by right-clicking on the image and selecting an option from the shortcut menu.



To save a single SAM image

1. Right-click the thumbnail and click Save on the menu.
2. In the Save File dialog, accept the default name for the file or enter another name, and click Save.

To save all SAM images

1. Right-click any of the images and click Save All on the menu.
2. In the Select Directory dialog, enter the name of the folder and click Select Folder.

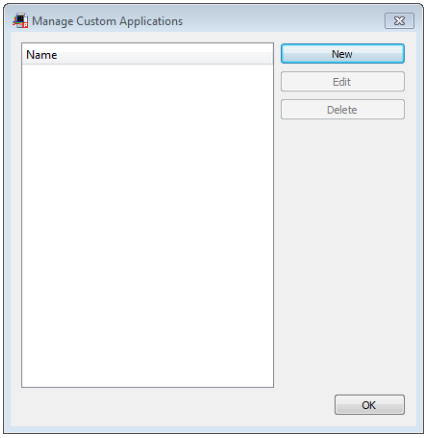
   The images are saved in the specified folder. The name of the file includes the user name, timestamp, and exposure time. For example: John Doe 2012-05-01 15 hr 44 min_Exposure_5.0sec.

Setting Up a Custom Application

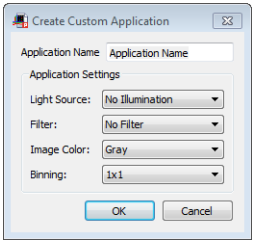
Use the Custom application to run an existing application with a new name or an application unlike existing applications. Selecting Custom on the Applications menu displays the Manage Custom Applications dialog box. If you have stored Custom Applications, they display here.

To create a custom application

1. Click New in the Manage Custom Applications dialog box.



The Create Custom Application dialog box appears.



2. Enter a unique application name.
3. Select a light source, filter, and image color from the lists.

4. Select a binning setting. Choosing a higher binning setting combines pixels to increase the amount of signal without increasing noise. While a higher setting provides optimal sensitivity for low-light applications such as chemiluminescence, it also reduces image resolution.
5. Click OK to create the custom application.

Application Tables

The following tables list the applications and primary filters for each light source.

Base System—UV, Standard Filter	
Ethidium bromide	Krypton
SYBR® Green	Coomassie Fluor Orange
SYBR® Safe	Pro-Q Diamond
SYBR® Gold	Pro-Q Emerald 300
GelGreen	Chemi
GelRed	Chemi Hi Res
Fluorescein	Chemi Hi Sens
OliGreen	Stain-free blot
PicoGreen	Colorimetric
GelStar	Rhodamine
Stain-free	Qdot 525
Oriole™	Qdot 605
Flamingo™	Qdot 625
SYPRO Ruby	

White Trans, Standard Filter	Blue Epi (catalog #170-8285) 530/30 Filter
Fast Blast™ stain	Pro-Q Emerald 488
Coomassie Blue	CY2
Silver stain	Alexa 488
Copper stain	DyLight 488
Zinc stain	

Red Epi (catalog #170-8283) 695/55 Filter	
CY5	DyLight 650
CY5.5	DyLight 680
Alexa 647	IRDye 680
Alexa 680	Qdots 705

Green Epi (catalog #170-8284) 605/50 Filter	
CY3	DyLight 549
Alexa 546	

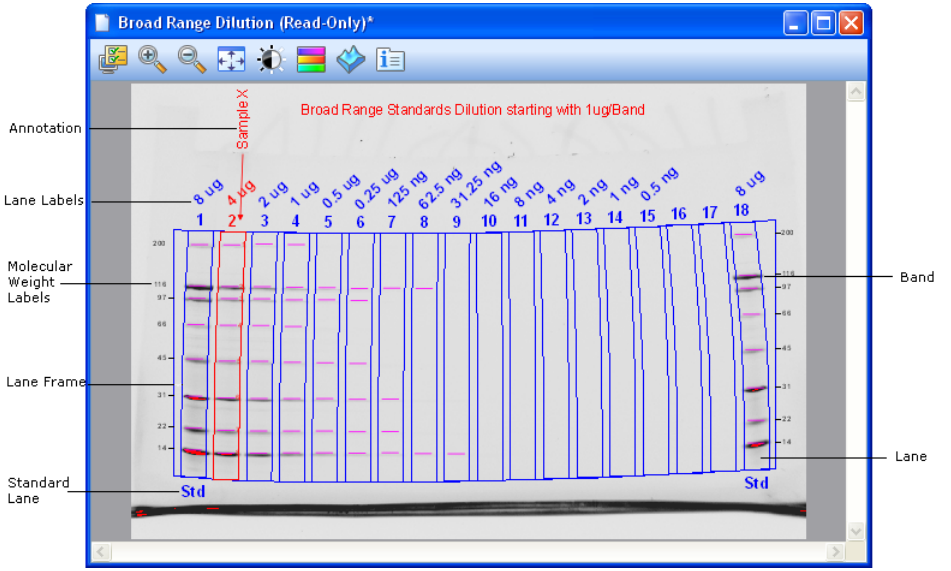
Xcita Blue™ (catalog #170-8182) Standard Filter[a]	
SYBR® Green (excision)	Fluorescein (excision)
SYBR® Safe (excision)	OliGreen (excision)
SYBR® Gold (excision)	PicoGreen (excision)
GelGreen (excision)	GelStar (excision)

a. Use the Xcita Blue screen to visualize gels without causing UV damage to the DNA. This is useful when you want to excise portions of the DNA.

5 Viewing Images

Once a gel has been imaged, the image appears in the workspace. Many controls are available to optimize viewing and to analyze the image.

Results Overview



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

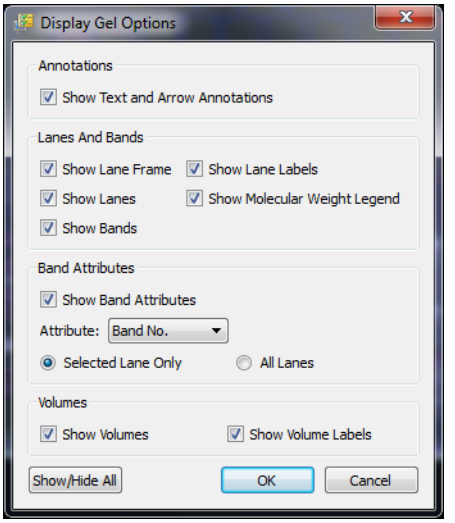
There are many ways to view the data associated with the results. You can view data as an analysis table, a lane profile, a standard curve, and a report.

Displaying Gel Images

Notice the display toolbar buttons above the gel image. Each of these tools is described in the following sections.

Display Gel Options

The figure below is a screenshot of the Display Gel Options dialog box. The sections that follow describe the settings in the dialog box.



ANNOTATIONS

You can choose whether to show text and arrow annotations that have been drawn on the image.

Lanes and Bands

You can turn on or off any image overlays, such as lane frames, lanes, bands, lane labels, and molecular weight legends.

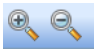
Band Attributes

You can show the following attributes for selected lanes or for all lanes.

- Band number
- Band label
- Molecular weight
- Relative front
- Volume
- Absolute Quantity
- Relative Quantity
- Band %
- Lane %

Volumes

If you have drawn volume boundaries on the gel, you can display the volume boundaries and their volume labels.

Zoom Tools 

The zoom tools resize the gel image. Click the magnifying glass with the plus sign to make the image larger; click the magnifying glass with the minus sign to make the image smaller.

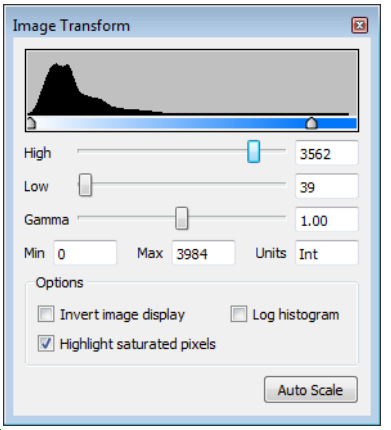
You can also zoom in on an area using the right mouse button. Right-click and drag to select the area you want to magnify. You can also resize the image with the scroll wheel on your mouse.

> **Tip:** You can return to the original view with one click by right-clicking anywhere on the image.

Fit in Window 

If you have zoomed in on an area of an image, clicking this button brings the entire image back into view.

Image Transform



Use the Image Transform dialog box to adjust image brightness and contrast, optimizing the image display so faint details can be seen. The minimum to maximum range varies depending on the light and dark values present in the image. These adjustments do not change the data; they change only the way the data are displayed. The human eye cannot see as great a range as 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, and the darkest is set to the maximum.

- The High progress indicator determines which intensity value is shown at the maximum gray scale (or other color) in the gel image.
- The Low progress indicator determines which intensity value is shown at the minimum gray scale (or other color) in the gel image.
- The Gamma progress indicator changes the gray scale curve. A value of 1 is linear. A value <1 redistributes a greater proportion of the gray scale to the first half of the intensity values. A value >1 redistributes a greater proportion of the gray scale to the second half of the intensity values.

You can also type numerical values in the text boxes next to the progress indicators. Clicking anywhere on the progress indicator bars moves the progress indicator incrementally.

Options:

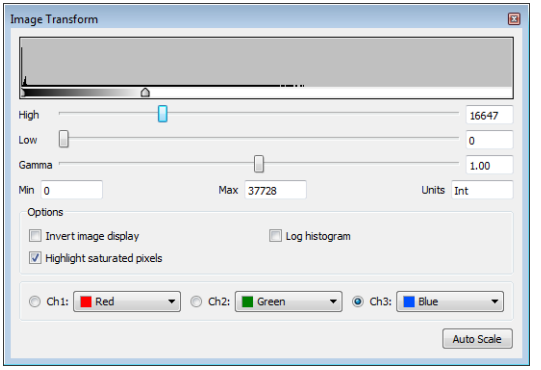
- **Invert image display** — inverts dark bands on a light background to light bands on a dark background. Light bands on a dark background are inverted to dark bands on a light background.
- **Highlight saturated pixels** — when this checkbox is selected, areas of the image with saturated signal intensity (above a measurable range) are highlighted in red.
- **Linear or logarithmic histogram** — this adjustment changes the y-axis on the histogram to display the number of pixels at each intensity value using either a linear or a logarithmic scale.

  Note: In multichannel images, you can individually highlight the saturated pixels on a channel in red. You cannot highlight the saturated pixels in a merged image.

For Multichannel Images

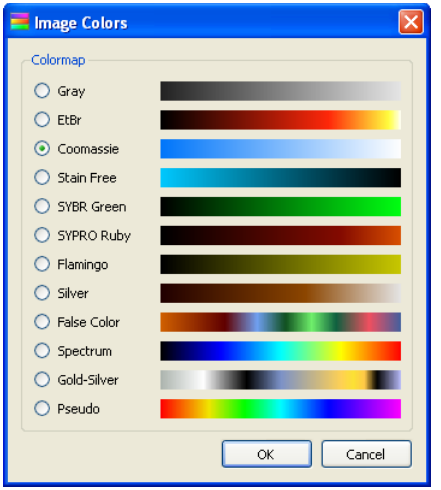
In the Image Transform dialog box, you can make changes to only one image at a time. When you work with a multichannel image, you can select each channel, in turn, at the bottom of the Image Transform dialog box and make any changes to the image.

In addition to the adjustments described in Image Transform on page 58, you can change the color of each channel. Changing the channel color in the Image Transform dialog automatically updates the title bar and the channel buttons in the multichannel image.



Changing the transform changes the transform of the selected channel and the change is reflected in the merged image.

Image Colors



You can choose a colormap for your image results file. Viewing the image with a different color scheme can make it easier to see all of the elements in the image, but it does not change your data.

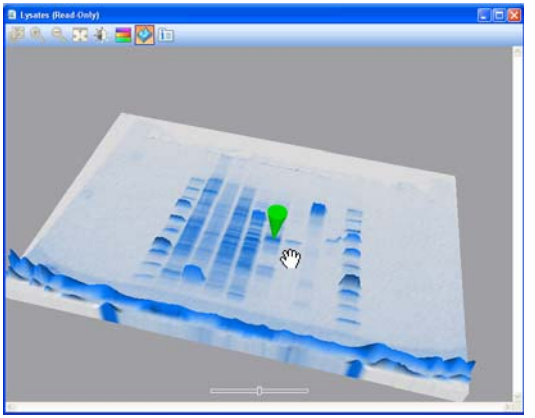
> **Note:** In multichannel images, colors can be changed only for the individual channels. You cannot change the colors of a merged image.

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 available colors include:

- Gray
- Flamingo™
- EtBr (Ethidium bromide)
- Silver
- Coomassie
- False color
- Stain-free
- Spectrum
- SYBR® Green
- Gold-silver

- SYPRO Ruby
- Pseudo

3-D Projection 



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

> **Note:** For multichannel images, you can view each channel separately in 3-D. A merged image cannot be displayed in 3-D.

To view the intensity of various bands

1. Select the 3-D button in the display toolbox above the image.
2. Click and drag the model to rotate it into your preferred view.
3. Bring the window into focus by clicking the image.
4. Press “C” to display an inverted green cone, which can be dragged around to evaluate the intensity of various bands.
5. Press “C” again to hide the tool.

Image Info 

The Image Info dialog box provides information about an active image.

> **Note:** For multichannel images, select a channel to display details for that image.

Image Details

Acquisition and image information appear in this tab.

