ChemiDoc™ MP System with Image Lab™ Software

Instruction Manual

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 detail 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/)

Alexa Fluor, Coomassie Fluor, Qdot, SYBR® and SYPRO are trademarks of Invitrogen Corporation. Coomassie is a trademark of BASF Aktiengesellschaft. Cy2 and Cy3 are trademarks of GE HealthCare. DyLight and Krypton are trademarks of Thermo Fisher Scientific Inc. Excel, PowerPoint, and Windows are trademarks of Microsoft Corporation. FireWire, iWork, Mac, Mac OS, and Numbers are trademarks of Apple Inc. GelGreen and GelRed are trademarks of Biotium, Inc. Intel Core and Pentium are trademarks of Intel Corporation. Mitsubishi is a trademark of Mitsubishi Companies. PulseNet International is a trademark of Centers for Disease Control and Prevention. Slo-Blo is a trademark of Littelfuse, Inc.

CHEF (U.S. Patent Number 5,549,796, issued to Stanford University) is exclusively licensed to Bio-Rad Laboratories, Inc.

Precision Plus Protein standards are sold under license from Life Technologies Corporation, Carlsbad, CA, for use only by the buyer of the product. The buyer is not authorized to sell or resell this product or its components.

SYBR is a trademark of Molecular Probes, Inc. Bio-Rad Laboratories, Inc. is licensed by Molecular Probes, Inc. to sell reagents containing SYBR Green I for use in real-time PCR, for research purposes only.

Bio-Rad Laboratories, Inc. is licensed by Invitrogen Corporation to sell SYPRO products for research use only under U.S. Patent Number 5,616,502.

IL v. 4.0

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 quantitation.

Bio-Rad ImageLab™ 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 is a high-resolution gel documentation system that allows fast, easy quantitation 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 you 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 quantitation 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 Appendix C 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 P93DW 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 Senstivity
- 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 (cm)	28 x 36
Maximum image area (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.

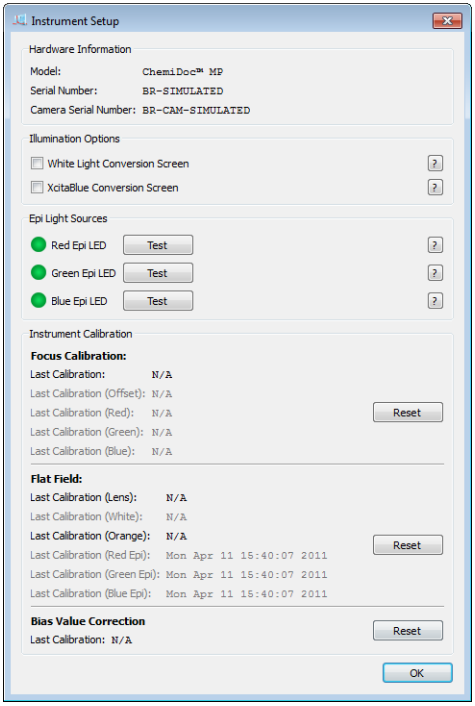
The instrument calibration wizard performs several procedures required to automate the system and prevent focus problems. Each of these calibrations affects your system as follows:

- **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 drop-down 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.

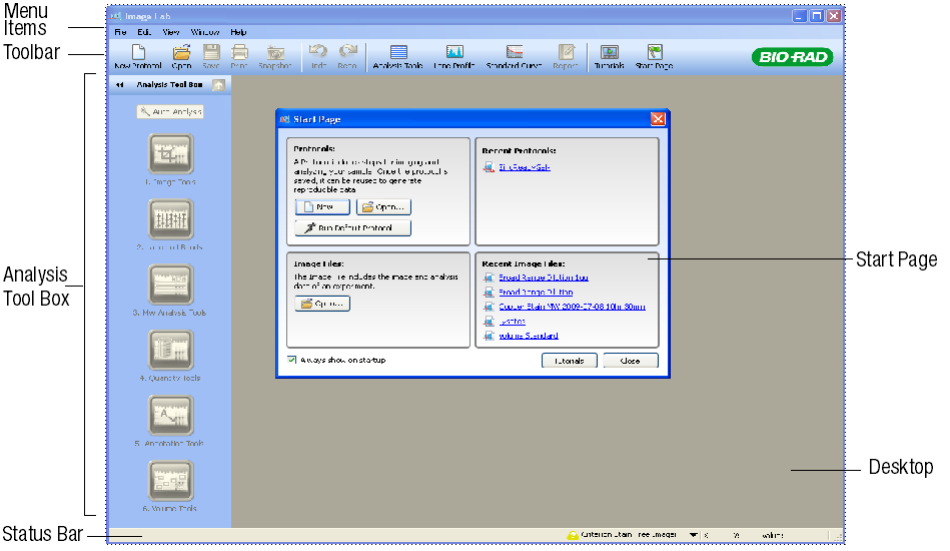
With Image Lab software you can view analyzed data, edit the analysis, and produce customized reports that show precisely the settings applied in order to ensure repeatable results.

Two types of files are generated by Image Lab software:

- Protocol files, which describe the parameters for imaging and analyzing your gel images. Protocol files are saved with a .ptl extension. Multichannel protocol files are saved with an .mptl extension
- 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.

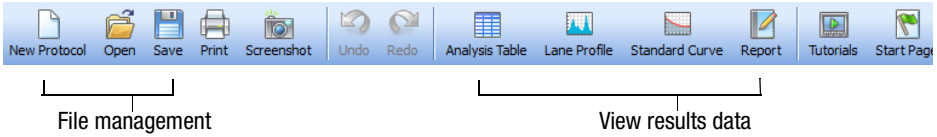
If many screens are open in the workspace, you can make one visible by clicking the title bar at the top of the selected screen. A list of open protocols and image files also appears in the Window menu; you can select one to bring it to the top.

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

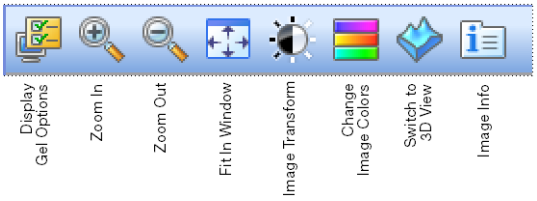
Results data associated with gel images can be viewed as an analysis table, a lane profile, a standard curve, or in a report. These views always display the analysis for the selected image.

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 60 for details.

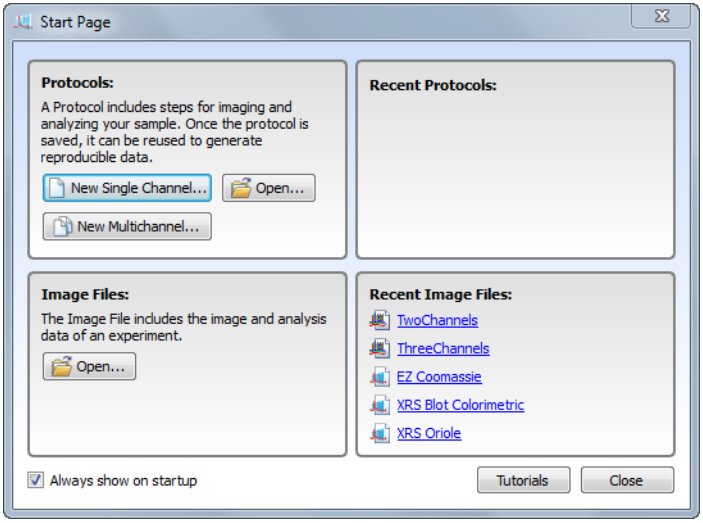
Display Toolbox

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.

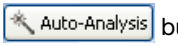


Start Page

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.

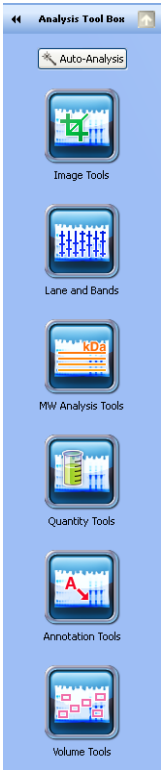


Image Tools enable you to flip, rotate, and crop images and to transform the image files.

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

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 quantitate bands, using either relative or absolute values.

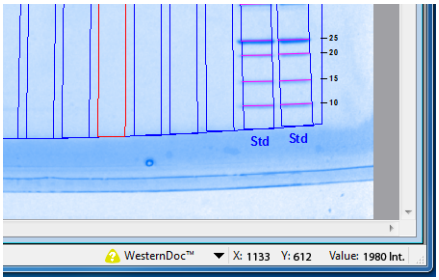
Annotation Tools are useful for drawing attention to any area of a gel.

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

These tools are describedon page 69.

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 29 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 96 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 98 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 95 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 55 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 can run 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 software, a protocol is any combination of settings for imaging, analyzing, and reporting that has been saved to run as a single workflow.

Image Lab software supports two kinds of protocol: single channel and multichannel. A single-channel protocol allows 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 allow you to choose up to three different applications (red, green, or blue), resulting in sequential imaging of a sample. These images can be combined into an RGB color composite image.

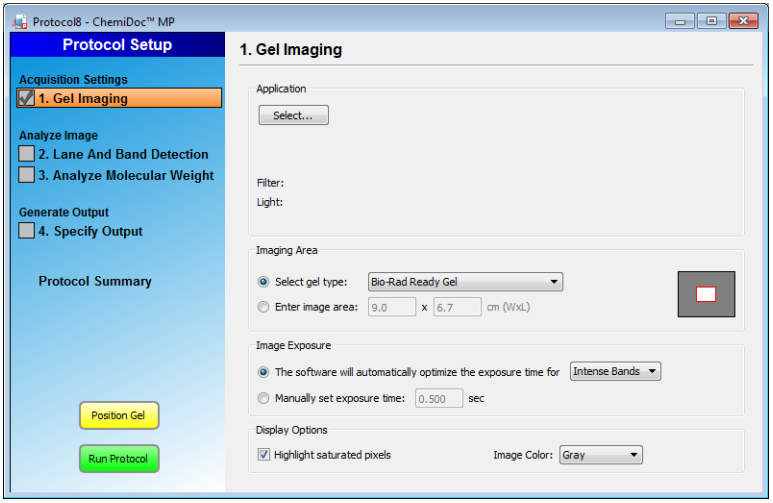
To access the Protocol Setup window:

Do one of the following:

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

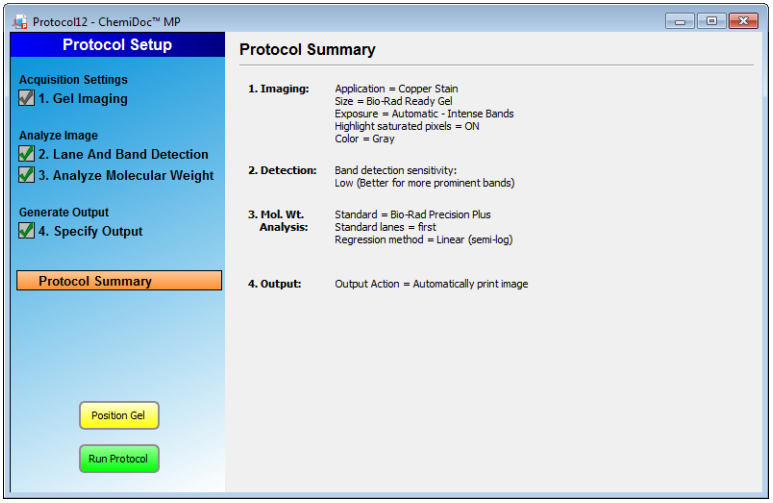
The appropriate Protocol Setup window appears for the type of protocol you selected.

