Gel Doc™ XR+ and ChemiDoc™ XRS+ Systems 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 details at
   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 (www.ijg.org/)

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IL v.4.0

Safety and Regulatory Compliance

Important Safety Information

Please read these instructions before attempting to operate the Gel Doc™ XR+ or ChemiDoc™ XRS+ imaging systems.

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 Gel Doc XR+ and ChemiDoc XRS+ systems 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 Gel Doc XR+ and ChemiDoc XRS+ imaging systems are warranted against defects in materials and workmanship for 1 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 their 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
- Switch off all the lights immediately after use
- Clean the transilluminator platen after use

Regulatory Notices

The Gel Doc XR+ and ChemiDoc XRS+ imaging systems are 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 Gel Doc XR+ or ChemiDoc XRS+ imaging system has a power supply that automatically chooses the correct voltage for your country or region.

Fuses

The universal hood of the Gel Doc XR+ or ChemiDoc XRS+ 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.

Table of Contents

1 Introduction

Gel Doc™ XR+ and ChemiDoc™ XRS+ gel documentation systems offer high performance and ease of use. They both contain a charge coupled device (CCD) camera to capture images in real time and allow you to accurately position your sample and generate optimized image data.

The Gel Doc XR+ and ChemiDoc XRS+ systems use the same lighttight enclosure (the universal hood), which contains built-in UV and white light illumination, but different CCD cameras. Both systems feature dynamic flat fielding technology for superior image uniformity and accurate quantitation.

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

Gel Doc XR+ Imaging System

Gel Doc XR+ imaging system 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 many options presented by the Image Lab software.

Features include:

- Detection of dynamic ranges that cover > 3 orders of magnitude
- Dynamic flat fielding specific to fluorescent and colorimetric applications

- Smart, application-based protocol setup using Image Lab software, which assists you by presenting appropriate filter and illumination sources for each application
- Ability to upgrade to the ChemiDoc XRS+ system

ChemiDoc XRS+ Imaging System

In addition to the gel documentation applications of Gel Doc XR+, the ChemiDoc XRS+ imaging system offers sensitive chemiluminescent detection. The system includes a supersensitive 16-bit CCD camera that is deeply cooled for the long imaging times required for faint sample detection and for accurate quantification of image data.

Features include:

- Suitability for imaging applications that require excellent sensitivity (chemiluminescent western blots)
- Smart, application-based protocol setup using Image Lab software, which assists you by presenting appropriate filter and illumination sources for each application
- Dynamic range > 4 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

Either the Gel Doc XR+ or the ChemiDoc XRS+ 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, included in the chapter which discusses your camera, 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

The universal hood 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 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 any touchpad input.

Image Lab Software

Each system ships with a full version of Image Lab software. In addition to controlling the imager systems, 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 to 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 10 for detailed instructions on the software.

Emission Filters

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

A standard filter (catalog #170-8081) 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 B 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 Gel Doc XR+ and ChemiDoc XRS+ systems: the Mitsubishi P93DW thermal printer, (catalog #170-8089).

Conversion Screens

White Light Conversion Screen

The white light conversion screen (catalog #170-8001) is a phosphor screen that produces white light transillumination when placed on top of the UV transilluminator.

White Light Transilluminator

An optional white light transilluminator (catalog #170-7950) may also be used with either system. The white light transilluminator plugs inside the universal hood and is controllable by the touchpad or the computer software.

XcitaBlue™ Conversion Screen

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

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 mainly determined by the size of the images you will 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 XP SP3 Professional
	Windows 7, 32- and 64-bit	Windows 7 Professional, 64-bit
	Mac OS X 10.6	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)

2 Gel Doc™ XR+ Imager

Refer to the Gel Doc XR+ Installation Guide (10016995) found in your Gel Doc XR+ Installation Kit (catalog # 170-8199) for instructions to install Image Lab software and to assemble and calibrate your system.

To recalibrate your system because you have acquired new accessories, refer to Chapter 4.

Gel Doc XR+ Imager Workflow

Following are the basic steps to acquiring, analyzing, and archiving an image using the Gel Doc XR+ system and Image Lab software:

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

Gel Doc XR+ Applications

The Gel Doc XR+ system is capable of running a wide variety of protocols. Those listed below are examples of the detection reagents that may be used to image your samples. Contact Bio-Rad technical support to determine whether your application can be imaged on this instrument.

See Chapter 6, Acquiring Images, for complete instructions on designing protocols.