Analysis Settings

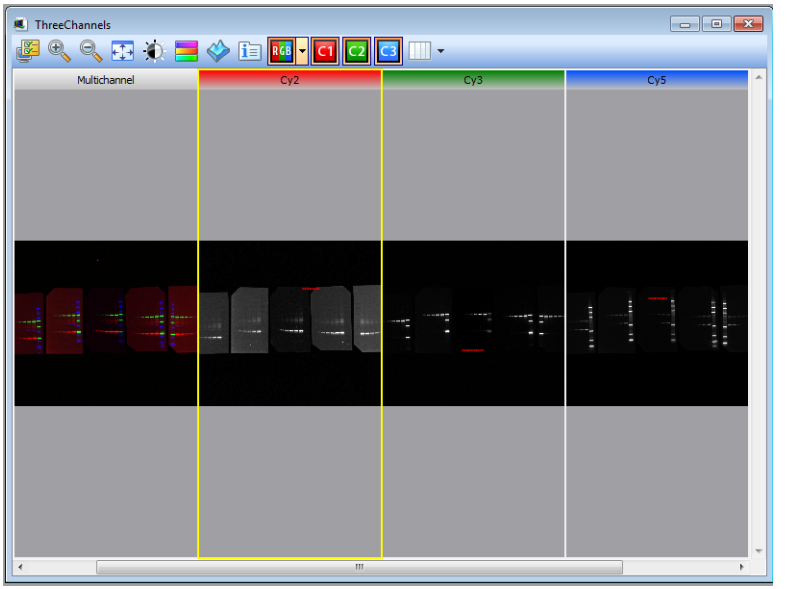
The settings that were used when the gel was analyzed are displayed here. For example, Band Detection and Molecular Weight Analysis will appear, if they were performed.

Notes

You can add notes, point out the types of samples used, and add any other information about the results. You can create custom labels for the lanes in your image. In a multichannel image, the custom labels are applied to all channels.

Displaying Multichannel Images

The Multichannel View includes a pane that displays the merged channels and panes for individual channels. Application names appear in the toolbar. A yellow border surrounds the active pane.



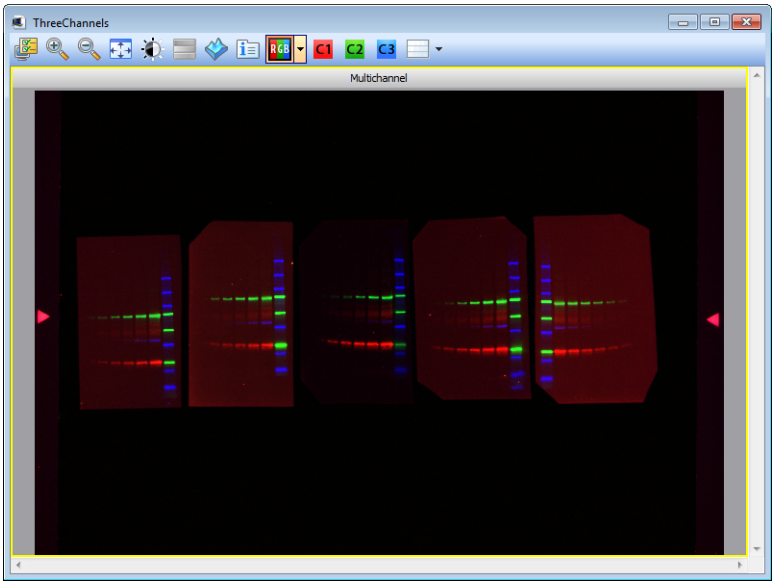
In the display toolbox above the image, additional controls are available for viewing the multichannel image.

Multichannel View 

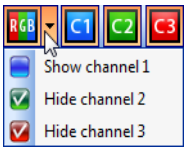
Click the Multichannel View button to display or hide the merged image panel in the multichannel display.

You can show or hide each channel using the channel buttons.

You can merge the three channels into a single multichannel pane.



You can also specify the channels to include in the merged view using the dropdown list.



Splitting Multichannel Images

You can work separately on the images that make up the multichannel image by splitting the multichannel image into individual image files. When you split a multichannel image, a new file is created for each channel (except the RGB channel). Each new file has the same name as the multichannel image; the application name is appended in parentheses. All acquisition settings and overlays are copied to the new files.

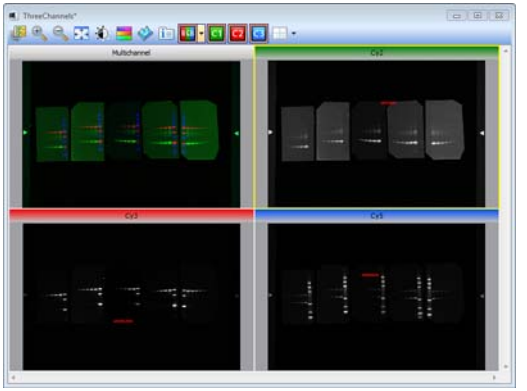
To split a multichannel image into separate files

1. Open a multichannel image.
2. Select Split Multichannel Image in the File menu. Each channel is displayed in its own window (except the RGB channel).
3. Save each image in its own file.

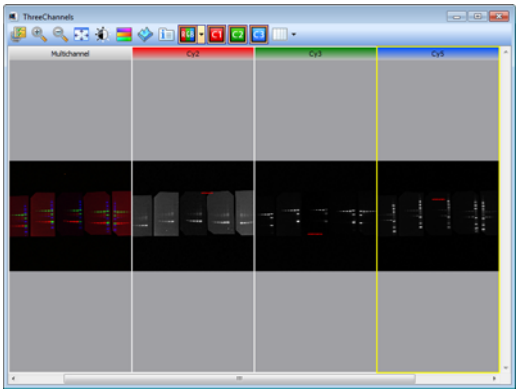
CHANGE LAYOUT 

You can choose a layout for the image panes. Clicking Change Layout shows a list of display options for the image panes. You can select from one of the four views that follow.

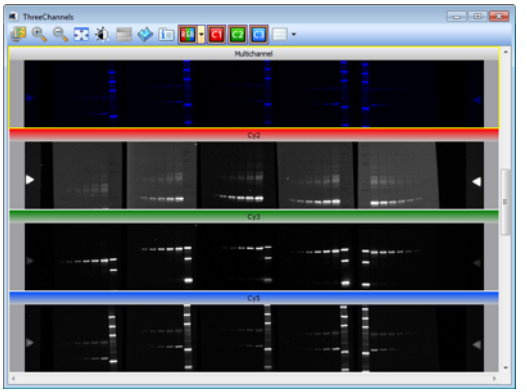
Grid View — by default, multichannel images appear in grid view.



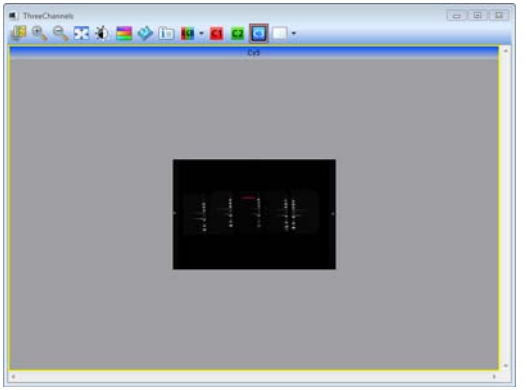
Vertical View



Horizontal View



Single View



Displaying Data

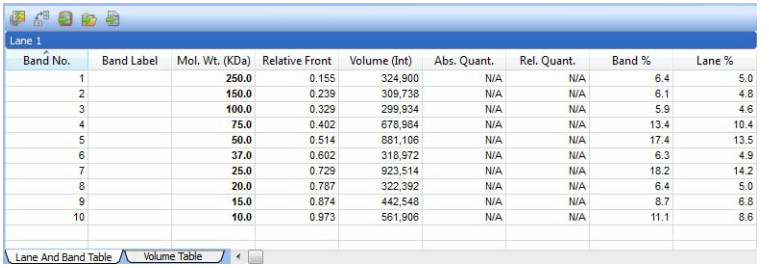
You can view the results from analyzed data associated with the gel images using an analysis table, a lane profile, a standard curve, or a report.

Use the buttons in the main toolbar to turn these views on or off. You can view your data with all views simultaneously.

Analysis Table Options 

Numerical data associated with an analysis can be viewed in an analysis table. Data from the Lane and Band analysis can be viewed in the Lane and Band tab. If volume analysis was performed, these data can be viewed in the Volume Table tab.

Clicking Analysis Table opens a tabular display of the data extracted from the results. The buttons above the table provide options for displaying and exporting analysis table data.



Lane 1

Band No.	Band Label	Mol. Wt. (KDa)	Relative Front	Volume (Int)	Abs. Quant.	Rel. Quant.	Band %	Lane %
1		250.0	0.155	324,900	N/A	N/A	6.4	5.0
2		150.0	0.239	309,738	N/A	N/A	6.1	4.8
3		100.0	0.329	299,934	N/A	N/A	5.9	4.6
4		75.0	0.402	678,984	N/A	N/A	13.4	10.4
5		50.0	0.514	881,106	N/A	N/A	17.4	13.5
6		37.0	0.602	318,972	N/A	N/A	6.3	4.9
7		25.0	0.729	923,514	N/A	N/A	18.2	14.2
8		20.0	0.787	322,392	N/A	N/A	6.4	5.0
9		15.0	0.874	442,548	N/A	N/A	8.7	6.8
10		10.0	0.973	561,906	N/A	N/A	11.1	8.6

Lane And Band Table | Volume Table

Set Window Size

To change the size of your Analysis Table window, move your cursor to the top of the window until it changes into a double-headed arrow. Click and drag the edge of the window until you can see all of the data.

> **Note:** Resizing the Analysis Table window is restricted when a Protocol window is open.

Display Data Options 

The Display Data Options dialog box displays three tabs: Measurements, Display, and Export.

Measurements — choose the measurements you want displayed in the table. Use the arrows to move the columns between the Not Displayed pane and the Displayed pane.

Default display settings — “Move selected lane to top by default” is on or off, depending on whether the checkbox is selected. This checkbox is selected by default.

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

Example — shows an example of how measurements will display with the selected Measurement and Precision settings.

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

Export delimiter — select a delimiter option for the exported file.

- Comma delimited
- Tab delimited
- Use other delimiter (user defined)

CHANGE ANALYSIS TABLE ORIENTATION 

This button toggles between two table orientations.

Horizontal — displays the lanes/volumes beside each other, so you can scroll through the table from left to right.

Vertical — displays the lanes/volumes on top of each other, so you can scroll through the table from top to bottom.

Copy Analysis Table to the Clipboard  — click this button to copy the analysis table to the clipboard so you can paste the analysis table into word processing or presentation applications. It is best to use the vertical table orientation when copying to an 8-1/2 x 11-inch page, to allow the columns enough room to display.

Export Analysis Table to a File  — when exported as a CSV file, the data file can be opened in a database application.

Export Analysis Table to a Spreadsheet  — you can use Excel's sorting and formula functions to manipulate your data. If you have Excel (PC or Mac) or Numbers (Mac) installed on your computer, it opens with your spreadsheet displayed.

Lane and Band Definitions

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 field of the Lane and Band table.

Molecular Weight — the molecular weight of the band is calculated based on the user-defined standard and regression method. Italic values indicate extrapolated values. When using nucleic acid gels, the size of the band is displayed in base pairs.

Relative Front — values between zero and one indicate the relative movement of the band from top to bottom.

Abs. Quant. — absolute quantification of the band.

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

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

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

Volume Definitions

Volume Number — a unique number is assigned to each volume.

Volume Label — software-generated labels for different types of volumes (U for unknown, B for background, S for standard). Label can be changed 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.

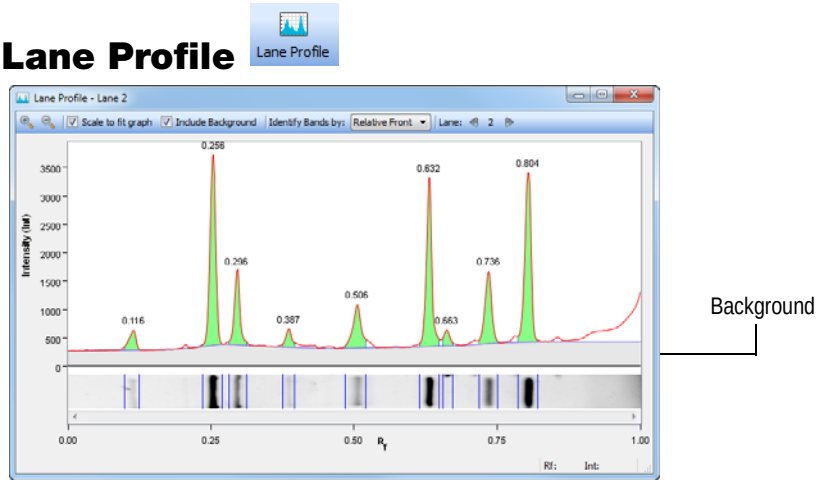
Maximum Value — intensity of the pixel with the maximum intensity inside the volume.

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 option shows a cross-section view of a single lane in the channel, rotated 90°.

To navigate in the Lane Profile window

- Use the Next and Previous buttons at the top of the window to page through the profiles of the lanes in your image.
- Click on a lane in your image to see its profile.
- To view the lane profiles of the different channels in a multichannel image, first click on the channel. The Lane Profile window updates and displays the lane profile of the selected lane in the channel. Then use the Next and Previous buttons to page through the lanes in the channel.

The title bar identifies which lane profile is in view (Lane 1, Lane 2, and so on). If the image is a multichannel image, the title also includes the name of the channel, for example, Lane 2 (Dylight 650).

There are several settings at the top of the window: “Scale to fit graph,” “Include Background,” and “Identify Bands by.” These settings, as well as the zoom tools, are global and they apply to all the profiles.

The zoom tools work the same way they work elsewhere in the software. For more information, see Zoom Tools on page 57.

In addition to the graph of the lane intensities, the Lane Profile tool also shows an image of the selected lane below the graph. The multichannel channel of multichannel images is always a gray scale 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 gray scale image.