The Protocol Setup Window

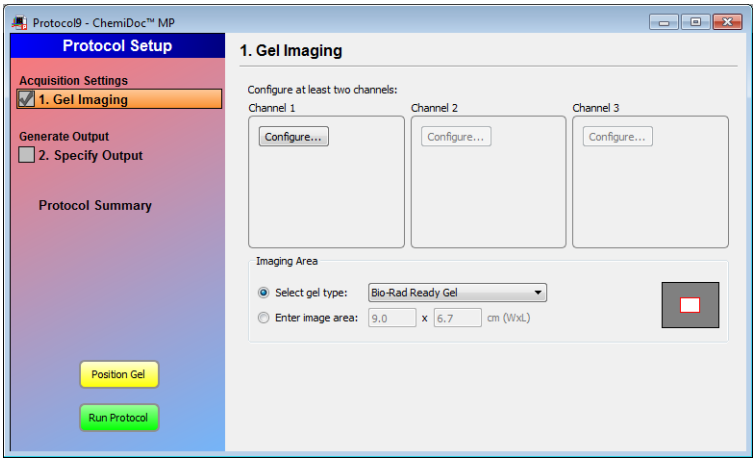


This is the Protocol Setup window for a single-channel protocol. The left pane displays headings. Under the headings are numbered protocol steps. You can enable or disable a step by selecting or clearing its checkbox. When you select a step, the right pane of the window displays the detailed settings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

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



The Multichannel Protocol Setup window has some different settings, but you work with it the same way as the Single-Channel Protocol Setup window.



Select a step in the left pane and configure that step's detailed settings in the right pane.

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

Creating a Single-Channel Protocol

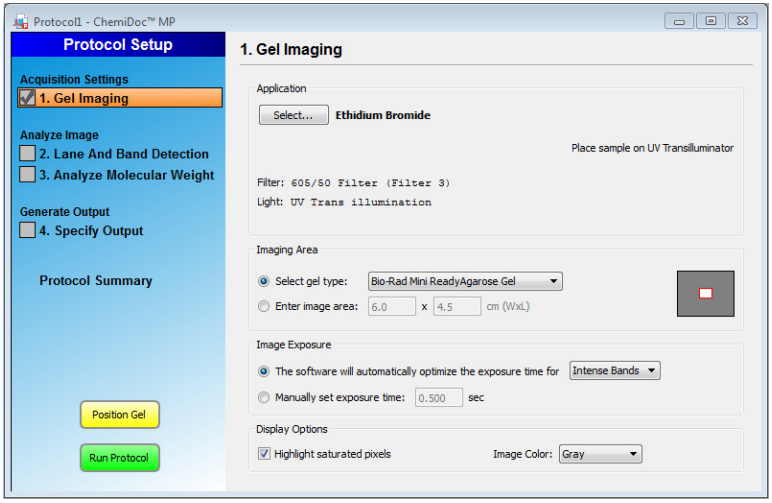
Setting up a single-channel protocol consists of three processes:

- Choosing acquisition settings
- Analyzing the image
- Generating 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.

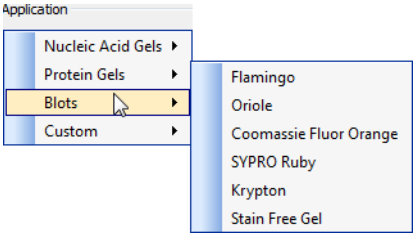
Acquisition Settings

Step 1. Gel Imaging



Application Options

1. **Choose an application** from a menu that appears when you click the Select button. 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.

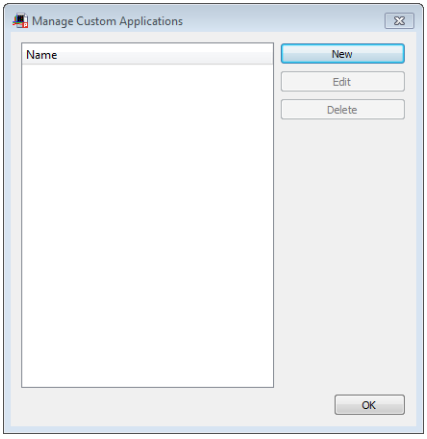


Note: For a list of applications with all required detection reagents, light sources, and any conversion screens or filters noted, see Application Tables on page 45.

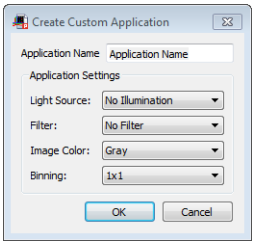
Note: If you select the Stain Free application you have the option of selecting the gel activation time. See Appendix A. Using the Criterion Stain Free™ System, for more information.

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 have stored Custom Applications, they display here.

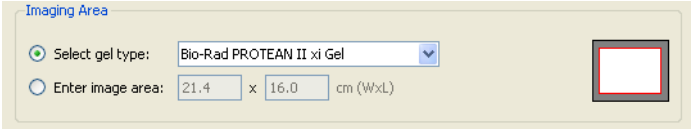
To create a new custom application, select New in the Manage Custom Applications dialog box.



The Create Custom Applications dialog box appears.



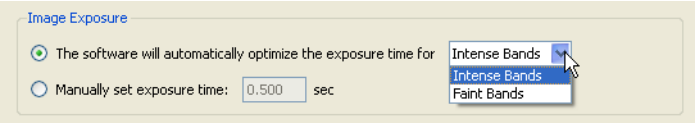
2. Choose a unique application name.
3. Select a light source, filter, and image color on the drop-down menus.
4. Select a binning setting. Choosing a higher binning setting combines pixels to increase the amount of signal without increasing noise. While a higher setting provides optimal sensitivity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



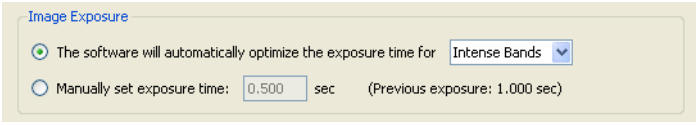
7. **Choose Image Exposure Time**—if you select the top radio button, Image Lab software acquires an image estimating an optimal exposure time. This option ensures the best use of the dynamic range.

- If you choose Intense Bands from the drop-down menu, exposure is optimized for all bands
- If you choose Faint Bands on the drop-down list, a longer exposure will make faint bands more visible, but more prominent bands may be overexposed
- Select the bottom radio button to manually override automated imaging that may cause saturation (overexposure) of more

prominent bands. Exposure time can range from 0.001 to 7200 seconds with the ChemiDoc™ MP system

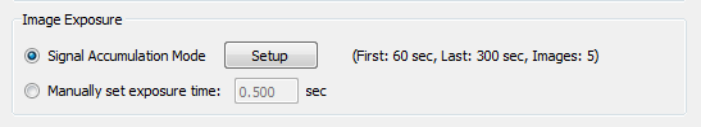


After imaging a gel with automatic exposure optimization, the exposure time displays in the protocol (see below) so you can manually adjust the exposure time if needed.



Note: You can also view the exposure time of the image later, in the Image Info window (see Image Info on page 55).

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

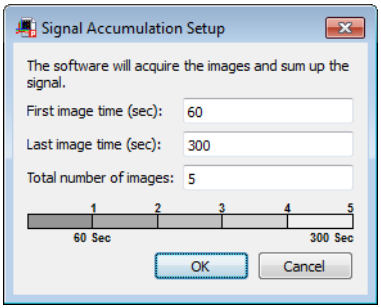


SAM is used to simplify capture of a good image from a chemiluminescent sample. This sample type often requires long integration times to obtain an image that represents the best range of signal.

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 seconds) 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 one-minute intervals, beginning at 1 min and ending at 5 min. The second one-minute image is added to the first one-minute image, resulting in a two-minute total integration time image. The third one-minute image is added to the previous image, and so on, until the last image is presented.

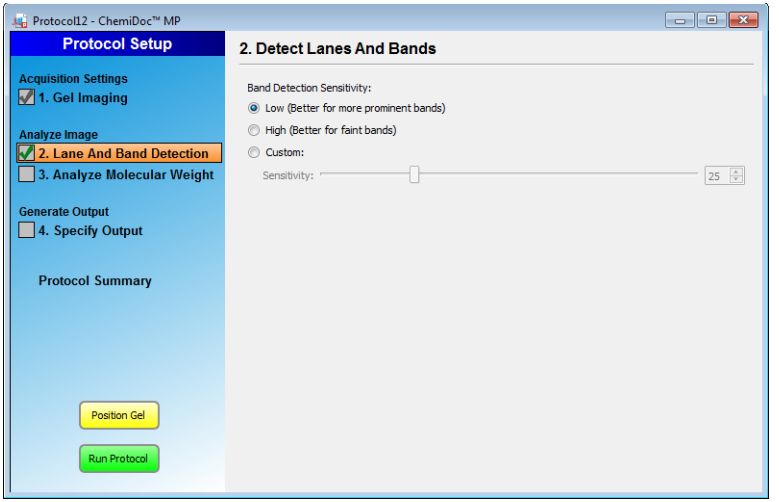
Although SAM is useful for determining the optimum imaging time for a chemiluminescent sample, it results in data that are not as accurate as data from a single image. Signal that is near the intensity of background noise becomes increasingly masked as the number of cumulative images grows. To identify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

8. **Set the Display Options**

- **Highlight saturated pixels**—select the Highlight saturated pixels checkbox to see any saturated pixels in red. This will show 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 54 to view the color choices dialog box

Analyze Image

Step 2. Detect Lanes and Bands



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

9. Select the Lane and Band checkbox in the left pane of the Protocol Setup window. The right pane displays lane and band detection options.

- **Low Band Detection Sensitivity**—this option sets detection at a low level 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 for images that are more faint. Extraneous bands can be removed later, using the Band Tools in the Analysis Toolbox. See Lane and Band Tools on page 72
- **Custom**—select a numeric value between 1 and 100 to choose the best detection sensitivity for your sample

Note: When Low Band Detection Sensitivity or High Band Detection Sensitivity is used, numerical values are set: low sensitivity = 25; high sensitivity = 75.