As you move your cursor over the profile, the current relative front (Rf) value and the average intensity (Int) value at the Rf value are displayed in the lower-right corner of the Lane Profile window.

Scale to Fit Graph

You can choose the highest point of the display to define the range of the graph. This provides the best view of the lane profile.

You may want to 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

When the Include Background checkbox is selected, the Lane Profile window shows the subtracted background under the blue line. The area used for band quantification appears in green under the red line.

When you clear the Include Background checkbox, the area of the lane profile that represents the background of the image does not display.

Identify Bands by

You can change how the bands are labeled by choosing from the options in the “Identify Bands by” list. By default, the bands are labeled with the band number.

You can display callouts of the following attributes:

- Band Number
- Band Label
- Molecular Weight
- Relative Front
- Volume
- Absolute Quantity

- Relative Quantity
- Band %
- Lane %
- Normalization Factor (Multichannel images only)
- Normalization Volume (Multichannel images only)

Adjusting Band Boundaries

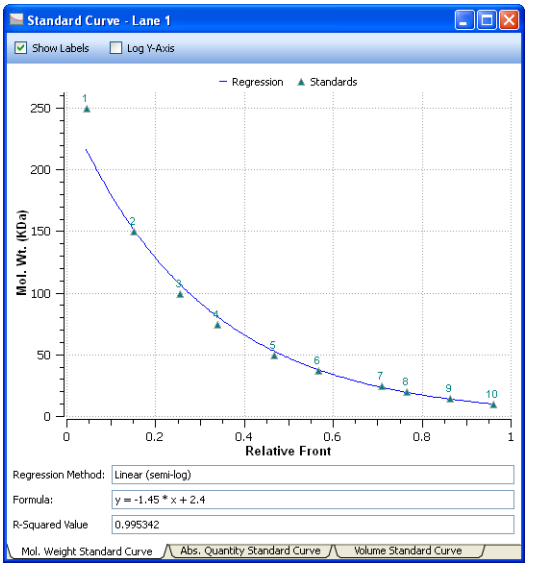
Below the profile of each lane, there is a strip of blue that displays the bands in the channel surrounded by a pair of vertical lines delimiting the boundaries of the bands. You can move the vertical lines and change the boundaries of the band.

To change the boundaries of a band

1. Hold your cursor over one of the boundary lines until a double arrow appears.
2. Drag the cursor to the desired position.

   Note: Boundary lines cannot overlap one another. Therefore, when you move a boundary line, you will not be able to move it beyond the next boundary line.

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 Mol. Weight Standard Curve, the Abs. Quantity Standard Curve, or a Volume Standard Curve.

Report 

See Chapter 7, Generating Reports for information about reports.

6 Analyzing Images

Analysis Toolbox tools are enabled once an image file is opened and in focus. An active or “in focus” window has a darker blue menu bar on a Windows PC. On a Mac, the window control icons display more brightly when a window is active. This distinction helps you to identify the active window among many open image files in your workspace.

Image Types

There are three types of images that are created using Image Lab:

- Single-channel images
- Linked multichannel images
- Unlinked multichannel images

The multichannel view in a multichannel image is a merged display of single channels. You can have up to three images in the multichannel image. The linked multichannel image is acquired using the multichannel protocol. Unlinked multichannel images are generated by combining several images using the Create Multichannel Image feature.

The Analysis Toolbox tools work differently on these images. Some tools, such as lane detection, are applied to one channel in a linked multichannel image and the changes are propagated to all the other channels. With unlinked multichannel images, lane detection must be applied separately to each channel.

Using Auto Analysis

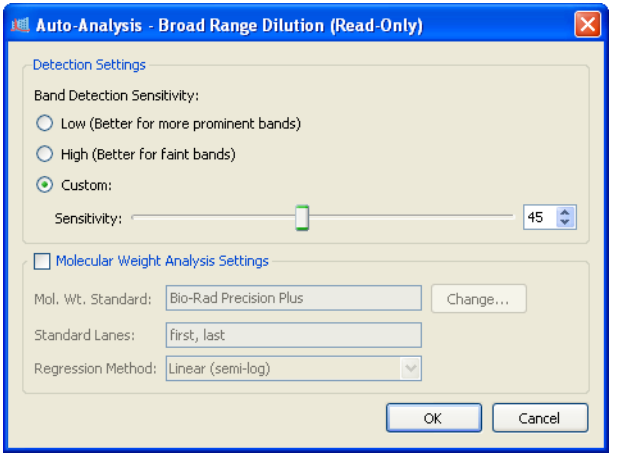
Auto analysis detects the lanes and bands and, optionally, calculates the molecular weight of the bands in your image. Clicking Auto Analysis in the Analysis Toolbox enables you to do the following:

- Analyze images obtained with protocols that did not include steps for detection and analysis
- Change your analysis parameters to reanalyze your images

  Note: If you change any settings for an analyzed gel, the initial analysis is overwritten. To preserve both analyses, save each image file with a different name.

Auto Detection Settings

Note: The Band Detection Sensitivity and Molecular Weight Analysis Settings are applied to all channels in a multichannel image. If you want to use different sensitivity levels to detect bands in each channel, use the Lane and Bands Tool.



The band detection sensitivity options are as follows:

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 Tool on page 80.

Custom — allows 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 or High Band Detection Sensitivity is used, the following numerical values are set: low sensitivity = 25; high sensitivity = 75.

Molecular Weight Analysis Settings

Molecular Weight Standard — choose any of the many Bio-Rad standards or other standards you have placed in your 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 Methods on page 43.

Analysis Toolbox Tools

All Analysis Toolbox tools customize the *analyzed* data in image files. These tools are available only when an image file is open. Click a specific image to select among many windows that may be open in your workspace. On a PC, the menu bar of the selected window becomes a darker blue than other windows. On a Mac, the window control icons display in brighter color when a window is active.

Note: Some tools delete the existing analysis.

To access a tool

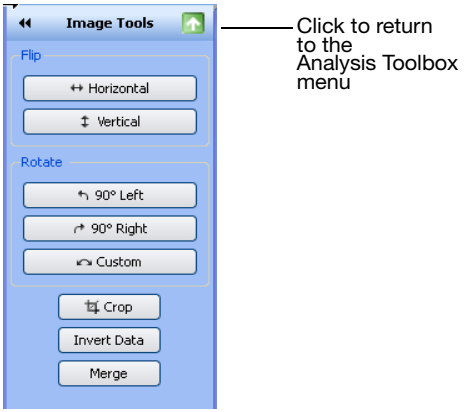
- Click any of the toolbox buttons.

To return to the Analysis Toolbox menu

- Click the green Up arrow to the right of the tool name.

Image Tools 

To display the image tools menu, click Image Tools.



You can manipulate your images using these image tools:

- **Flip** — you can flip the gel image horizontally or vertically.
- **Rotate** — you can rotate the gel image 90° using the Left or Right buttons.
- **Crop** — you can trim the outer edges of your image.
- **Merge** — you can merge chemiluminescent blot image with a colorimetric image of the same blot.

The sections that follow describe how to use these tools in greater detail.

Correcting a Slanted Gel

To correct a slanted gel

1. Click Custom.
2. Rotate the red arrows that appear over the gel to any degree between 0 and 360° by dragging them.
3. Right-click the gel image and choose Rotate to set your gel in the new position. You will be prompted to Cancel or Continue, in case you want to reset the rotation.

Cropping a Gel Image

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

To crop a gel image

1. Click Crop. 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.
4. (Optional) Type a name for the crop settings in the dialog box that appears and click OK.
5. Right-click and select Crop or Cancel. Selecting Crop 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 in the dialog box that appears and click Load. The red box resizes and the crop specifications appear on the image.
4. Right-click and select Crop. The image is cropped to the area specified in the crop settings you selected.

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 and click Delete Crop Settings.
3. Select the crop settings in the dialog box that appears and click Delete.

Inverting Data

Invert Data is used for negative stains and zymograms. Intensity values of bands must be greater than background to perform analysis on the gel. View the gel as a 3-D projection to determine if the data must be inverted.

Merging Images

Use this button to merge a chemiluminescent blot image with a colorimetric image of the same blot. If you have used 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 immuno-detection. These two images can then be merged into a combined image with both signals.

Note: Merging images can have an adverse effect on quantification. If accurate quantification is required, perform analysis on the original, separate images. Only images of the same size can be merged.

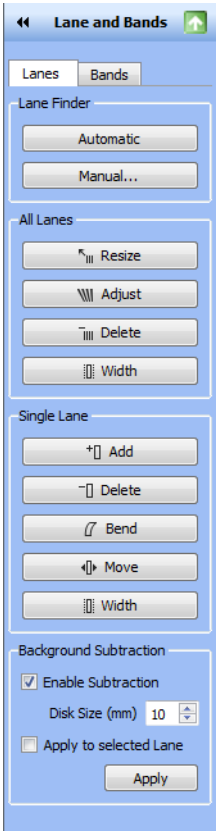
Lane and Bands Tool 

The Lane and Bands tool enables you to identify the lanes and bands in your images.

To open the Lane and Bands tool

- In the Analysis Tool Box, click Lane and Bands tool, then select the tab for the Lanes tools or the tab for the Bands tools.

The following is a screen shot of the Lanes tools.



Detecting Lanes

To detect the lanes in the image

Choose one of the following methods to find the lanes in your images:

- Click Automatic if the gel image is fairly typical.
- Click Manual to detect a specific number of lanes or if automatic lane detection did not find all the lanes.

Detecting Lanes in MultiChannel Images

To use automatic lane detection with multichannel images, select one of the channels and click Automatic. Automatic lane detection works differently in multichannel images depending on whether the channels are linked or unlinked.

- For linked multichannel images, the lane detection is based on the selected channel, and the detected lanes are applied to all channels.
- For unlinked multichannel images, lane detection is applied to only the selected channel. You must individually select each channel and apply automatic lane detection.

The lanes in unlinked multichannel images may fall in slightly different places on each channel, and you may want to have more control over where the lanes fall. You can detect the lanes in one channel of an unlinked multichannel image using either the automatic or manual lane detection tools. Then you can copy the lanes into the other channels and position the lanes so that the lanes in each channel are matched to one another. For more information on copying lanes, see Copying Lanes on page 84.

Using the All Lanes and Single Lane Tools

To use the All Lanes and Single Lane tools

- Click the lane tool first, then click the lane that you want to apply the change to.

More specific information on how to use each lane tool is described in the following sections. Tool actions in linked multichannel images are applied across all channels. Tool actions in unlinked multichannel images are applied in only the selected channel.

All Lanes Tools

The All Lanes tools are described below:

- **Resize** — a square anchor point appears in each corner of the image. Drag the handles of the red squares to resize all lanes.
- **Adjust** — if the gel image is irregular, you can adjust the orientation of all lanes by dragging a single corner of the lane frame. The Adjust All Lanes tool does not resize lane width.

  You can add additional anchor points to the top or bottom borders of the rectangle by clicking the lane frame. Remove any unneeded anchor

point by right-clicking it. By dragging these anchor points, you can adjust for “smiling” gels.

- **Delete** — deletes all lanes.
- **Move Lane Frame** — you can move the entire lane frame when resizing or adjusting the lanes. Click Resize or Adjust and make the changes to your lanes. Then click anywhere in the frame and move it to the desired location.

  Tip: On the PC, you can hold down the Shift key or the Ctrl key and use the arrow keys on your keypad to move the lane frame. On the Macintosh, hold down the Shift key or the Command key and use the arrow keys.
- **Width** – Drag one of the anchor points on any lane to change the width of all lanes at once. In a multichannel image, the width of all of the lanes in all channels are changed.

  Note: The All Lanes Width tool makes the width of all lanes uniform. Therefore, if you used the Single Lane tool to change the width of an individual lane, this change is overridden by the All Lanes Width tool.

  Tip: You can use the plus key (+) on your keyboard to increase the lane width and the minus key (-) to decrease the lane width.

Single Lane Tools

The Single Lane tools are described below.

- **Add** — adds a lane to a gel image. Click Add, then click within the lane frame where you want to place the new lane. The lanes are automatically renumbered.

  Note: To add a lane outside the frame, add a lane inside the frame and click Move to expand the lane outside the frame’s boundaries.
- **Delete** — deletes a lane. Click Delete, then click either the lane or its lane number. The lanes are automatically renumbered.
- **Bend** — bends a lane to better fit the gel image. Click Bend, then drag one of the square anchor points to fit the image.

  Note:
  - To add additional anchor points, left-click within the lane. Drag these anchor points to adjust the lane to fit the gel image.
  - To remove and anchor points, right-click on the anchor point.
- **Move** — moves a lane to a new position on a gel image. Click Move, then click the lane you want to move. Drag it to a new

location. The lanes are renumbered according to their new position.

- **Width** — changes the width of a lane. Click Width, then click within the lane. Click on the anchor points to adjust the lane width.

Copying Lanes

You can copy the lanes from one channel into any other channel. This tool is useful if you want to create a multichannel image from unlinked images, and you want to identify and match the lanes in your channels.

To use the copy lanes feature, you must open the Lane and Bands tool. The entire frame and all the lanes are copied; individual lanes cannot be copied.

To copy lanes between channels

1. Open the Lane and Bands tool.
2. Select the channel that contains the lanes you want to copy.
3. From the Edit menu, select Copy.
   The message, Copying All Lanes, is displayed briefly.
4. Select the channel that you want to copy the lanes into and from the Edit menu, select Paste.
   A channel can contain only one lane frame at a time. Therefore, if you paste lanes into a channel that already contains a lane frame, you are prompted to confirm the deletion of the existing lanes. If you click Yes, the existing lanes are deleted and replaced with the copied lanes.
   Note: If you copy lanes into a channel of a linked multichannel image, the lanes are copied into all channels of the image, including the Multichannel channel.