STEP 3. ANALYZE MOLECULAR WEIGHT

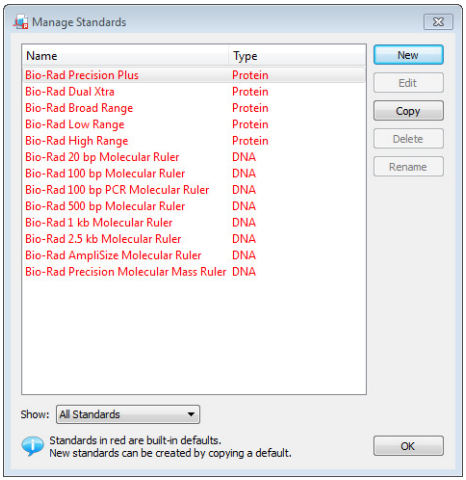
10. To analyze molecular weight automatically, select Analyze Molecular Weight in the left pane of the Protocol Setup window. When this checkbox is selected, the software calculates the molecular weight for each band based on the specified standard.

11. 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.

STANDARDS

Determining molecular weight depends on selection of 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 117 for all standards available from Bio-Rad and their catalog numbers.

Clicking Change opens the Manage Standards dialog box, in which you can add other third-party standard samples and edit the list.



STANDARD LANES

12. 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, and Last.

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

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.

Table 1.

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 are not calculated.

13. 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 65 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 99 for more information about molecular weight.

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

Output Settings

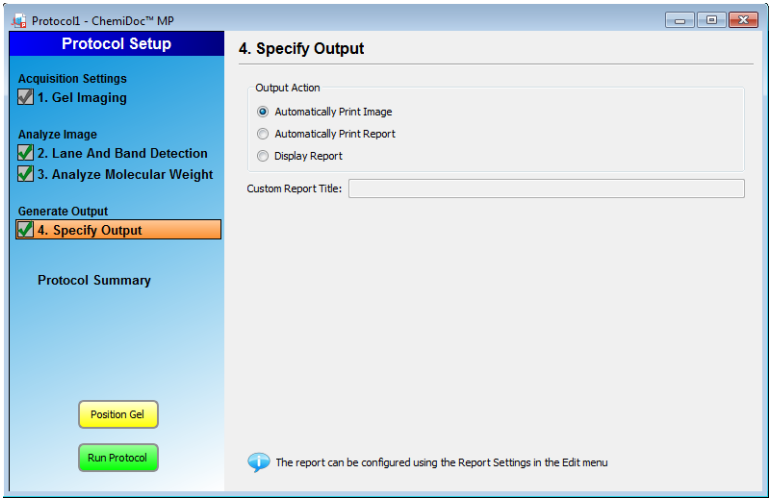
Image Lab software prints to the default printer unless you select otherwise.

You have three choices for specifying output:

- You can automatically print the image; this is useful for gel documentation, especially if a thermal printer is connected. See Appendix D. Mitsubishi P93/P95 Thermal Printer Settings on page 121 for instructions on setting up the Mitsubishi P93/P95 thermal printer
- You can automatically print the report, including all accompanying analysis information

  Note: You cannot print a report on a thermal printer.
- You can display the image on your computer

Step 4. Specify Output

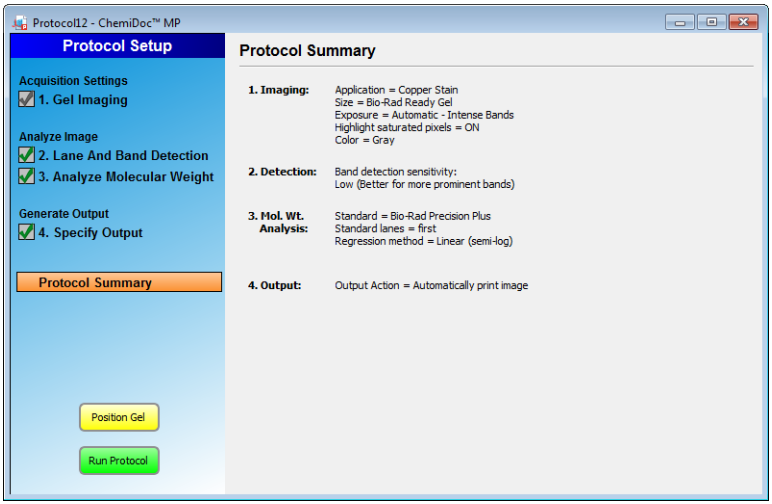


14. Select Specify Output in the left pane of the Protocol Setup window to display output options.
15. In the right pane, you can choose whether to automatically display or to print an image or a report after a protocol runs.

The report can be customized with the options in Edit > Report Settings. See Report on page 91 for information about reporting options.

Review Protocol Settings

16. Click Protocol Summary in the left pane of any Protocol Setup window to see a quick review of all protocol settings.



17. Click Save in the left pane to save the protocol.

Creating a Multichannel Protocol

Setting up a multichannel protocol consists of two parts:

- Choosing acquisition settings
- Generating output

The two processes are listed in the left pane of the Protocol Setup window. A numbered step in each process appears under these headings.

To select an option under a protocol step:

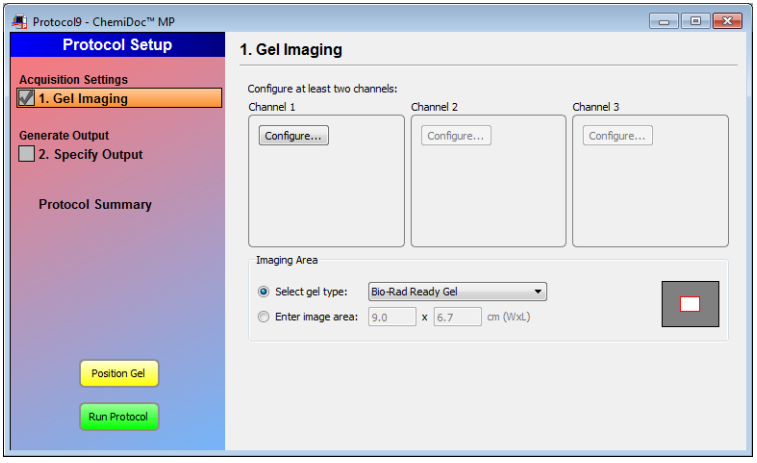
- Select the accompanying checkbox. Options for that step appear in the right pane of the window

To disable any step:

- Clear its checkbox

Acquisition Settings

Step 1. Gel Imaging

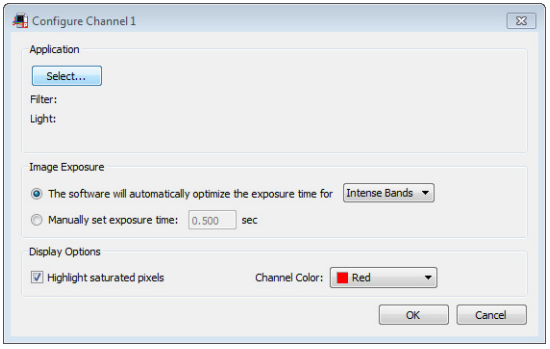


You must configure acquisition settings for at least two channels to create a valid multichannel protocol. Each channel can be assigned a color (red, green, or blue) so you can easily identify each channel.

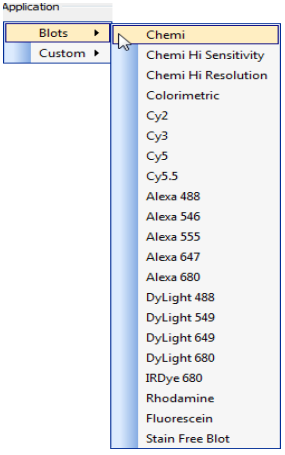
Configure each channel separately.

Application Options

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. **Choose an application**—click Select and choose an application from the drop-down list that appears.

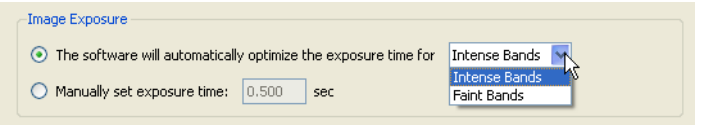


Note: Use the Custom application to run an existing application with a new name or an application unlike existing applications. See Setting Up a Custom Application on page 43 for more information.

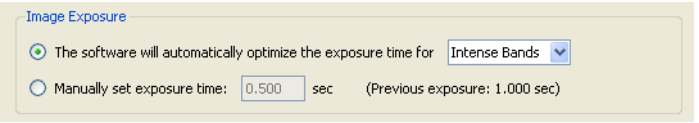
For a list of applications with all required detection reagents, light sources, and any conversion screens or filters noted, see Application Tables on page 45.

3. **Choose Image Exposure Time**—if you select the top radio button, Image Lab software 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 drop-down list, exposure is optimized for all bands
- If you choose Faint Bands from the drop-down list, a longer exposure will make faint bands more visible, but more prominent bands may be overexposed
- If you select the bottom radio button to manually override automated imaging, this may cause saturation (overexposure) of more prominent bands. Exposure time can range from 0.001 to 7200 seconds with the ChemiDoc™ MP system

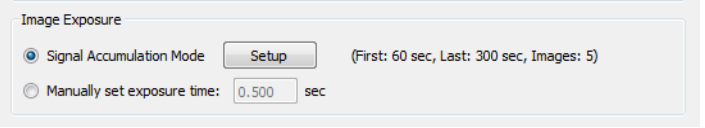


After imaging a gel with automatic exposure optimization, the exposure time displays in the protocol so you can manually adjust it if needed.



Note: You can also view the exposure time of the image later, in the Image Info window (see Image Info on page 55).

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

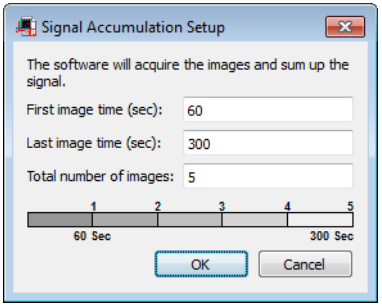


SAM is used to simplify capture of a good image from a chemiluminescent sample. This sample type often requires long integration times to obtain an image that represents the best range of signal.

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 seconds) 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 one-minute intervals, starting at 1 min and ending at 5 min. The second one-minute image is added to the first one-minute image, resulting in a two-minute total integration time image. The third one-minute image is added to the previous image, and so on, until the last image is presented.

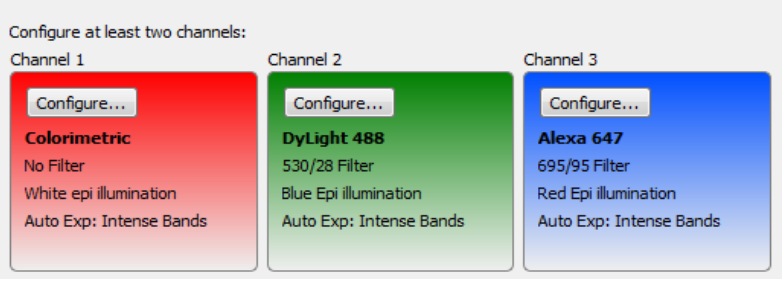
Although SAM is useful for determining the optimum imaging time for a chemiluminescent sample, it results in data that are not as accurate as data from a single image. Signal that is near the intensity of background noise becomes increasingly masked as the number of cumulative images grows. To identify 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 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.