Once the lanes are copied into the channel, you can manipulate individual lanes using the lane tools so that they are correctly positioned.

Using Lane Background Subtraction

Perform lane-based background subtraction by selecting Enable Subtraction in the Background Subtraction field. Use the Lane Profile view to see the subtracted lane background.

Rolling Disk — specifies the size of a hypothetical rolling disk (between 1 and 99 mm) that removes background levels along the length of the lane. The size of the disk determines how closely the background level follows the intensity profile.

A large disk follows the profile trace less closely, touching fewer points along the trace and removing less background. A smaller disk more closely follows the profile trace, removing more background.

A disk radius that is too large will result in poor background removal. A disk radius that is too small may subtract actual data. For most samples, a size of ≤10 mm is usually appropriate. You can perform this task several times until you are satisfied with the amount of background removed. Use the Lane Profile tool to evaluate the appropriate disc size for background subtraction.

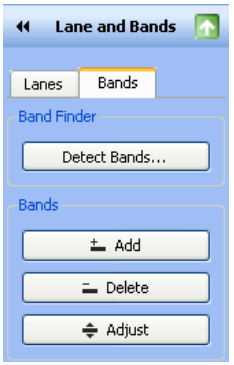
Apply to selected Lane — when you select this box, the level of background subtraction specified with the previously described buttons is applied only to the selected lane. This option enables you to set different background subtraction levels for each lane.

Detecting Bands

Bands are detected for individual images. For multichannel images, you must detect the bands for each channel, one at a time. This applies whether the multichannel image is linked or unlinked.

> **Note:** Band detection on the multichannel channel is disabled. The multichannel view is a composite of the other channels. Detecting bands in this view is not particularly useful because quantification of overlapping bands will result in combined values from multiple channels.

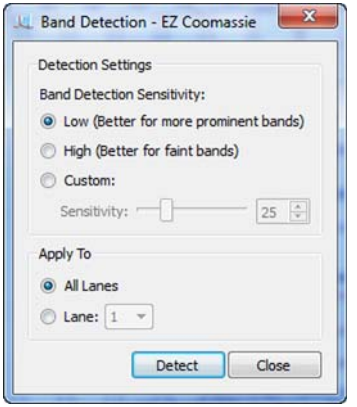
The following is a screen shot of the Bands tools.



To detect bands in the image

- Click Detect Bands. The Band Detection dialog box opens in which you can select band detection sensitivity and the lanes to which it applies.

  Note: With multichannel images, you can leave the Band Detection dialog box open as you select each channel one by one and detect the bands in each channel. You do not have to close and reopen the dialog box for each channel.



Editing the Detected Bands

Several tools are available to optimize the bands in your images.

- **Add** — adds a faint band. Click Add, then click anywhere within the lane. Image Lab then looks for a faint band close to where you clicked. You can darken your entire image to view faint bands more easily using the Gamma slider in the Image Transform dialog box.

  Note: For instructions, see Image Transform on page 58.
- **Delete** — deletes a band from the image. Use this feature to remove bands that are not relevant to your analysis. Click Delete, then click on the band you want to remove.
- **Adjust** — adjusts the height of a band. Click Adjust. Two boundary lines appear around each band. Move the cursor over a boundary line until you see a double-headed arrow. Move the boundary line up or down; the center recalculates and the band appears there.

  Note: You can also adjust band boundaries in the Lane Profile view.

Normalizing Volume Data

There are several reasons why you might want to normalize the volume data in multichannel images:

- The lanes are loaded with the same volume; however, 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 the gel to the membrane.

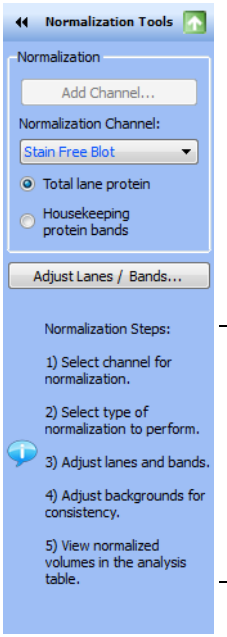
In these instances, you can correct for these differences by normalizing the volume data. There are two ways to normalize volume data:

- **Total Lane Protein Normalization** – one lane in the normalization channel is used to calculate the normalization factor.
- **Housekeeping Protein Bands Normalization** – a single band of a housekeeping protein is used to calculate the normalization factor. In order to get accurate results, the housekeeping protein must be stable and impervious to the treatments in your experiment. It must be the same in pre-treatment and post-treatment.

In both cases, the normalization factor is then used to calculate the normalized volumes in the lanes for all channels.

Using the Normalization Tool 

Click Normalization on the Analysis Tool Box.



The Normalization Steps guide you through the steps to normalize your volume data.

The Normalization Steps outline the recommended steps to follow. You will need to review your lanes and bands and possibly make adjustments to them.

> **Tip:** From the Normalization tools pane, click Adjust Lanes/ Bands to quickly navigate to the Lane/Bands Tool.

See Optimizing Normalization of Volume Data on page 89 for an explanation of each step in the process.

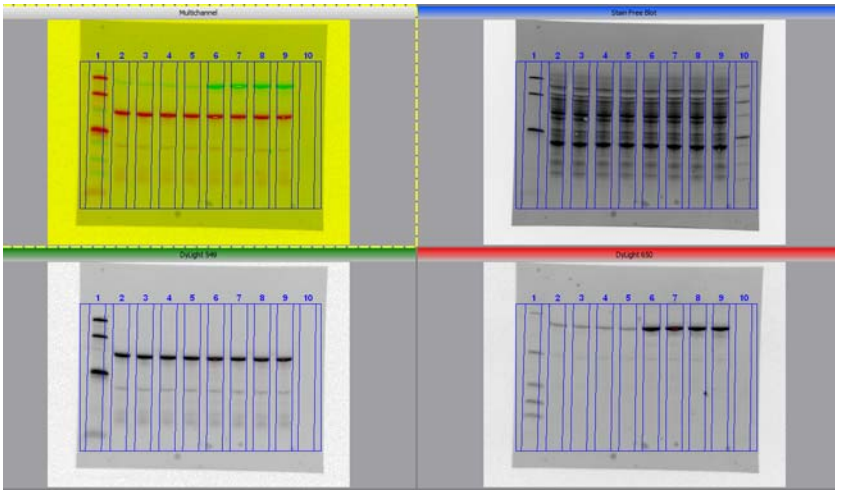
Optimizing Normalization of Volume Data

The following are the steps you should follow to get the best results when normalizing your volume data:

1. Detect the Lanes in the Channels (page 89).
2. Make Adjustments to the Lanes (page 90).
3. Detect the Bands (page 91).
4. Subtract any Extraneous Background (page 92).
5. Remove Compromised Data (page 92).
6. View the Data in the Analysis Table (page 93).

Detect the Lanes in the Channels

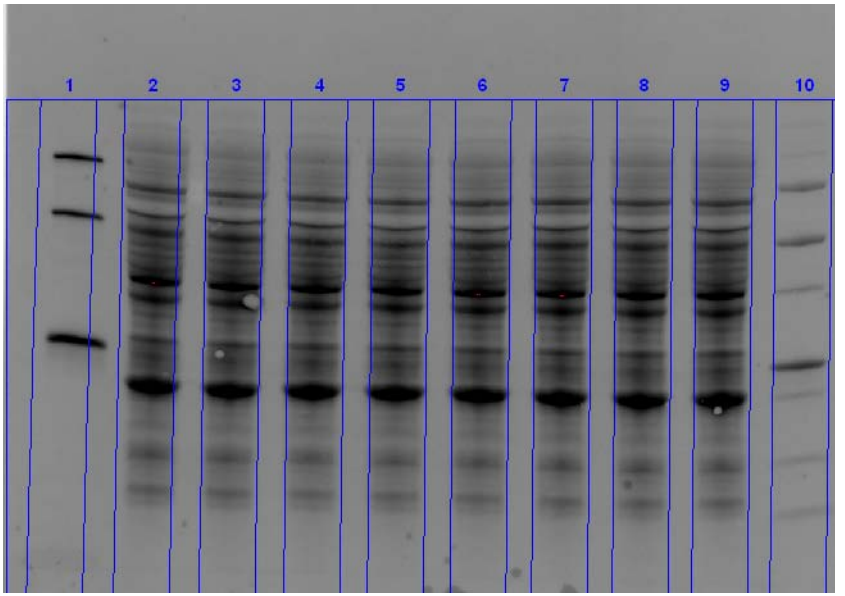
Start by detecting the lanes in your multichannel image. You can either use the automatic or manual lane detection method. For more information on when to use each method, see Lane and Bands Tool on page 80. If your multichannel image consists of unlinked images, you will need to pay particular attention to the lanes that are detected to ensure that the corresponding lanes are correctly mapped for each channel. For example, you may want to use the Copy Lanes tool to map your lanes. For more information on Copy Lanes, see Copying Lanes on page 84.



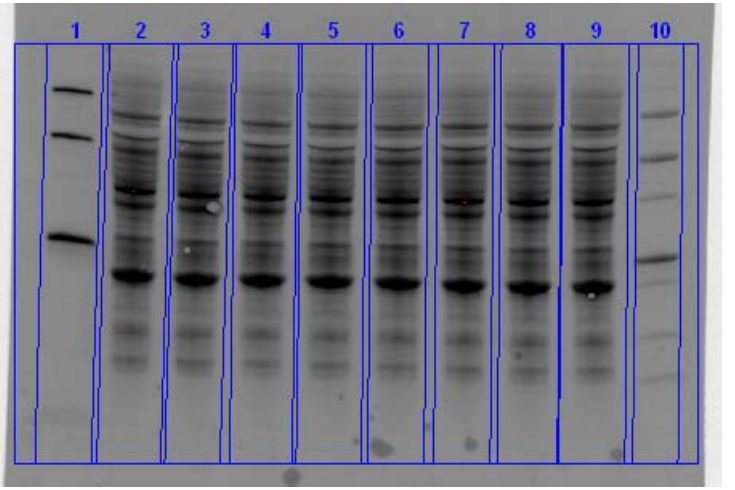
Make Adjustments to the Lanes

Regardless of which method you use to detect your lanes, you should review the lane borders to ensure that each lane encompasses all the protein in a particular lane, and that the lane does not overlap into another lane. For example, in the following figure, lanes were detected using the automatic lane finder. Some of the lanes exclude material that should be included.

Note: Image Lab automatically excludes standard lanes from normalization.



Use the Lanes tools to make the necessary adjustments to your lanes. For more information on these settings, see Using the All Lanes and Single Lane Tools on page 82. In this example, the lanes were slightly widened to include the relevant material. The corrected image follows.

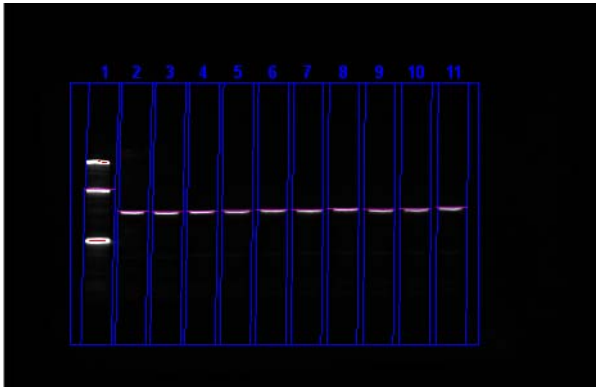


Detect the Bands

Use the band detection tool to detect the bands in the channels. If you are using the total lane protein method to normalize your data, it is recommended that you do not use the band detection tool on the normalization channel, because this will generate large amounts of superfluous data in the analysis table. For more information, see Detecting Bands on page 85.

If you are using a housekeeping protein to calculate the normalization factor, you must isolate this protein in your image. Remove all bands other than the housekeeping protein from your image. The normalization channel should

have only one detected band in each lane. Use the Delete Bands tool to exclude all other bands from the calculation.



Subtract any Extraneous Background

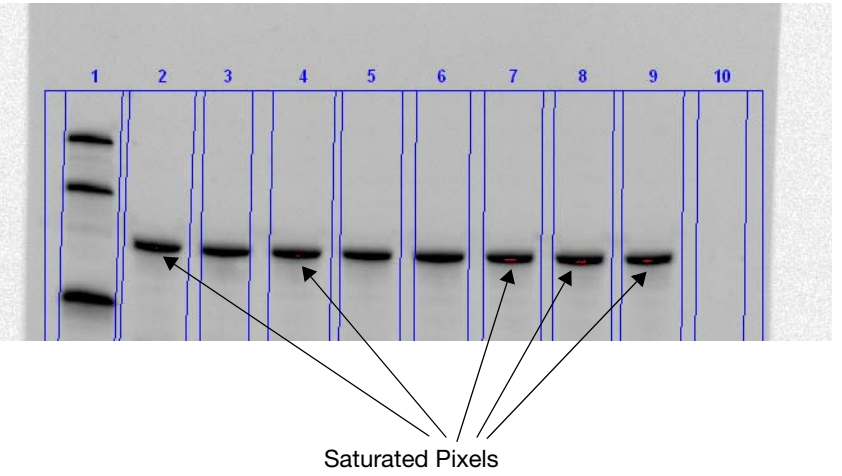
The lane volume data is background subtracted. Review the background subtraction being applied to each lane by using the Lane Profile tool. Verify an equivalent background profile is being subtracted from each lane in the normalization channel so the normalization factor is accurate. For more information on the lane Background Subtraction tool, see Volume Background Subtraction on page 112.

Remove Compromised Data

There are several reasons why lanes should be excluded from your analysis:

- **Empty lanes** — 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. If the first nonstandard lane is not a valid lane (for example, an empty lane), delete it from the channel.