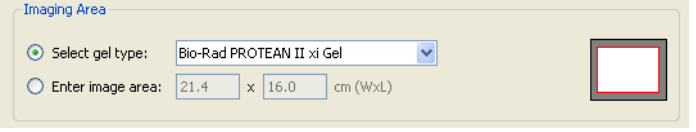
Channel Color—select a color to display the sample image. Assigning each channel a different color makes it easy to identify each channel.Once you set up the first channel, the second channel box becomes active.

5. Repeat steps 1 through 5 to set up the second channel as well as the third, if applicable. The software determines which applications are available based on your selection of other channels. 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. **Choose the Imaging Area**—select from a 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.



Output Settings

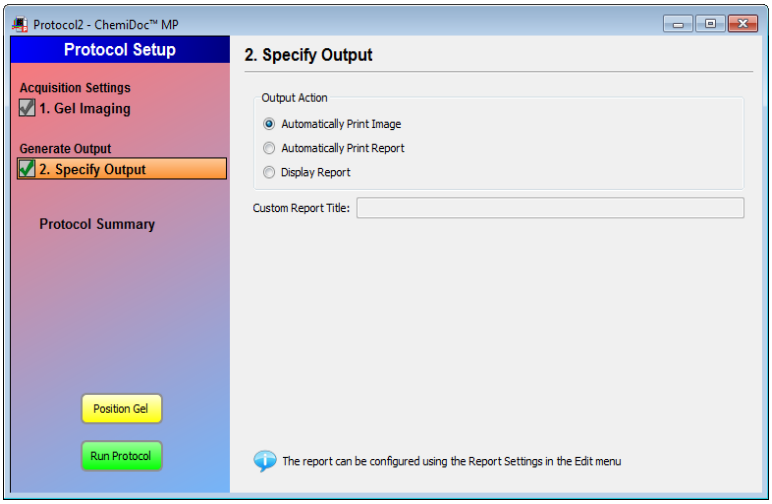
Image Lab software prints to the default printer unless you select otherwise.

You have three choices for specifying output:

- You can automatically print the image; this is useful for gel documentation, especially if a thermal printer is connected. See Appendix D. Mitsubishi P93/P95 Thermal Printer Settings on page 121 for instructions on setting up the Mitsubishi P93/P95 thermal printer
- You can automatically print the report, including all accompanying analysis information

  Note: You cannot print a report on a thermal printer.
- You can display the image on your computer

STEP 4. SPECIFY OUTPUT



7. Select Specify Output in the left pane of the Protocol Setup window to display output options.
8. In the right pane, you can choose whether to automatically display or to print an image or a report after a protocol runs.

   The report can be customized with the options in Edit > Report Settings.

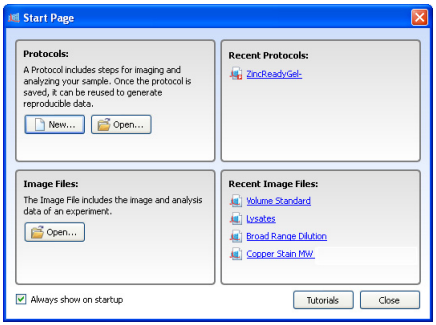
For information about reporting options, see Report on page 91.

Choosing a Protocol

To open a saved protocol:

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

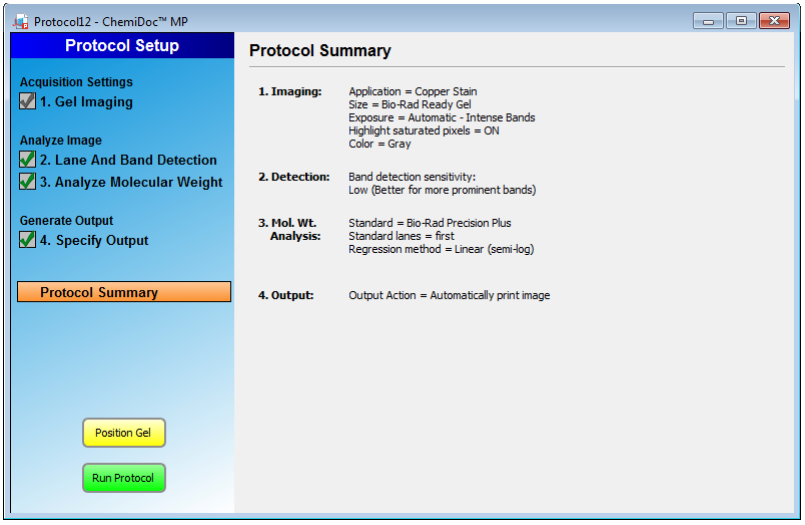
You can also choose recently used protocols or image files from the lists on the Start Page.



Editing Protocols

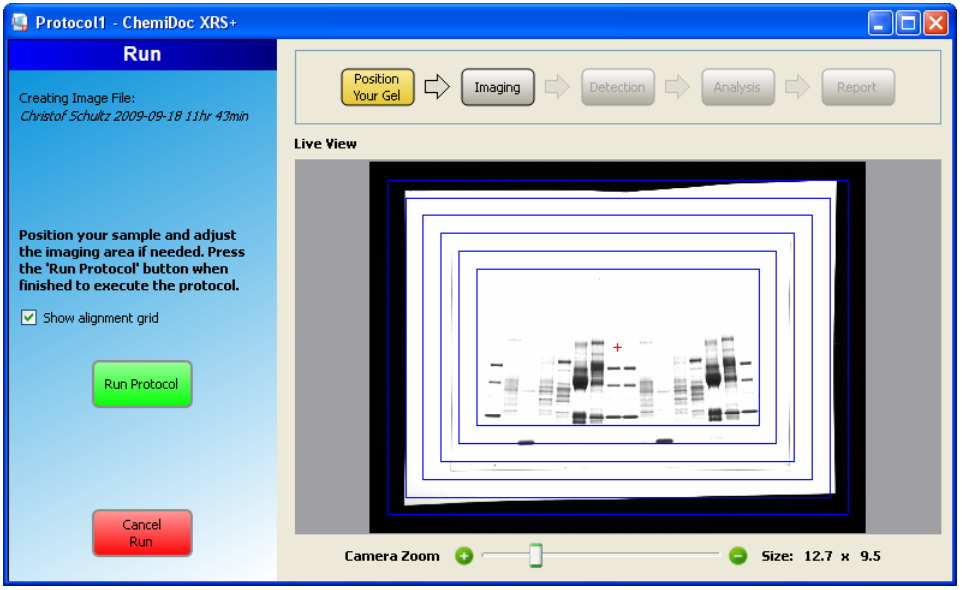
Protocols can be changed and/or renamed using the many tools in Image Lab software.

Open any saved protocol. You are presented with the same set of menus and choices described in Creating a Single-Channel Protocol on page 24.



Make and save your changes without renaming the protocol to replace an older one. Choose Save As and type a different name to make a second protocol.

Position Gel



To position a gel:

1. Click the yellow Position Gel button in the Protocol Setup window shown on page 41.
2. Place a gel on the imager stage and view the gel in Image Lab software.
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, Appendix C. Accessories for more information.

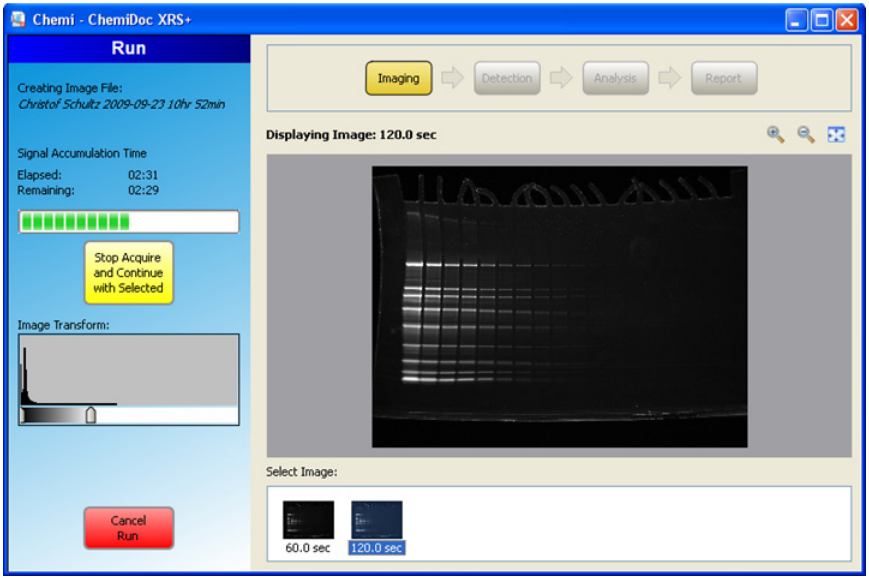
Running a Protocol

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.

If you enabled SAM in the protocol, the most recent image appears by default in the main window.



To end the protocol:

- Click Cancel Run

To save and analyze an image at any time:

1. Right-click the thumbnail and select Save on the pop-up menu.
2. Click Stop Acquire and Continue with Selected.

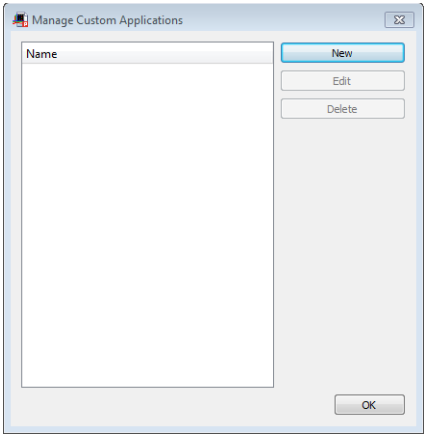
This choice stops the acquisition and continues the protocol using the selected image. All other acquired images are discarded.

Setting Up a Custom Application

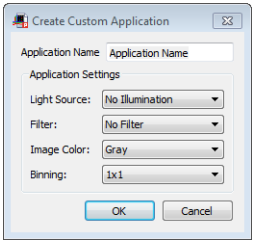
Use the Custom application to run an existing application with a new name or an application unlike existing applications. Selecting Custom on the Applications menu displays the Manage Custom Applications dialog box. If you have stored Custom Applications, they display here.

To create a custom application:

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



The Create Custom Applications dialog box appears.



2. Choose a unique application name.
3. Select a light source, filter, and image color on the drop-down 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.

Creating a Multichannel Image from Single Images

You can create a multichannel image from existing single images and from single channels in other multichannel images. Only images with the same aspect ratio can be combined in a multichannel image.

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, select Create Multichannel Image. The open images appear listed in the left pane of the Create Multichannel Image dialog box.
3. Drag a file for each channel into the appropriate channel box in the right pane. When you select the first file, the list of remaining files includes only files with the same aspect ratio.
4. (Optional) Specify a color for each channel in the accompanying drop-down lists. The resulting multichannel image appears in the Image Preview section of the dialog box.
5. Click OK to save the multichannel image.