- **Lanes contain saturated pixels**— check your images for saturated pixels, indicated by red. These points cannot be read and cannot be used in quantification.



- **Poor transfer quality** — check the quality of the transfer. If the transfer is poor with splotchy or blurred areas, delete these 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. The 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.

Tip: Omit the normalization channel from the Analysis Table using the Multichannel View button. (See Displaying Multichannel

Images on page 63 for information on displaying and hiding channels.)

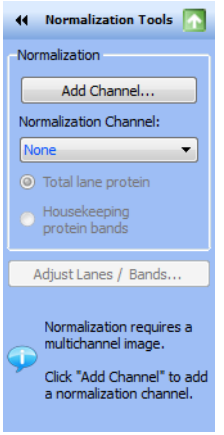
			Lane 2				
ıe (Int)	Norm. Factor	Norm. Vol. (Int)	Channel	Band No.	Volume (Int)	Norm. Factor	Norm. Vol. (Int)
930,610	1.000	5,930,610	DyLight 650	1	21,880,420	0.173	3,785,924
457,858	1.000	6,457,858	DyLight 549	1	4,009,850	0.173	693,816
058,452	1.000	7,058,452					
899,044	1.000	9,899,044					
443,154	1.000	11,443,154					
497,458	1.000	15,497,458					
215,490	1.000	11,215,490					
368,178	1.000	20,368,178					
841 124	1.000	15 841 124					

Adding a Channel to a Single Image

The Normalization tool can be used only with multichannel images. If you have a single image, there is an Add Channel feature in the Normalization tools pane that guides you through the process of creating a multichannel image. Once the multichannel image is created, you can use the tool to get normalized values.

To use the Normalization tools with a single image

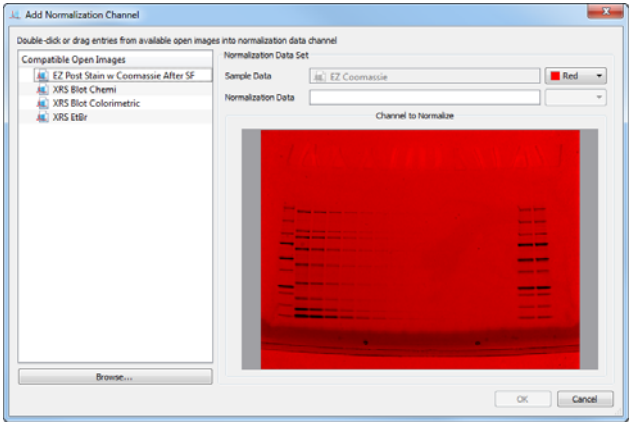
1. Click Normalization in the Analysis Toolbox.



2. Click Add Channel to open the Add Normalization Channel window.

   Note: The image that is selected when you click Add Channel is the first image in your multichannel image.

   The selected image appears as the first image in the Sample Data field.



3. To add a second image, double-click on an image from the Compatible Open Images list.

   If no images appear in the list or you want to use a different image, click Browse to navigate and select the desired image.

   Note: Image Lab checks to see if the image has the same aspect ratio as the Sample Data image. If the aspect ratio is not compatible with the Sample Data image, it will not appear in the list of Compatible Open Images. The aspect ratios may not be compatible if they were created using different instrument models (for example, Gel Doc EZ and ChemiDoc MP) or if the images are cropped at different aspect ratios.

   Tip: You can move an image to the Normalization Data box by dragging it from the Compatible Open Images list, and you can remove it by dragging it back to the list.

4. Click OK.

   The Auto-Analysis window appears prompting you to detect the lanes and bands in your images.

5. In the Auto-Analysis window, select the level of sensitivity and click OK.

6. Follow the Normalization Steps listed in the pane to get the best results.

See Optimizing Normalization of Volume Data on page 89 for an explanation of each step in the process.

Molecular Weight Analysis Tools

Molecular Weight Analysis Tools enable you to determine molecular weight (or base pairs, if 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 each channel in your image.

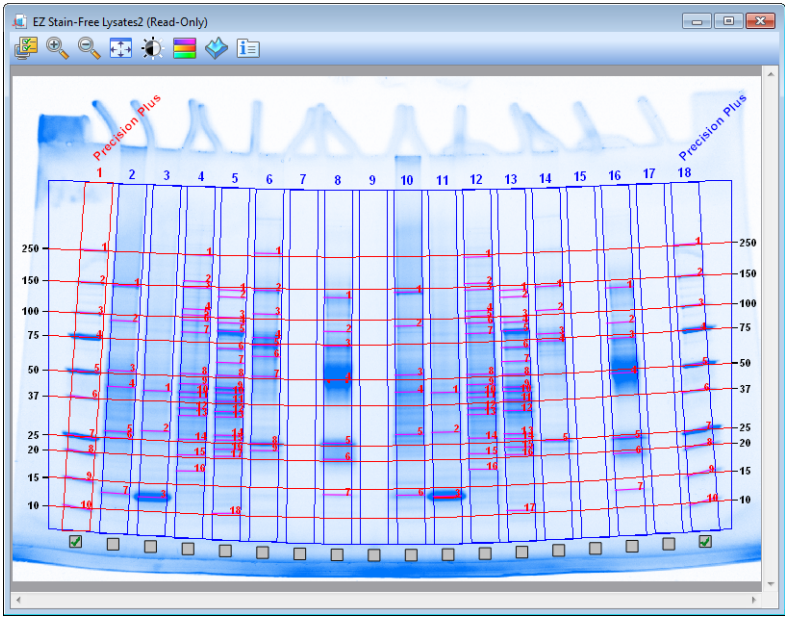
You can view each band's molecular weight in the molecular weight column of the Lane and Band tab in the Analysis Table view.

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

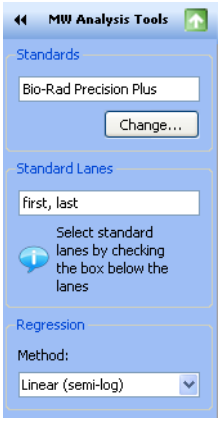
Lane And Band Table | Volume Table

You can also display the molecular weight of the bands on the gel image by opening the Display Gel Options window and selecting Mol. Wt from the Band Attributes section. (See Displaying Gel Images on page 56 for information on displaying band attributes.)

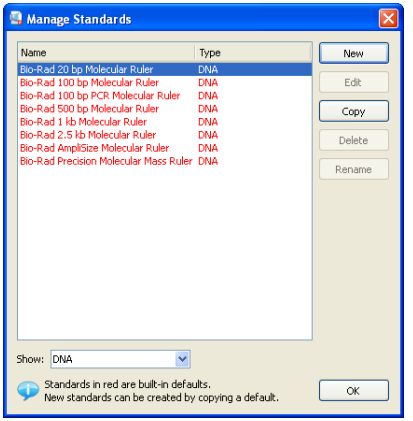


Molecular Weight Standard

You can change the standards that determine the molecular weight of test samples.



Click Change to access the Manage Standards dialog box, where you can choose another standard or add your own standards.



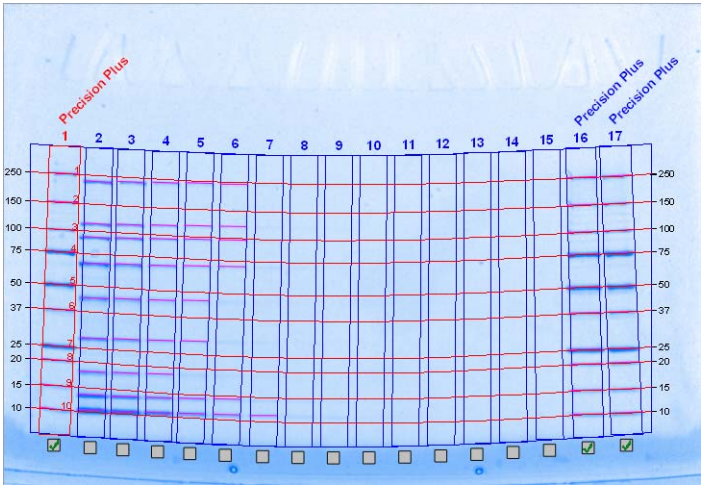
Standard Lanes

Standard samples are placed in the first and last lanes by default. 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 Lane and Bands view, *Std* appears below the lanes, identifying them as standard lanes. In the Molecular Weight Analysis view, these lanes are indicated with a check mark below the lane.

Molecular Weight Analysis in Single-Channel Images

The screen shot below shows a single channel with the molecular weight calculated. There are three standard lanes on both ends of the frame (lanes 1, 16, and 17). Standard lanes are identified with a check mark in the boxes below the lane. The molecular weights of the bands in the standard lanes appear on either side of the lane frame. The red lines running from one end of the lane frame to the other end identify the location of the bands in the standard lanes. You can use these lines to see where the bands in the other lanes fall relative to the bands in the standard lanes.

Note: You can use the lane labels to identify your standard lanes.



The screen shot below shows the molecular weight for the first four lanes in the image. The values for the molecular weight in any standard lane appear in bold. In this example, the values for the first lane are in bold. (Lanes 16 and 17 are not included in the screen shot, but their values would also be in bold.) The molecular weight of the bands in the remaining lanes are 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**						

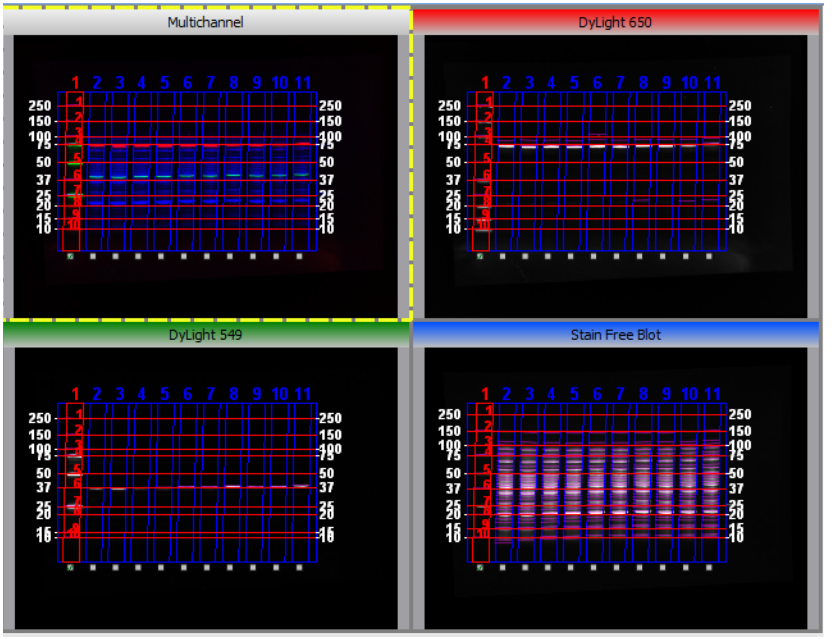
Molecular Weight Analysis in Multichannel Images

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, then 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 is used to detect the bands in this standard lane and 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.

With unlinked multichannel images, each channel is treated independently as if it were a single-channel image. You select a standard lane for each channel and the molecular weight is calculated for only the bands in that channel.

In the following screen shot, 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 are displayed in all channels.



The molecular weights of the bands in the three channels are calculated based on standards established in lane 1 and are displayed in the analysis table.

Lane 1			Lane 2			Lane 3			Lane 4
Channel	Band No.	Mol. Wt. (KDa)	Channel	Band No.	Mol. Wt. (KDa)	Channel	Band No.	Mol. Wt. (KDa)	Channel
DyLight 650	1	250.0	DyLight 650	1	85.8	DyLight 650	1	86.6	DyLight 650
DyLight 650	2	150.0	DyLight 650	2	73.6	DyLight 650	2	72.3	DyLight 650
DyLight 650	3	100.0	DyLight 549	1	37.0	DyLight 549	1	36.8	DyLight 549
DyLight 650	4	75.0	Stain Free Blot	1	146.7	Stain Free Blot	1	142.5	Stain Free Blot
DyLight 650	5	50.0	Stain Free Blot	2	110.9	Stain Free Blot	2	108.4	Stain Free Blot
DyLight 650	6	37.0	Stain Free Blot	3	100.7	Stain Free Blot	3	100.0	Stain Free Blot
DyLight 650	7	25.0	Stain Free Blot	4	89.4	Stain Free Blot	4	87.3	Stain Free Blot
DyLight 650	8	20.0	Stain Free Blot	5	78.7	Stain Free Blot	5	76.2	Stain Free Blot
DyLight 650	9	15.0	Stain Free Blot	6	75.0	Stain Free Blot	6	73.2	Stain Free Blot
DyLight 650	10	10.0	Stain Free Blot	7	65.4	Stain Free Blot	7	64.2	Stain Free Blot
DyLight 549	1	250.0	Stain Free Blot	8	58.7	Stain Free Blot	8	58.0	Stain Free Blot

Channel number

Regression Methods

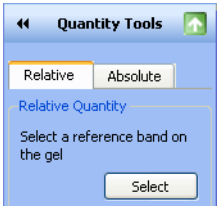
There are four regression methods. See Regression Methods on page 43.

Quantity Tools

You can quantify bands in test samples automatically using either the Relative or Absolute tabs under Quantity Tools.