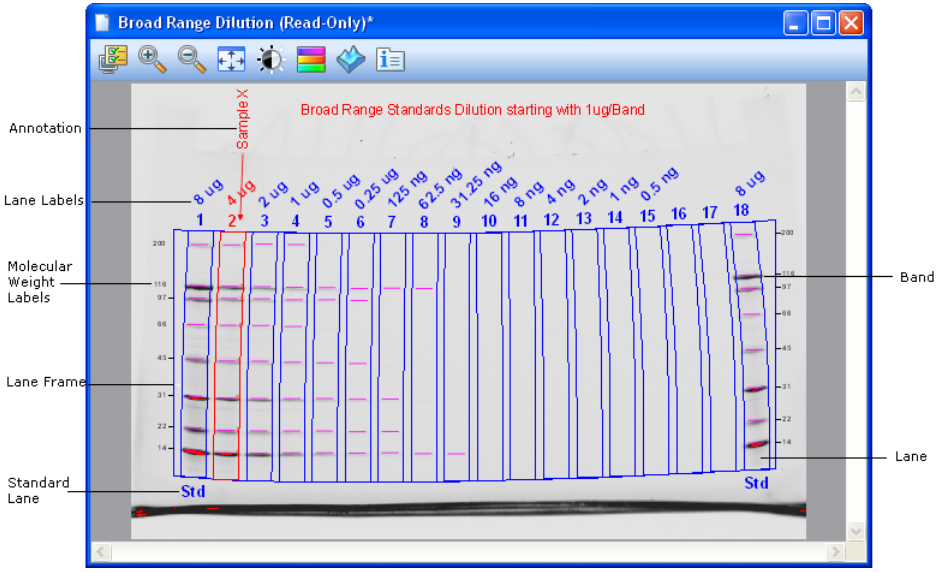
To replace a channel in a multichannel image:

1. Open the multichannel image and the new image you want to use.
2. In the File menu, select Create Multichannel Image. The open images appear listed in the left pane of the Create Multichannel Image dialog box.
3. Drag the channels you want to use from the multichannel image into the appropriate channel boxes.
4. Drag the new image you want to use into one of the channel boxes.
5. Click OK to save the new multichannel image.

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



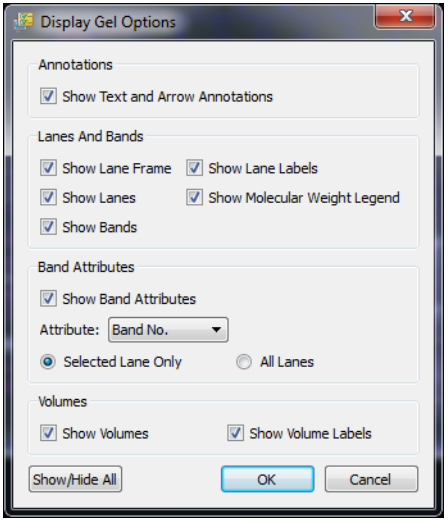
The screen shows a gel image with band and lane detection as well as annotations. The labels represent overlays that you can show or hide.

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 



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; the one with the minus sign to make the image smaller.

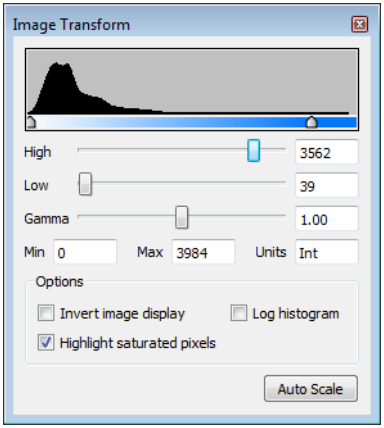
You can also zoom with the right mouse button. Click and drag to select an area you want to magnify. Right-click again to return to the original view.

You can also resize the image with the scroll wheel on your mouse.

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 detail 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:

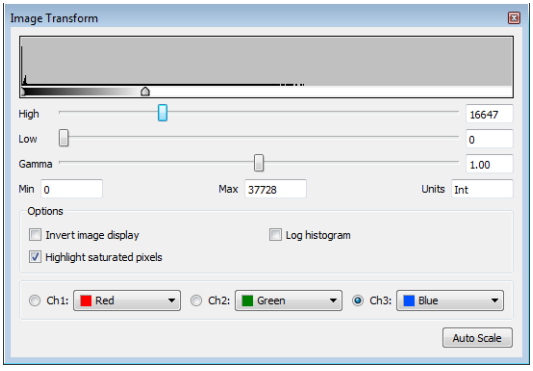
- **Invert Image display**—inverts dark bands on a light background to light bands on a dark background and vice versa

- **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.

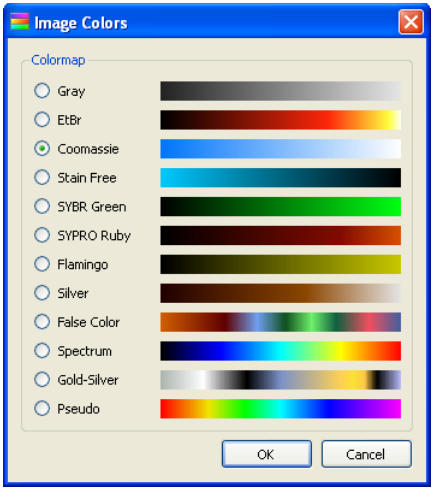
For Multichannel Images

Image Transform displays only one channel and can transform only one image at a time. When you are working with a multichannel image, you can select a channel at the bottom of the Image Transform dialog box. You can also change the color of each channel. Doing so automatically updates the title bar and the channel buttons in the image display.



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

Image Colors



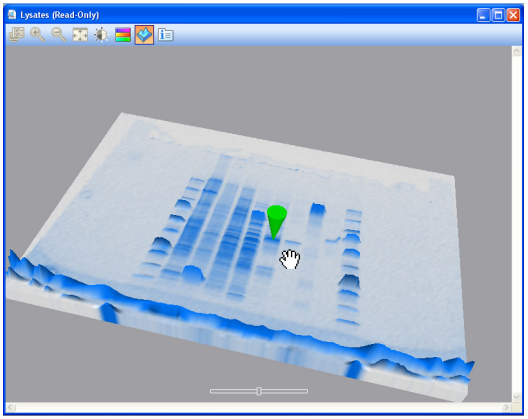
You can choose a colormap for your image results file. Viewing the image with a different color scheme can make it easier to see all of the elements in the image, but it does not change your data.

> **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. Available colors include:

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

3-D Projection 



Clicking the 3-D 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.

Image Info 

The Image Info dialog box provides information about an active image. Click Image Info in the display toolbox to display three tabs of information categories about the image.

Image Details

Acquisition and image information appear in this tab.

> **Note:** For multichannel images, channel settings also appear in this tab. Select a channel to display its details.

Analysis Settings

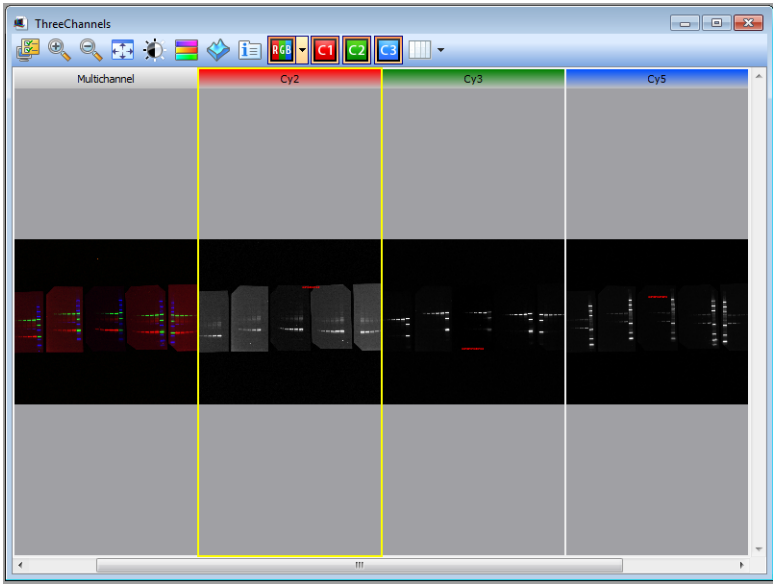
The settings that were used when the gel was analyzed are displayed here. For example, Band Detection and Molecular Weight Analysis will appear, if they were performed.

Notes

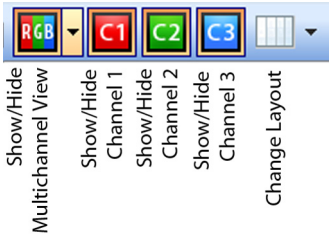
You can add notes, make custom labels for each of the lanes, point out the types of samples used, and add any other information about the results.

Displaying Multichannel Images

Multichannel View includes a pane for the merged display of all 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.

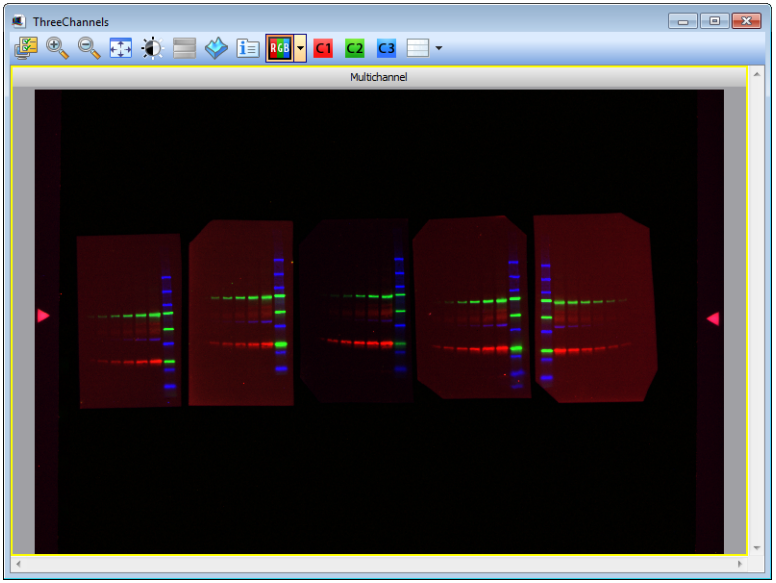


Multichannel View 

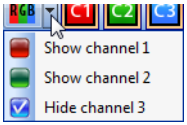
Click the Multichannel View button to display or hide the merged image panel in the multichannel display.

You can show or hide channels individually using the three 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 drop-down 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 separate 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.

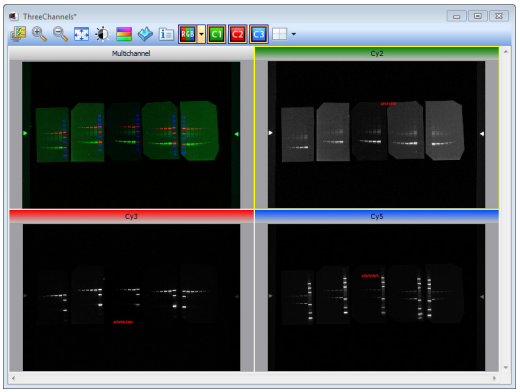
To split a multichannel image into separate files:

1. Open a multichannel image.
2. Select Split Multichannel Image in the File menu. A separate file appears for each channel in the multichannel image (except the RGB channel).
3. Save each file.