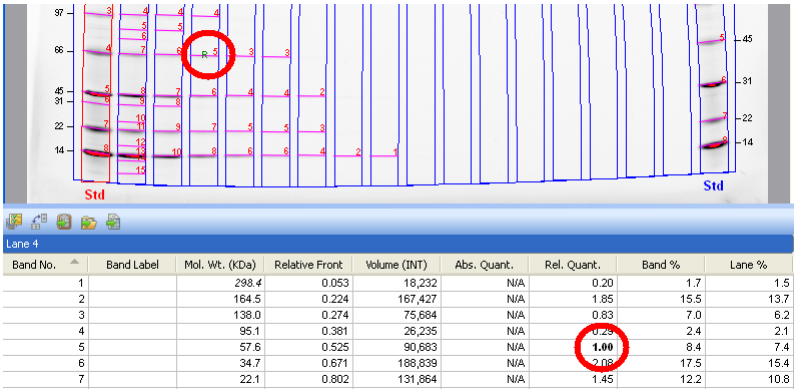
Relative Quantity Tab

To compare the relative quantities of bands, first select the Relative tab.



To select a reference band from an image and quantify all other bands relative to it

1. Click the Select button.
2. 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

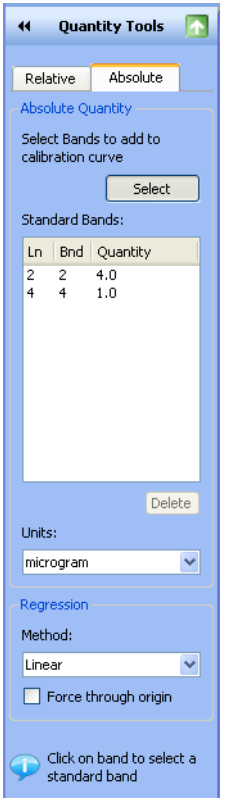
To review the relative band quantities

- 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. Values above 1.00 indicate that the band quantity is greater than the reference band. Values below 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 determine the absolute quantities of bands, first select the Absolute tab.



To calculate the absolute quantities of the bands

1. Click Select.
2. Select at least two standard (known) bands and assign quantity values. The values display 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. To do so, select the entry in the Standard Bands field by clicking it and then clicking Delete.
3. Select a unit of measure from the choices listed in the Units dropdown list.
4. Select a regression method from the dropdown list.

   Keep the following guidelines in mind while making your choice.

   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.

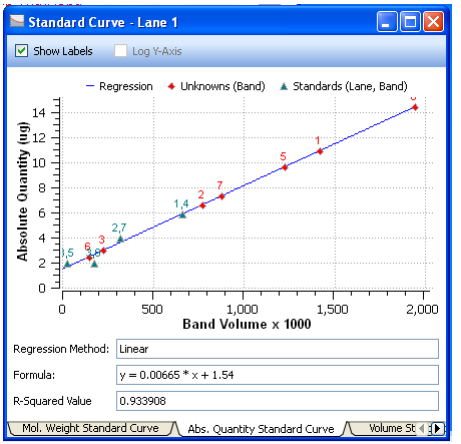
   Cubic spline — generates a smooth curve that connects each data point. At least four standard points are required to use this method of least-squares polynomial fits.

Regression Method	Minimum Number of Standard Bands	Minimum Number with Force Through Origin Option
Linear	2	1
Point-to-point	2	1
Cubic spline	5	4

5. Click Standard Curve in the toolbar, select the Absolute Quantity Standard Curve tab, and a calibration curve displays. Standards are

represented by green triangles. Unknown values are represented by red triangles.

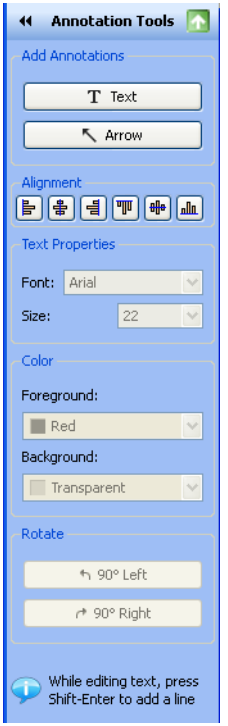
Note: Selecting the Force Through Origin checkbox always starts the standard curve graph at 0,0, regardless of the best curve fit.



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 draw attention to areas of interest.



Add Annotations

- **Text** — you can add text annotations to gel images to draw attention to important details. First click Text, and then click an area you want to emphasize. A text box appears with a dotted-line border. Type your comment in the text box. Drag the box to change its position.

  Note: While editing text, press SHIFT+ENTER to add text. This adds a new line in the annotation.

- **Arrow** — to add an arrow, first click Arrow. Click the area where you want the arrow to start and then drag to stretch the arrow point to the location you want to emphasize. To move the arrow, click the middle and drag it. 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

Alignment Buttons — these buttons enable you to align multiple annotations, such as lane numbers, which you have manually added.

To select annotations — you can select several annotations by holding down the CTRL key (Command key on the Mac) and clicking each one or by dragging a selection box around them.

To copy annotations — you can copy annotations within or between images. First select the annotation you want to copy. Then press CTRL+C to copy the annotation and CTRL+V to paste it.

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

Text Properties

You can change the size and type font of your text annotations. To select multiple items for change, hold down the CTRL key (Command key on the Mac) and click each item.

- **Font** — first click the text box you want to change. Then open the dropdown Font menu to show all fonts installed on your system. Click one of the font names to change the font of the text annotation.
- **Size** — first click the text box you want to resize. Click the dropdown Size list to increase or decrease the size of the text. You can set the font size between 6 and 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, hold down the CTRL key and click each item.

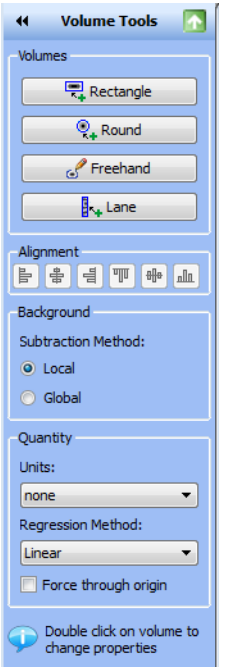
- **Foreground** — click a text annotation or arrow. This activates the Foreground field, so you can select a foreground color from the dropdown list.
- **Background** — click a text annotation. This also activates the Background field so you can select a background color from the dropdown list.

Rotate

You can rotate text annotations 90° to the left or right by clicking the Rotate buttons.

Volume Tools 

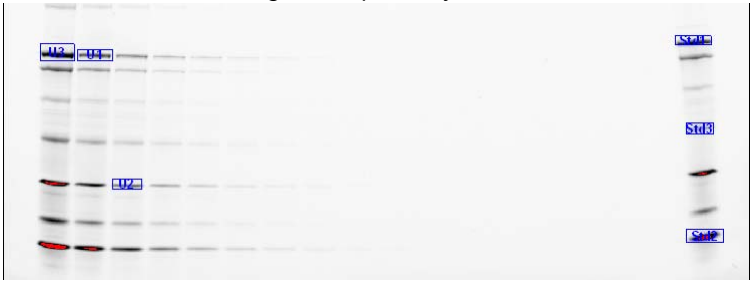
Volume tools enable you to manually quantify features on a sample image when automated lane and band analysis is not appropriate or possible, such as in dot blots.



Note: The analysis table displays the color-coded volume drawn for each channel of 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 a rectangle, circle, freehand, or lane shape by clicking the appropriate button under the Volumes field.

A default label appears within the shape drawn. The volume label can be one of three types—U for unknown, Std for standard, or B for background—along with a number that is assigned sequentially.



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. See Volume Definitions on page 70.

  Note: In multichannel images additional column bars, channel numbers, and volumes are color-coded based on their channel association.

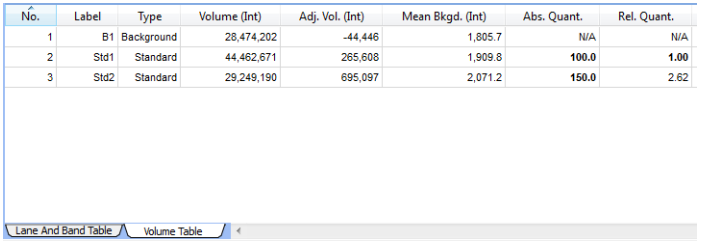
VOLUME TYPES

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. See Absolute Volume Quantity on page 113.

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



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 pixels may be included inside the volume. These background pixels usually have an intensity value that you do not want to include in your volume quantification. There are two ways of calculating this background intensity for your 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 may 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. This opens the Volume Properties dialog box.
3. Select the Background option button.

Notes:

- 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 those background volumes are used to calculate the average background. Your background volume(s) will have default names 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 your data object, you obtain a negative value for your adjusted volume in the analysis table. If this happens, select a new background region with less intensity than your 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 above 1.0 indicate that the volume is greater than the reference volume. Values below 1.0 indicate the volume is less than the reference volume.

Absolute Volume Quantity

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

If you have drawn your volume around an object of known quantity, you can use it to calculate the quantity of your unknown volumes. The quantities of your 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. This opens 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.

Standard volumes will have the default names S1, S2, and so on, based on the sequence of their creation.

To review the regression curve

- Open the Standard Curve window and select the Volume Standard Curve tab.

Regression Methods

Three regression methods are available to generate the volume quantification curve used for absolute quantity: linear, point-to-point, and cubic spline. To display the standard curve, click the Standard Curve button in the toolbar and select the Volume Standard Curve tab in the Standard Curve dialog box. See Regression Calculation Methods to learn how each of these methods is calculated.

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

> **Note:** Selecting the Force Through Origin checkbox always starts the standard curve graph at 0,0, regardless of the best curve fit.

Alignment

Align volumes by using the appropriate alignment button. To select several volumes, CTRL-click each one or drag them to use a selection box.

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

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

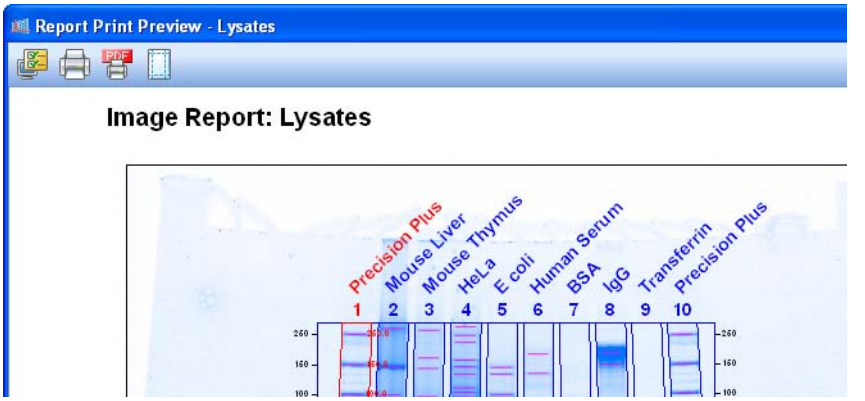
7 Generating Reports

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.

The print report settings can be chosen with the Report Settings dialog box in the Edit menu or by clicking Report in the main toolbar.

Report 

Click Report to produce a Print Preview of your report.

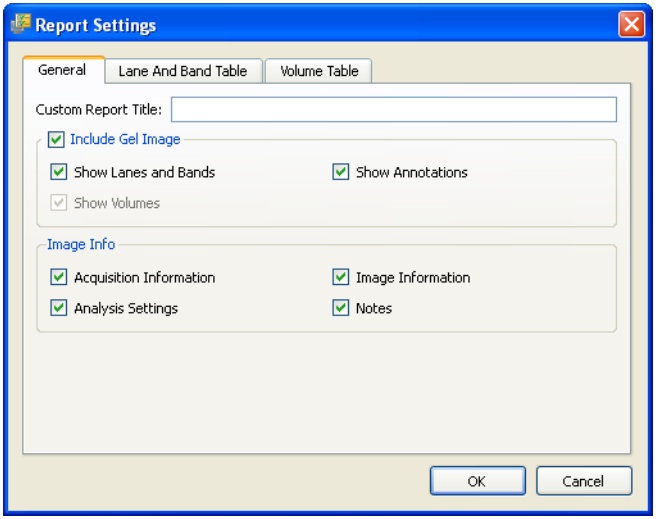


Clicking Print sends all of the above information to a printer.

Use the following dialog boxes to remove information from reports. Doing so does not delete the data from the analysis.

General Tab

By default all information is included, but you can customize reports here.



Clear any of the following settings to exclude information from a report.

Custom Report Title — you can provide a custom report title for the report.

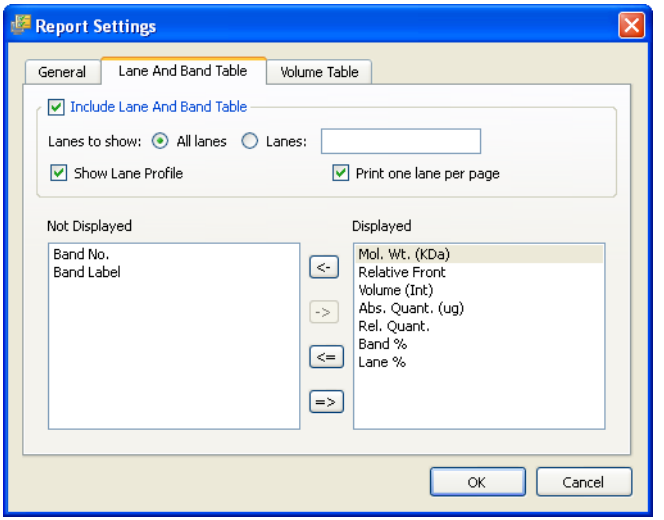
Include Gel Image — the following options determine which overlays display on the gel image.

- Show Lanes and Bands
- Show Volumes
- Show Annotations

Image Info

- Acquisition Information
- Analysis Settings
- Image Information
- Notes

Lane and Band Table Tab



Clear any of the following settings to exclude information from the report.

Include Lane and Band Table

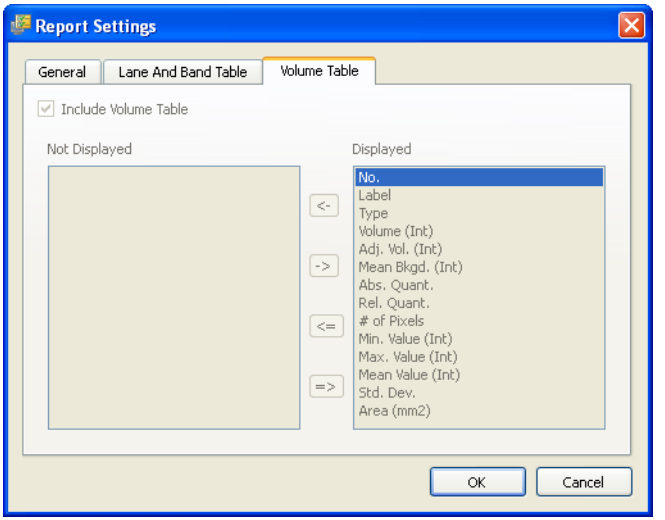
Lanes to show

- All lanes or only user-defined lanes
- All lanes on one page, or one lane per page (add page break after each lane)

Show Lane Profile — includes the lane profile for each lane.

Not Displayed/Displayed — move items that you do not want to include in the report to the left column.

Volume Table Tab



Include Volume Table — clear to exclude this information from your report.

Not Displayed/Displayed — move items that you do not want to include in the report to the left column.

Print Report 

Click the Print Report icon to print your report.

Print Report to .pdf File 

The Print Report to .pdf File icon brings up a Save dialog box so the .pdf file can be saved on your system.

Adjust the Printer Settings 

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

8 Exporting Results

The most convenient way to archive complete information about experiments is to produce reports. However, you may want to export only gel images or analysis table data for analysis in different programs, such as Quantity One®, FPQuest™, or InfoQuest™FP software. Or you may need exported files for presentation or publication.

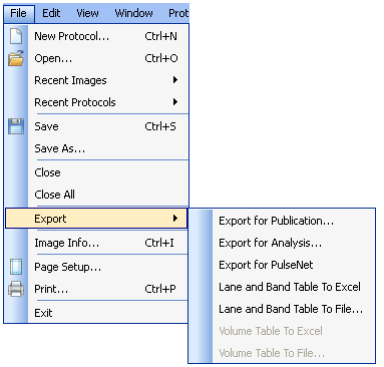
Exporting Gel Images

Image Lab™ software includes features for exporting gel images several ways.

- You can export displayed image data to a publication (choose Export for Publication).
- You can export raw image data as a 16-bit TIFF file (choose Export for Analysis).
- You can export image data to PulseNet; doing so reduces the image to an 8-bit TIFF file, limits its resolution, and restricts its file size to 300 Kb.
- You can export lane and band tables as well as volume tables to a spreadsheet program or to a file.

The options to export gel images are available using the Export option in the File Menu.

The available export options are described in the following sections.

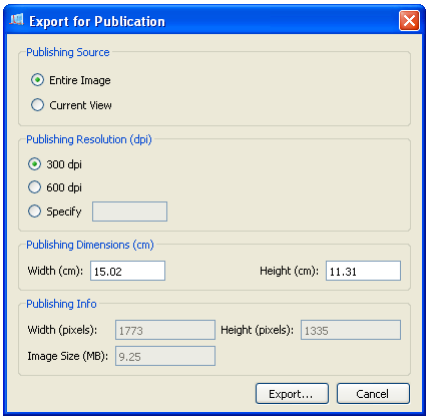


Exporting Gel Images for Publication

Use this format only to export visual information to presentation or word processing software, such as PowerPoint or Word.

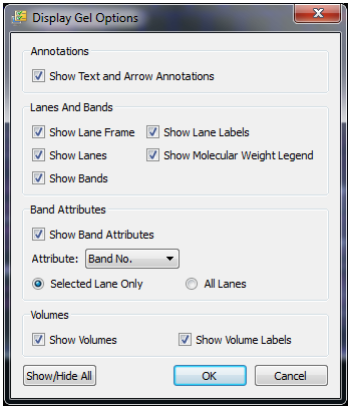
When you select File > Export > Export for Publication, you can export a displayed image to a file. You can select from .bmp, .png, .jpg, and TIFF formats. The gel displays with any lanes, bands, and annotations that appear on the screen.

> **Note:** For a multichannel image, select the image pane you want to publish before exporting the image for publication. Image Lab software exports the active pane.



Note that you can select the entire image or the current view, select the resolution or specify a custom resolution, specify the publishing dimensions, and view the resulting published image size and dimensions.

You can zoom in on an area in a current view to export only that area, or you can export the entire image. You can exclude annotations or overlays by clicking Display Gel Options in the toolbar to access the appropriate settings.



Exporting Gel Images for Analysis

When you select File > Export > Export for Analysis, you can export full image data (raw data only) for analysis as a 16-bit TIFF file.

> **Note:** 16-bit TIFF images are not compatible with all image viewers.

The image may 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, FPQuest, or InfoQuestFP software.

> **Note:** For multichannel images, Image Lab software exports the separate channel images, but not the multichannel image. Each exported channel image is saved with its application name appended to the filename you selected.

Exporting Gel Images to PulseNet International

When you select File > Export > Export for PulseNet, Image Lab software reduces the image to an 8-bit TIFF 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 Macintosh) installed on your computer, choosing File > Export > Lane and Band Table to Excel opens a table directly in the spreadsheet program. You can then use the Save As option to produce other formats.

Exporting Volume Tables to File

Choosing Export > Volume Table to File exports as a CSV file so the data file can be opened in a database application.

Screenshot Tool Export

Use the Screenshot tool, available in the toolbar, to capture a displayed image to the clipboard or to save it to a file (.bmp, .gif, or .png).

Analysis Table Export

You can export table analysis data from the File menu or by using the export buttons at the top of the Analysis Table window.

The Analysis Table window has several buttons to export data to different formats, depending on how the data are to be presented.

Copy Analysis Table to the Clipboard 

Click this button to copy the analysis table to the clipboard and then paste the analysis table into word processing or presentation applications. It is best to use the vertical table orientation when copying to an 8.5 x 11-inch page to accommodate the columns.

Exporting Analysis Table to a File 

This button exports an analysis table as a CSV (comma-separated values) file, so your data file can be opened in a database application.

Export Analysis Table to a Spreadsheet 

This button enables you to use Excel's sort and formula functions with your data. If you have Excel (or Numbers on a Mac) installed on your computer, the data open in the spreadsheet program.

9 Maintenance

This chapter includes instructions for maintaining the universal hood in proper working condition by replacing parts.

UV Transilluminator Lamp and Starter Replacement

> **Note:** The UV filter surface should always be kept clean from the chemical agents used as gel dyes. Use protective gloves when touching the UV transilluminator cover.

Depending on usage, the UV bulbs and starters last for many years. Replace bulbs when you notice them flickering. If a bulb does not turn on when it is new or moved, replace the bulb starter and test the bulb again.

Three types of bulbs are available. The catalog numbers are listed in Ordering Information on page 118. The standard bulb is 302 nm. Optionally, the 254 nm bulb is used for cross-linking of protein, and the 365 nm bulb is used to minimize denaturing of DNA.

To replace the lamps

1. Turn off the power.
2. Disconnect the power cord from the universal hood.

3. Remove the four screws located on the left/right sides of the transilluminator cover.
4. Remove the cover with the UV glass by sliding it forward, then lifting up.
5. Place it on a nonabrasive surface so that the glass does not get scratched or damaged.

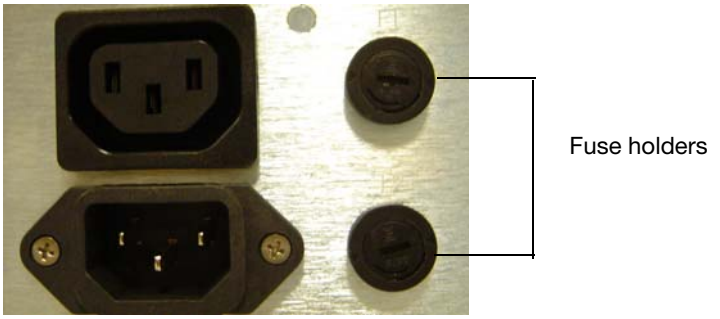
   Note: Do not put the UV cover directly on the bench. Wear gloves when touching the lamps.

6. Rotate the lamp until it becomes loose and the pins come to a vertical position.
7. Remove the lamp. Install the new lamp by rotating so that the pins are horizontal and the lamp is tight.

8. Refer to the pictures above to replace a starter. Remove the starter by rotating it counterclockwise, and then pull it out.
9. To replace a starter, insert it into the holder and rotate clockwise.
10. Reassemble the cover and retighten the screws on both sides.

Fuse Replacement

Always unplug the instrument before changing or checking the fuses.



This unit is protected by 2 fuses (5 x 20 mm, 2 A Slo-Blo). The fuses are located in fuse holders housed in the power entry module. This module is located on the right side of the back of the universal hood.

To replace the fuses

1. Unplug the main power cable from the power outlet.
2. Use a flat screwdriver to turn the slotted front of each fuse holder counter clockwise; the holder pops out so you can extract the fuse.
3. Remove the blown fuses and replace them with two new ones (catalog #900-8935).
4. Slide each fuse holder into the power entry module until it snaps in place.

A Using the Criterion Stain Free™ System

The Criterion Stain Free system comprises the ChemiDoc™ MP imager, Image Lab™ software, and three types of precast gels:

- Criterion™ TGX Stain-Free™
- Criterion Stain Free
- Mini-PROTEAN® TGX Stain-Free™

The stain-free system eliminates the time-consuming staining and destaining steps required by other protein detection methods. Stain-free gels include unique trihalo compounds that allow rapid fluorescent detection of proteins with the ChemiDoc MP imager—without staining.

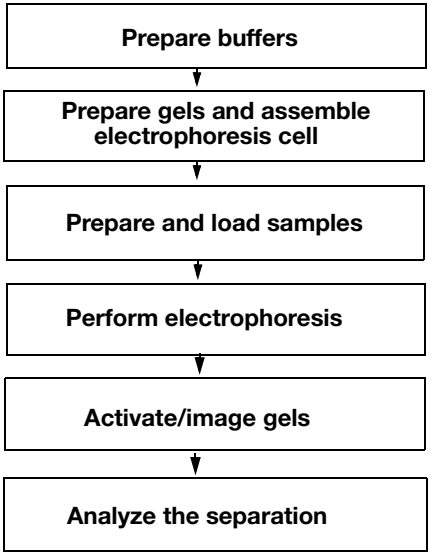
The trihalo compounds in the gels react with tryptophan residues in a UV light–induced reaction to produce fluorescence, which can be easily detected by the ChemiDoc MP imager within gels or on low fluorescence PVDF membranes. Activation of the trihalo compounds in the gels adds 58 Da moieties to available tryptophan residues and is required for protein visualization. Proteins that do not contain tryptophan residues cannot be detected using this system. The sensitivity of the stain-free system is comparable to staining with Coomassie Brilliant Blue for proteins with a tryptophan content >1.5%; sensitivity superior to Coomassie staining is possible for proteins with a tryptophan content >3%.

Benefits of the stain-free system include:

- Elimination of staining and destaining steps for faster time to results
- No background variability within a gel or between gels (as is often seen with standard Coomassie staining)
- Elimination of the need for acetic acid and methanol in staining and destaining, which reduces organic waste
- Visualization of transferred or blotted proteins on low fluorescence PVDF membranes

Stain-Free Workflow

For detailed information about the Activate/image gels step, refer to Chapter 4, Acquiring Images, on page 25. For all other workflow steps, refer to the Criterion™ Precast Gels Instruction Manual and Application Guide or to the Mini-PROTEAN® Precast Gels Instruction Manual and Application Guide.



Electrophoresis with Stain-Free Gels

Stain-free gels are made and packaged without SDS so they can be used for both SDS and native PAGE applications.

To perform electrophoresis with these gels, prepare the sample and running buffers, set up the electrophoresis cell, and perform the run.

Imaging Gels

Use unstained standards with stain-free gels, as some prestained standards are not detected by the stain-free system. To monitor electrophoresis, use a 1:1 mixture of unstained and prestained standards.

Setting up a protocol for stain-free gels is very similar to setting up protocols for other applications. Follow the instructions in Creating a Protocol, on page 29. Choose one of the following activation times based on your sample and the purpose of your experiment:

- **Gels used in blotting** — use 1 min activation for optimal results when using western blotting followed by immuno detection.
- **Good sensitivity** – use 2.5 min activation when samples are abundant and when a fully optimized signal-to-noise ratio is not necessary.
- **Best sensitivity** – use 5.0 min activation for detection of proteins that are in low concentration and for the best quantification of the maximum number of bands. Because the reaction is near completion after 5 min, this method offers an optimal signal-to-noise ratio.

  Note: If the gel has already been activated for 2.5 min, activating it for another 2.5 min may improve it; but activating an image for more than 5 min will not.

Imaging Blots

To blot stain-free gels, use standard blotting procedures as described in the instruction manual you are using. Use only PDVF membranes with low background fluorescence, as membranes other than low fluorescence PDVF can result in high background or low sensitivity with the ChemiDoc MP imager.

To assess transfer efficiency, be sure to activate and visualize the gel using the ChemiDoc MP imager before transfer.

B Troubleshooting

Follow these suggestions to troubleshoot your ChemiDoc™ MP system.