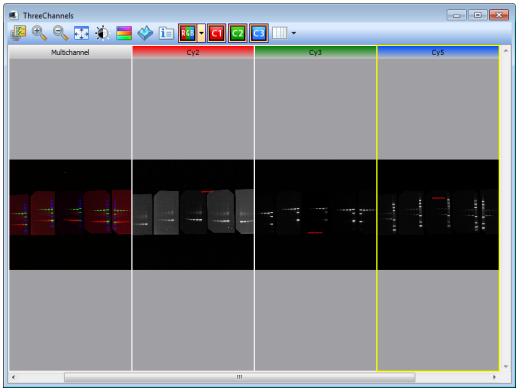
CHANGE LAYOUT 

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:

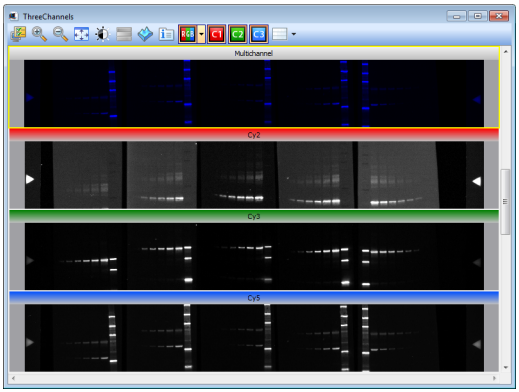
Grid View—by default, multichannel images appear in grid view.



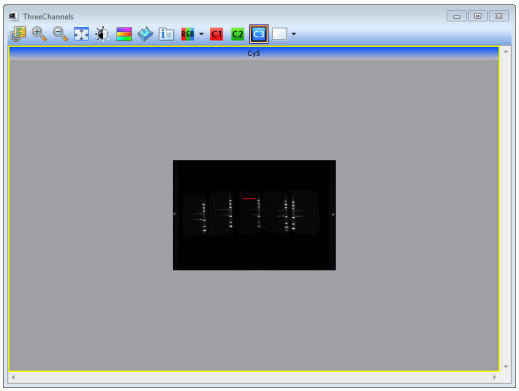
Vertical View



Horizontal View



Single View



Displaying Data

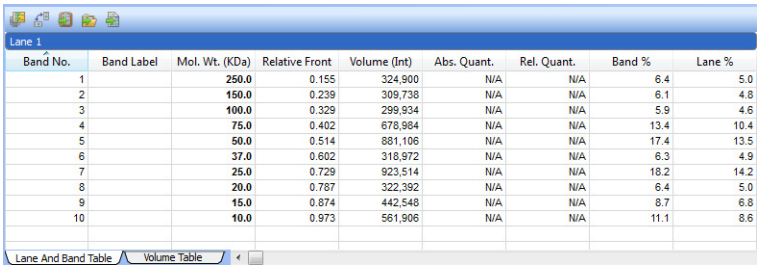
The results from analyzed data associated with the gel images can be visualized using an analysis table, a lane profile, a standard curve, or a report.

Buttons to toggle these views on and off are located in the main toolbar. All of the views can be seen at one time.

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 offer several ways of 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 to the top of the window until the cursor changes into a double-headed arrow. Left-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 

This dialog box displays three tabs: Measurements, Display, and Export.

Measurements—choose the measurements you want to display by removing measurements you want to exclude from the Displayed box. To do so, click the left-arrow button for each measurement you want to exclude; this moves it to the Not Displayed box.

Default display settings—Move Selected Lane to Top 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 both the Lane and Band table and the Volume table.

Example—see how your measurement will display with the number of decimal places you choose in Per Measurement Precision.

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.

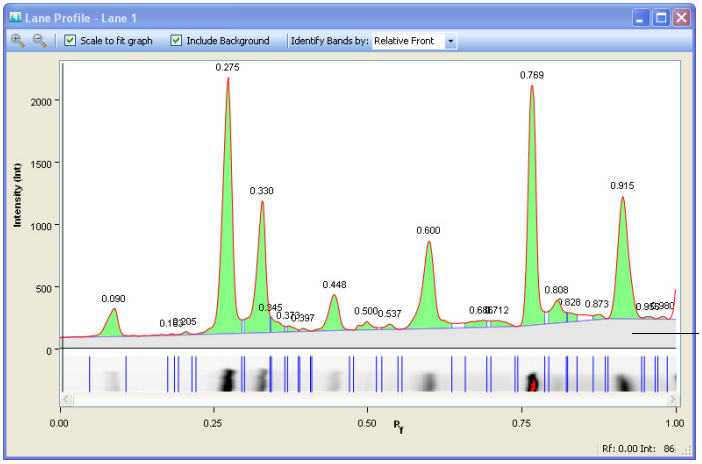
Maximum Value—intensity of the pixel with the maximum intensity inside the volume.

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 of the selected lane, rotated 90°. You can select other lanes by clicking them while the Lane Profile window is open.

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.

You can see the current Rf (relative front) value and the intensity at the cursor in the lower-right corner of the Lane Profile screen.

You can change how the bands are labeled by choosing from the options in the drop-down list. By default, the bands are labeled with the band number.

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

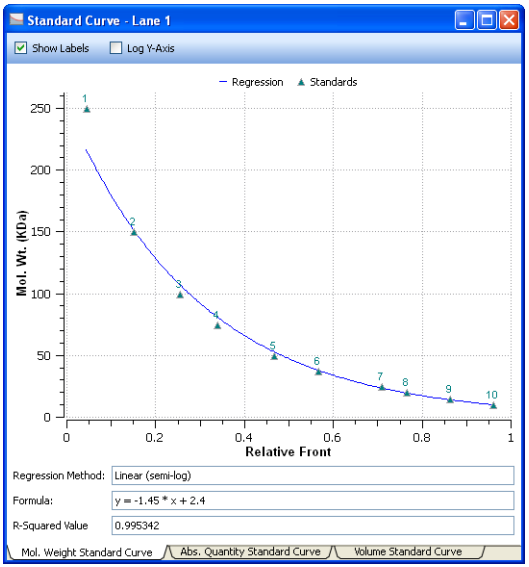
When you clear the Include Background checkbox, the area of the lane profile that represents the background of the image does not display.

IDENTIFY BANDS BY

You can display callouts on the Lane Profile to indicate these attributes:

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

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.

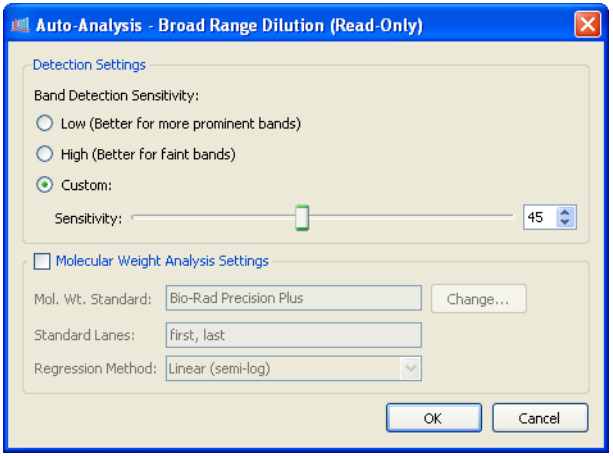
Auto Analysis Settings

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.

DETECTION SETTINGS



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 Band Tools on page 72.

Custom—allows you to set a numerical 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. For more information, refer to Standards on page 30.

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 32.

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:

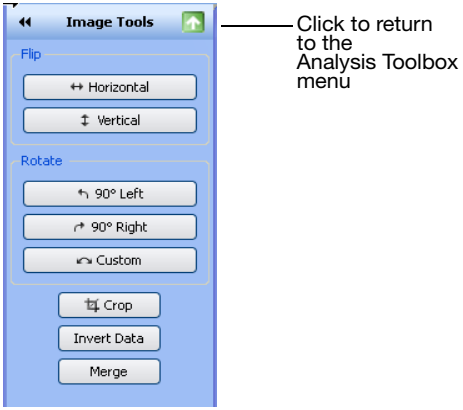
- Click any of the toolbox buttons

To return to the Analysis Toolbox menu:

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

Image Tools

To show the image tools menu, click 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.

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.

CROP

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.

Invert 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.

Merge

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.

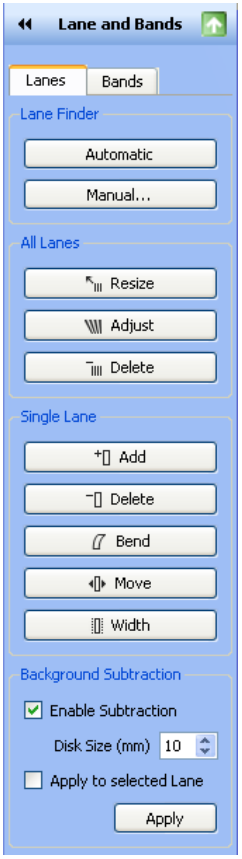
> **Note:** Merging images can have an adverse effect on quantitation. If accurate quantitation is required, perform analysis on the original, separate images. Only images of the same size can be merged.

Lane and Band Tools

Click Lane and Band Tools to select a tab, one for the lane tools and one for band tools.

LANE TAB

You can choose how lanes are detected by selecting Automatic or Manual Lane Finder.



- 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. You can then drag the handles on the corners of the red square to resize the lane frame

All Lanes

Resize—you can resize all lanes by dragging the handles on the corners of the red square to fit the gel image.

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 tool does not resize lane width.

You can also add more anchor points on 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 so-called smiling gels.

Delete—you can delete all lanes.

Single Lane

Add—you can add a single lane to a gel image. First click Add, and then click within the lane frame where you want to place the new lane. The lanes are 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—you can delete a single lane. First click Delete, and then click either the lane or its lane number. The lanes are renumbered.

Bend—you can bend a single lane to better fit the gel image. First click the Bend button and then drag one of the square anchor points to fit the image.

You can add more anchor points within the lane by left-clicking it. Drag these anchor points to adjust the lane to fit the gel image. Remove anchor points by right-clicking them.

Move—you can move a single lane to a new position on a gel image. First click Move, and then click the lane you want to move. Drag it to a new location. The lanes are renumbered according to their new position.

Width—you can change the width of a single lane.

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—you can specify 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 much background is subtracted.

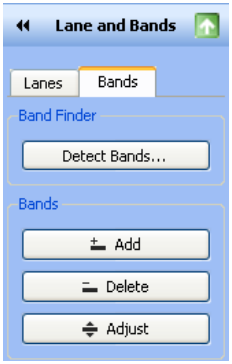
A large disk follows the profile trace less closely, touching fewer points along the trace and removing less background. A smaller disk more closely follows the profile trace, removing more background.

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.