Problem	Possible Cause	Solution
ChemiDoc MP System		
Camera does not respond/camera not found	• Camera power may be turned off. • Camera cables may not be seated properly. • Software driver for the camera is missing. • Computer power-saving modes may be interfering with the camera driver. • Cables may be defective. • 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 camera driver is not present, reload the camera driver from the Image Lab™ software CD. • Disable power-saving modes on the computer. • Replace cables. • Replace camera.
Horizontal stripes in image when using the UV mode	• Emission filter not in proper position	• Move filter lever so that the filter slider positions the filter under the camera lens.
Image is not visible on the monitor	• Incorrect monitor settings • Lens cap attached	• See your computer manual for settings. • Remove lens cap.
Image is not bright enough	• Wrong emission filter in use. • For chemiluminescence, emission filter is in front of lens.	• Verify correct filter for application. • Verify no emission filter is in front of lens.

Problem	Possible Cause	Solution
Printout does not look like the monitor image	• Monitor settings are wrong • Printer settings are wrong	• Refer to monitor instructions to correct settings. • Refer to the printer manual to correct settings.
Light leakage into the darkroom	• The lens body is not seated properly against the gasket on the hood's adapter plate.	• Loosen the thumbscrew and seat the lens properly against the gasket on the hood's adapter plate.
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 focus using the proper accessory.
Lens limits seem artificially restricted	• Camera lens not seated properly on lens mounting plate	• Reseat camera on lens mounting plate.

C Accessories

Calibrating Accessories

If you are installing accessories along with your original system installation, calibrate your system with a one-time Instrument Calibration wizard. Complete instructions are in the installation guide that arrives with your system. If you acquire new conversion screens, light sources, or filters for a ChemiDoc™ MP imaging system after your original system installation, you will have to recalibrate your system to use them.

See Chapter 2, System Calibration, for instructions on how to calibrate newly acquired accessories.

Installing Optional Accessories

Epi Light Modules

Epi light modules are available for the ChemiDoc MP system in three colors: red (catalog #170-8283), blue (catalog #170-8285), and green (catalog #170-8284). For installation instructions, see Installing an Epi Light, an instruction sheet that accompanies each module.

UV/White Light Conversion Screen

This optional white light conversion screen (catalog #170-8001) converts the UV light generated in the universal hood to white light. Your system must be calibrated to use the white light conversion screen.

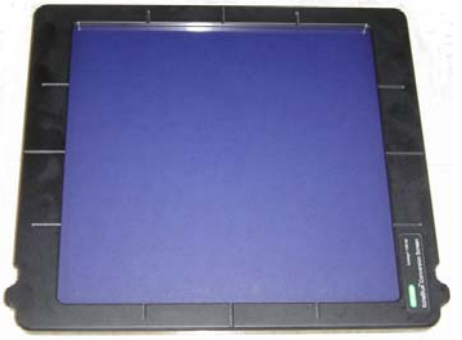
To calibrate, go to the Edit > Instrument Setup dialog box and select the appropriate checkbox. The Image Lab™ software guides you through the calibration.

To use the white light conversion screen

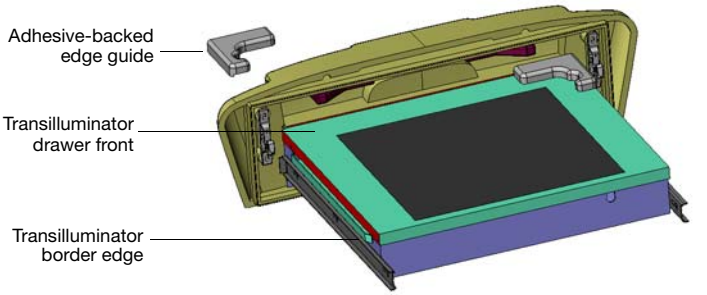
1. Center the conversion screen on the imager stage.
2. Center your samples on top of the conversion screen.
3. Image the gel using your preferred application.

XcitaBlue™ Conversion Screen

The optional XcitaBlue conversion screen kit (catalog #170-8182) converts UV to blue light, which enables you to visualize DNA samples while protecting them against UV damage.



The XcitaBlue conversion screen is held in place by adhesive-backed edge guides. After the edge guides are installed, the conversion screen remains centered and will not slide, even if you close the drawer rapidly.



To install the XcitaBlue conversion screen

1. Perform a trial placement first, without removing the paper tape. Place the edge guides in each corner of your transilluminator, as shown. The

edge guides should touch the inside of the drawer front and fit over the edge of the metal transilluminator border (shown in red).

2. Calibrate your system to use this accessory by going to Edit > Instrument Setup. Select the XcitaBlue Conversion Screen checkbox under Illumination Options. The software prompts you to calibrate the focus with height offset.
3. To visualize a sample using the XcitaBlue conversion screen, place the screen between the edge guides.
4. Center the gel on top of the XcitaBlue conversion screen, and proceed with normal image capture. Use the gel alignment template kit to center your gels easily and consistently.

Gel Alignment Template Kit

The Bio-Rad gel alignment template kit (catalog #170-8184) allows four sizes of standard agarose gels to be centered quickly and easily and ensures the consistent placement of each gel.

7 x 7 cm | 15 x 7 cm | 7 x 10 cm
15 x 10 cm

The kit contains:

- Magnetic locator frame
- Instruction sheet
- Alignment guides for the following gel trays:

  Sub-Cell® GT UV-transparent mini-gel tray, 7 x 7 cm

  Sub-Cell GT UV-transparent wide mini-gel tray, 15 x 7 cm

Sub-Cell GT UV-transparent mini-gel tray, 7 x 10 cm

Sub-Cell GT UV-transparent gel tray, 15 x 10 cm

The gel alignment templates fit exactly into the XcitaBlue conversion screen frame (catalog #170-8183).

To install and use the gel alignment template kit

1. Place the locator frame over the transilluminator with the magnetic side down. Match the corners of the magnetic locator frame with the edges of the transilluminator. The UV symbol on the frame will be in the same orientation as the UV symbol on the imager.
2. Place the gel alignment template that matches the size of your sample tray or agarose gel into the magnetic locator frame.
3. Place your gel or gel tray into the open area of the template.

   Note: No recalibration is necessary to use the gel alignment template kit.

Orange Fluorescence Reference Plate

The orange fluorescence reference plate allows you to apply UV flat fielding corrections to your ChemiDoc MP imager. Corrections are made for all UV illumination sources, filters, and the camera lens.

The orange fluorescence reference plate can be used to correct for image nonuniformities for red and orange gels, including:

- Ethidium bromide
- GelRed
- Flamingo™ fluorescent gel stain
- Coomassie Fluor Orange
- SYPRO Ruby protein blot stain
- Krypton
- Qdot 625

The orange fluorescence reference plate (catalog #170-8008) can be used with several of the Bio-Rad® Molecular Imager® series of products, including:

- ChemiDoc MP system with Image Lab software
- ChemiDoc XRS+ system with Image Lab software
- ChemiDoc XRS+ system with Quantity One® software

When you order the reference plate, you receive:

- Orange fluorescence reference plate, overall dimensions: 29.5 x 29.5 cm; viewing surface: 27 x 27 cm
- Instruction sheet (10017296)

Calibrating the System to Use the Orange Fluorescence Reference Plate

Your system must be calibrated to use the orange fluorescence reference plate.

To calibrate, go to the Edit > Instrument Setup dialog box and select the appropriate checkbox. Image Lab software guides you through the calibration.

Ordering Information

The following table contains catalog numbers and descriptions for all parts available for the ChemiDoc MP system, plus all optional accessories and replacement parts. For more information, see the Bio-Rad catalog.

Catalog #	Description
Molecular Imager Series of Products (includes Universal Hood III, Camera, Cables, and Accessories)	
170-8280	ChemiDoc MP system
Installation Kits	
170-8282	ChemiDoc MP installation kit
Universal Hood	
170-8281	Universal Hood III
Imaging Cameras	
170-8255	ChemiDoc MP camera with motorized zoom lens
Image Lab Software	
170-9690	Image Lab software, Windows/Mac
Optional Accessories	
170-8001	UV/White light conversion screen (UV to white light)
170-8182	XcitaBlue (UV to blue light) conversion screen kit, without standard detection filter
170-8283	Kit, Red LED Module
170-8284	Kit, Green LED Module
170-8285	Kit, Blue LED Module
170-8183	XcitaBlue (UV to blue light) conversion screen kit, with standard detection filter
170-8008	Orange fluorescent reference plate
170-8089	Mitsubishi P93W Printer, 100/240 V, USB
170-3759	Bio-Rad fluorescent ruler
170-3760	Gel cutter ruler
170-8184	Gel alignment template kit
Replacement Parts	
170-8026	Image Lab focus calibration target
170-8027	Image Lab flat fielding disc
170-8185	XcitaBlue viewing goggles
170-7581	Mitsubishi thermal printer paper, 4 rolls

Catalog #	Description
170-7813	Sample holders for gels
100-2784	UV transilluminator lid (includes UV filter glass)
1001-4106	Thumbscrew for camera
170-8081	Filter, standard emission, 62 mm
100-1370	UV bulb starter, quantity 3
930-2242	Multicolor target
	Lamps
100-1361	UVB lamp, 302 nm (1 each)
170-8097	302 nm lamp kit, (6 lamps)
170-8098	254 nm lamp kit, (6 lamps)
170-6887	365 nm lamp kit, (6 lamps)
	Fuses
900-8935	Fuse T 2 A, 250 V, quantity 10
900-0234	Fuse T 4 A, 250 V, quantity 10
	Universal Hood III
100-2787	Universal Hood feet, quantity 4
170-8068	UV Shield for Universal Hood
	Connection Cables
931-0071	Cable, USB, Type A to B, 10 ft
901-0064	Cable, USB, Type A to B, 6 ft
Optional Analysis Software	
170-9600	Quantity One 1-D Analysis software
170-9300	FPQuest™ software
170-9310	InfoQuest™FP Basic Fingerprint software
Protein Standards	
161-0363	Precision Plus Protein™ Unstained Standards. 1 ml
161-0373	Precision Plus Protein™ All Blue Standards, 500 pl
161-0374	Precision Plus Protein™ Dual Color Standards, 500 pl
161-0375	Precision Plus Protein™ Kaleidoscope™ Standards, 500 pl
161-0385	Precision Plus Protein™ WesternC™ pack, 50 applications
161-0318	Prestained SDS-PAGE standards, broad range, 500 pl
161-0317	Unstained SDS-PAGE standards, broad range, 200 pl

Catalog #	Description
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
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
170-8356	EZ Load precision molecular mass ruler (base pairs/ng of sample)
Pulsed Field Standards and Markers	
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
170-3633	CHEF DNA size marker, 3.5-5.7 Mb

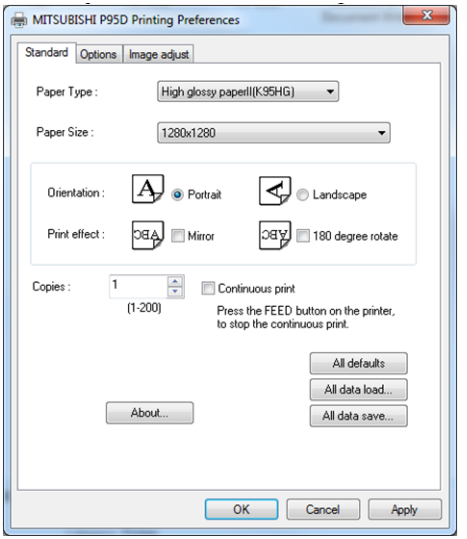
D Mitsubishi P93/P95 Thermal Printer

Setting up a Thermal Printer on Windows

The printer driver can be found on the Image Lab™ software installation CD in the Misc directory.

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.
5. Click OK to apply your changes.

Setting up a Thermal Printer on a Mac

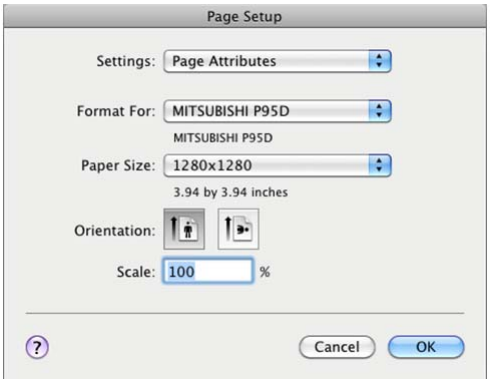
The printer driver can be found on the Image Lab software installation CD in the Misc directory.

To set up a thermal printer on a Mac system

1. Install the printer driver.
2. Connect the printer to the computer.

To configure the correct paper size

1. Start Image Lab software.
2. Select File > Page Setup.



3. In the Settings dropdown list, select Page Attributes.
4. In the Format For dropdown list, select the Mitsubishi printer.
5. In the Paper Size dropdown list, select 1280 x 1280.
6. In the Settings dropdown list, select Save as Default.
7. Click OK to save the settings.

E Regression Calculation Methods

Each regression method calculates a standard curve. Some of the 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 (semilog): 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 may 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 (semilog): 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.

Glossary

Aspect ratio: The ratio of the width to the height of an image.

CCD: (Charge-coupled device) A light-sensitive silicon chip used as a photodetector in ChemiDoc™ MP and ChemiDoc™ XRS+ camera systems.

Colormaps: Different color representations of a gel image.

Electrophoresis: A technique for separating molecules based on the differential movement of charged particles through a matrix when subjected to an electric field.

Example precision: The number of decimal places chosen for displaying a measurement.

Flat fielding: An average intensity computation that compensates for nonuniformities generated by an instrument.

Histogram: A graphed representation of the brightness, or gray value, of an image.

Native charge density: The inherent electrical charge of a protein without the addition of SDS.

pI: Isoelectric point; the pH at which a protein molecule carries no net charge.

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.

Quantitative imaging: Determines the quantity of a protein's components through analysis of the pixel values in a digital image of the sample.

UV-B: The range of ultraviolet light used by the system.

UV transilluminator: The part of the imager that transmits UV light through a sample.

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10022469 Rev C US/EG *12-0722 0412 Sig 1211*