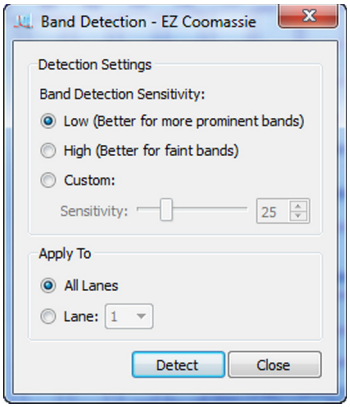
Apply to selected Lane—when you select this box, the level of background subtraction specified with the previously described buttons is applied only to the selected lane. This option enables you to set different background subtraction levels for each lane.

BANDS TAB

This tab enables you to detect bands or reset band detection settings. Click a button in the menu, and then click a lane in the image.



Detect Bands—this option opens a dialog box in which you can select band detection sensitivity and the lanes to which it applies.



Add—use this option to add faint bands. First click Add, and then add a new band by clicking anywhere inside the lane.

> **Note:** You can darken your entire image to view faint bands more easily using the Gamma slider in the Image Transform dialog box. For instructions, see Image Transform on page 52.

Delete—you can delete bands that are not relevant to your analysis. First click Delete, and then click any bands you want to remove.

Adjust—you can adjust the height of a band. First click Adjust. Two boundary lines appear around each band. Move over a boundary line until you see a double-headed arrow. Move the boundary line up or down; the center recalculates; the band appears there.

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

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.

You can view each band's molecular weight in the molecular weight column of the Lane and Band tab in the Analysis Table view or in the gel image by selecting Mol. Wt. from the Band Attributes section of the Display Gel Options window:

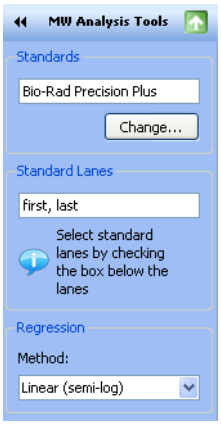
Lane 1 - Precision Plus

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

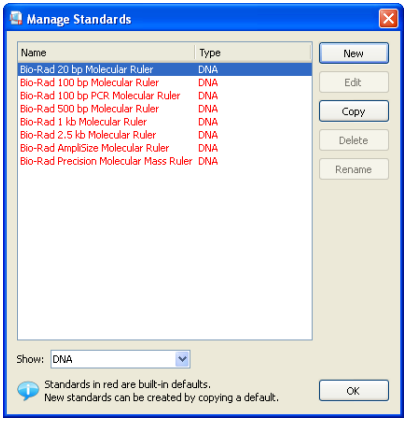
Lane And Band Table | Volume Table

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 third-party 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. Standard lanes are labeled Std below the lane.

Regression Methods

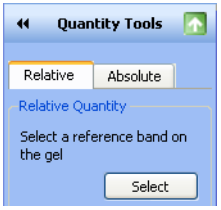
There are four regression methods. See Regression Methods on page 32.

Quantity Tools

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

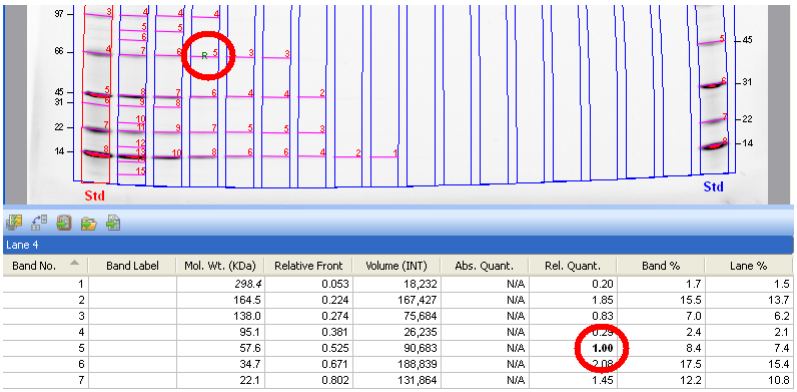
Relative Quantity Tab

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



To select a reference band from an image and quantitate 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.



Lane 4

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 drop-down list.

4. Select a regression method from the drop-down 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.

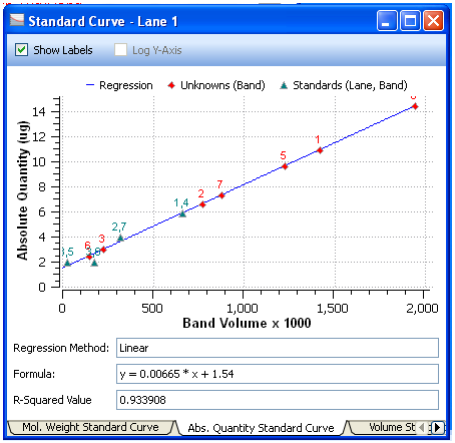
   Cubic spline—generates a smooth curve that connects each data point. At least four standard points are required to use this method of least-squares polynomial fits.

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 drop-down 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 drop-down Size list to increase or decrease the size of the text. You can set the font size between 6 and 72 points using the drop-down 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 drop-down list.

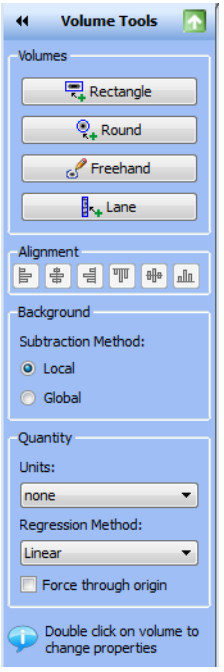
Background—click a text annotation. This also activates the Background field so you can select a background color from the drop-down list.

ROTATE

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

Volume Tools

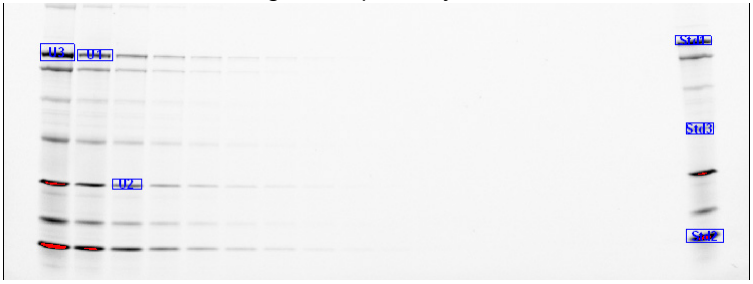
Volume tools enable you to manually quantitate features on a sample image when automated lane and band analysis is not appropriate or possible, such as in dot blots.



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 quantitate 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.



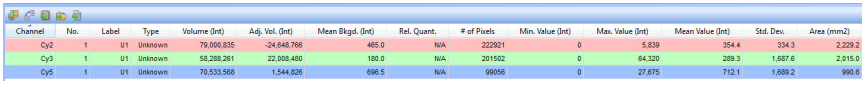
Each new volume you create initially has a red border, which indicates that the volume is selected. When you click elsewhere on the image, the border changes to blue, indicating that the volume is no longer selected.

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 63

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



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

Volume Types

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

Unknown volumes are volumes you want to quantify.

Standard volumes are used for absolute quantities. See Absolute Volume Quantity on page 89.

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 quantitation. 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 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 quantitation 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 Appendix E. 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.

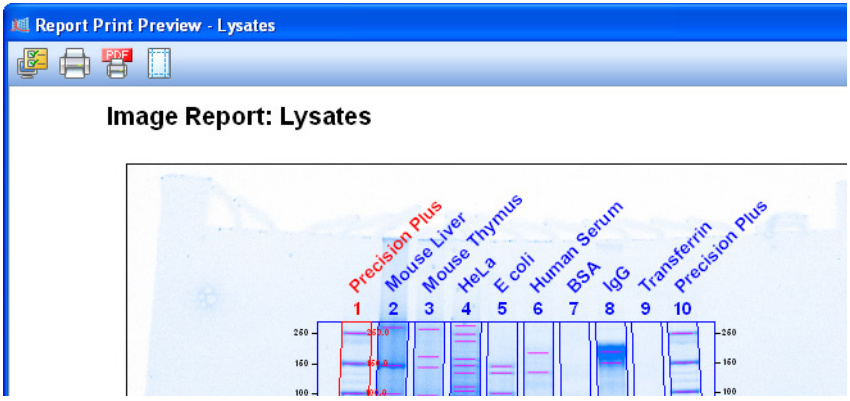
7 Generating Reports

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.

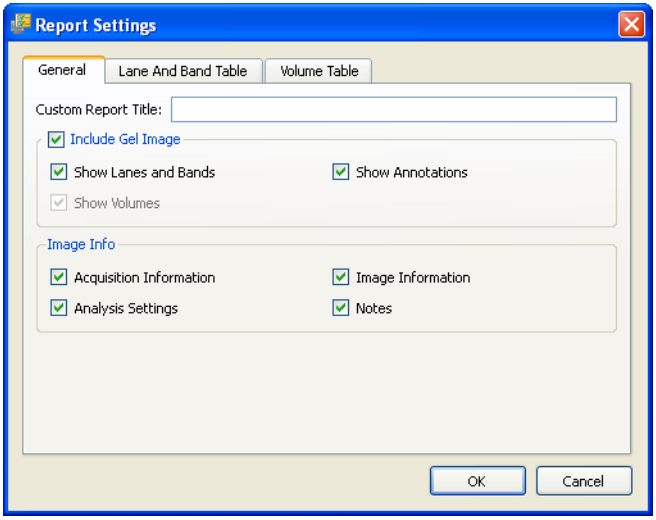


Clicking Print sends all of the above information to a printer.

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.

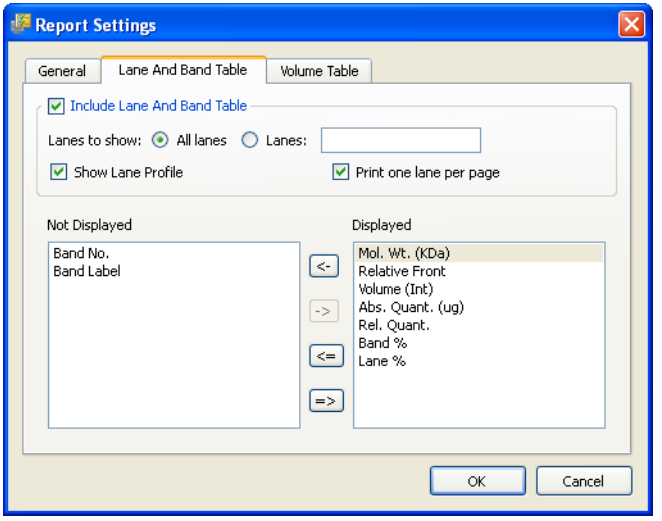
Include Gel Image—the following options determine which overlays display on the gel image.

- 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

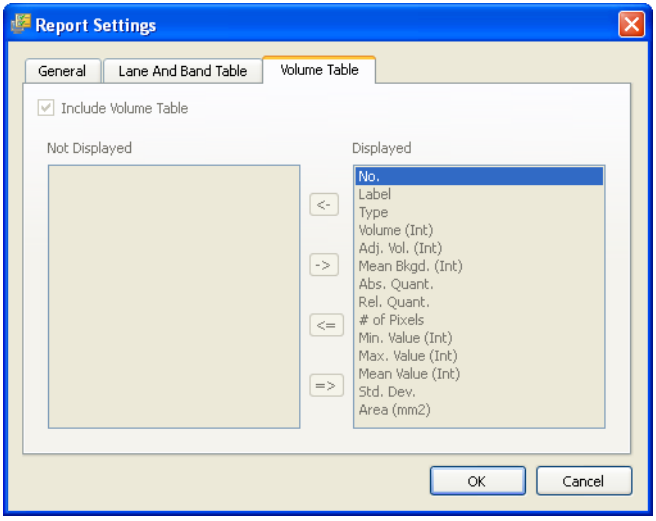
Lanes to show:

- 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.

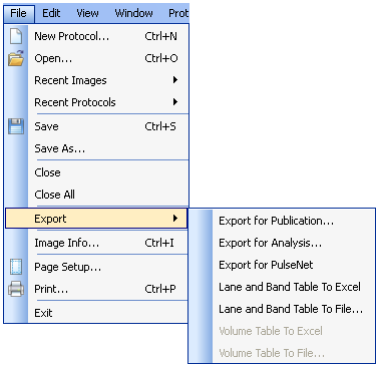
Exporting Gel Images

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.

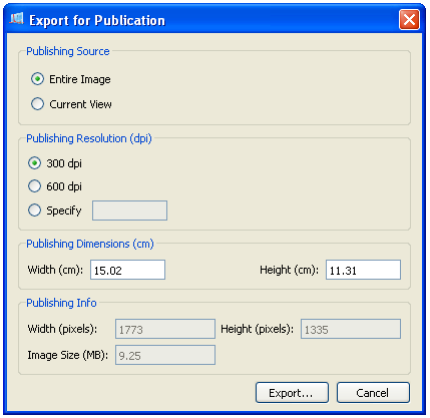


Exporting Gel Images for Publication

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

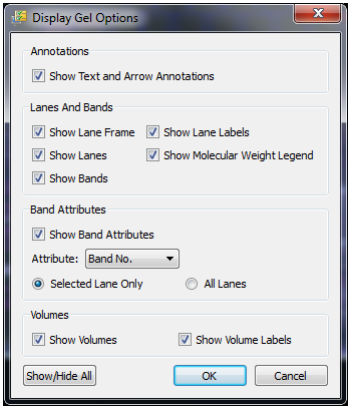
When you select File > Export > Export for Publication, you can export a displayed image to a file. You can select from .bmp, .png, .jpg, and TIFF formats. The gel displays with any lanes, bands, and annotations that appear on the screen.

> **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.

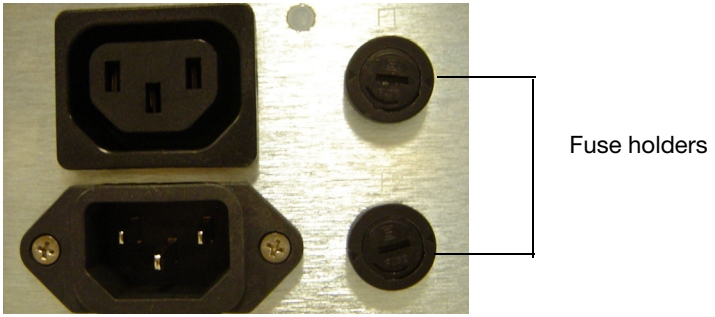
   Note: Do not put the UV cover directly on the bench. Wear gloves when touching the lamps.

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.

Appendix 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%.

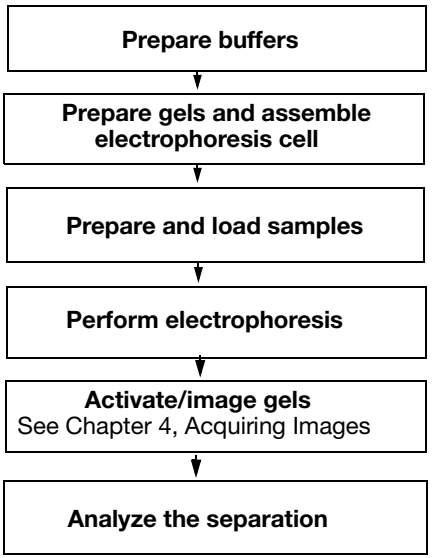
Benefits of the stain-free system include:

- 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 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, except where otherwise noted.



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 in many ways the same as setting up protocols for other applications. Follow the instructions in Creating a Single-Channel Protocol, on page 24, but after step 3, set the activation options for the gel as follows.

Set the activation options: Visualizing proteins requires activation of the gel.

Choose an activation time option based on your sample and the purpose of your experiment:

- Use 2.5 min activation when samples are abundant and when a fully optimized signal-to-noise ratio is not necessary
- 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 five minutes, 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.

Continue setting up the protocol at step 7 under Application Options, on page 24.

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.

Appendix 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

Appendix C. Accessories

Calibrating Accessories

If you are installing accessories along with your original system installation, you 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.

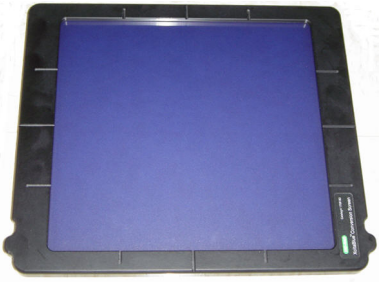
To use the white light conversion screen:

1. Center the conversion screen on the imager stage.
2. Center your samples on top of the conversion screen
   .

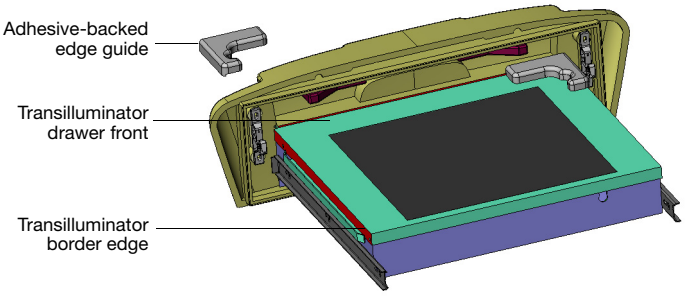
3. Image the gel using your preferred application.

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.



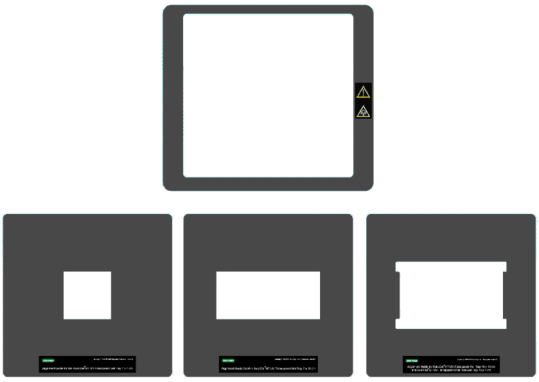
To install the XcitaBlue conversion screen:

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. Remove the paper tape from the bottom surface of each edge guide.
3. Press each edge guide into position carefully, as it is difficult to reposition them once the adhesive surfaces touch.
4. 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.
5. To visualize a sample using the XcitaBlue conversion screen, place the screen between the edge guides.

6. 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
170-7813	Sample holders for gels
100-2784	UV transilluminator lid (includes UV filter glass)

Catalog #	Description
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
Nucleic Acid Standards	
170-8351	EZ Load™ 20 base pairs molecular ruler
170-8352	EZ Load 100 base pairs molecular ruler
170-8353	EZ Load 100 base pairs PCR molecular ruler

Catalog #	Description
170-8354	EZ Load 500 base pairs molecular ruler
170-8355	EZ Load 1 kb molecular ruler
170-8205	2.5 kb molecular ruler
170-8200	AmpliSize® molecular ruler
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

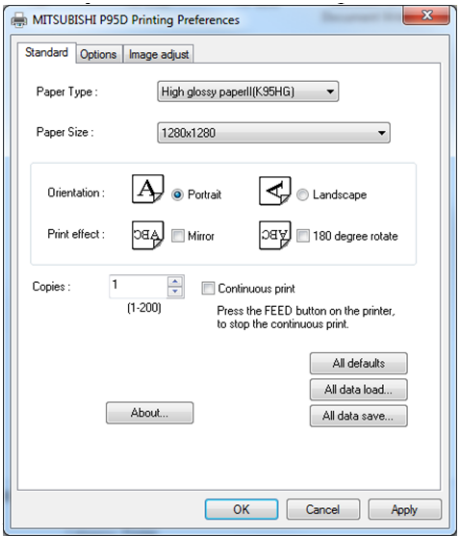
Appendix D. Mitsubishi P93/ P95 Thermal Printer Settings

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.

Mac

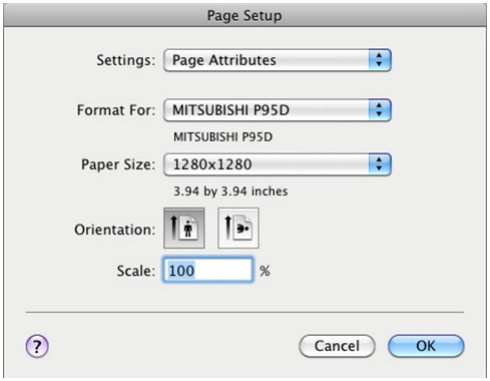
The printer driver can be found on the Image Lab software installation CD in the Misc directory.

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

Appendix 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

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.

Index

A

B

C

D

E

F

G

H

I

L

M

N

O

P

Q

R

S

T

U

V

W

X

Z

Bio-Rad
Laboratories, Inc.

Life Science
Group

Web site *www.bio-rad.com* ***USA*** *800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *01 877 89 01*
Belgium *09 385 55 11* ***Brazil*** *55 31 3689 6600* ***Canada*** *905 364 3435* ***China*** *86 21 6169 8500*
Czech Republic *420 241 430 532* ***Denmark*** *44 52 10 00* ***Finland*** *09 804 22 00* ***France*** *01 47 95 69 65*
Germany *089 31 884 0* ***Greece*** *30 210 777 4396* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 1 459 6100*
India *91 124 4029300* ***Israel*** *03 963 6050* ***Italy*** *39 02 216091* ***Japan*** *03 6361 7000* ***Korea*** *82 2 3473 4460*
Malaysia *60 3 2117 5260* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *0318 540666*
New Zealand *64 9 415 2280* ***Norway*** *23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700*
Russia *7 495 721 14 04* ***Singapore*** *65 6415 3170* ***South Africa*** *27 861 246 723* ***Spain*** *34 91 590 5200*
Sweden *08 555 12700* ***Switzerland*** *061 717 95 55* ***Taiwan*** *886 2 2578 7189* ***Thailand*** *66 2 6518311*
United Kingdom *020 8328 2000*

10022469 Rev B *0711 Sig 0211*