Nucleic Acid Gels

- Ethidium bromide
- SYBR® Green
- SYBR® Safe
- SYBR® Gold
- GelGreen
- GelRed
- Fast Blast™

Protein Gels

- Coomassie Blue
- Copper stain
- Zinc stain
- Flamingo™ fluorescent gel stain
- Oriole™ fluorescent gel stain
- Silver stain
- Coomassie Fluor Orange
- SYPRO Ruby
- Krypton

Blots

- Colorimetric
- Qdot 525
- Qdot 565
- Qdot 625
- Cy2
- Alexa Fluor 488
- DyLight 488
- Fluorescein

Gel Doc XR+ Technical Specifications

Applications	
Chemiluminescence	No
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, self-powered or conversion screen. Optional XcitaBlue™ UV/blue conversion screen.
Illumination control	3 modes Trans-UV, Trans White, Epi-White
Detector	CCD
Pixel size, H x V in microns	4.65 x 4.65
Cooling system	Not applicable
Camera cooling temperature	Not available
Filter holder	3 positions (2 for emission filters, 1 for using no filter)
Emission filters	1 included (standard), 3 optional
Dynamic range	>3.0 orders of magnitude
Pixel density (gray levels)	4,096
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, and reporting
Workflow reproducibility	100% repeatability via recallable protocols; from image capture to quantitative analysis and reports
Autofocus (patent pending)	Pre-calibrated focus for any zoom setting
Image flat fielding (patent pending)	Dynamic; pre-calibrated and optimized per application
Autoexposure	2 user-defined modes (intense or faint bands)

*Using the optional XcitaBlue kit (catalog #170-8182) is highly recommended for SYBR® Safe DNA applications because the UV to blue conversion screen allows you to visualize DNA samples while protecting them from UV damage.

3 ChemiDoc™ XRS+ Imager

Refer to the ChemiDoc XRS+ Installation Guide (10006999) found in your ChemiDoc Installation Kit (catalog # 170-8299) for instructions to install Image Lab™ software and to assemble and calibrate the ChemiDoc XRS+ system.

To recalibrate your system because you have acquired new accessories, refer to Chapter 4.

ChemiDoc XRS+ Imager Workflow

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

1. Select an existing protocol or customize a new one.
2. Position the sample to be imaged.
3. Run a 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 6 Acquiring Images for instructions.

ChemiDoc XRS+ Applications

The ChemiDoc XRS+ system is capable of running protocols to image all the detection reagents from the Gel Doc XR+ list, plus the others added to this list. 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 Acquiring Images on page 29, for detailed instructions on designing protocols.

Nucleic Acid Gels

- Ethidium bromide
- SYBR® Green
- SYBR® Safe
- SYBR® Gold
- GelGreen
- GelRed
- Fast Blast™ stain

Protein Gels

- Coomassie Blue
- Copper stain
- Zinc stain
- Flamingo™ fluorescent gel stain
- Oriole™ fluorescent gel stain
- SYPRO Ruby
- Silver stain
- Coomassie Fluor Orange
- Krypton

Blots

- Chemiluminescent
- Colorimetric
- Qdot 525
- Qdot 565
- Fluorescein
- Qdot 625
- Cy2
- Alexa Fluor 488
- DyLight 488

ChemiDoc XRS+ 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, self-powered or conversion screen. Optional XcitaBlue™ UV/blue conversion screen.
Illumination control	5 modes (Trans-UV, Epi-White, and No Illumination for chemiluminescence are standard) Trans White and XcitaBlue conversion screens are optional.
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	3 positions (2 for filters,1 without filter, for chemiluminescence)
Emission filters	1 included (standard), 3 optional
Dynamic range	>4.0 orders of magnitude
Pixel density (gray levels)	65,535
Dynamic flat fielding	Application-specific, for all applications
Instrument size, L x W x H in cm	36 x 60 x 96
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, and reporting
Workflow reproducibility	100% repeatability via recallable protocols; from image capture to quantitative analysis and reports
Autofocus (patent pending)	Pre-calibrated focus for any zoom setting or sample height
Image flat fielding (patent pending)	Dynamic; pre-calibrated 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 SYBR® Safe DNA applications because the UV to blue conversion screen allows you to visualize DNA samples while protecting against UV damage.

4 System Calibration

When your system is installed, system calibration is performed with a calibration wizard. See the Installation Guide (10016995 for the Gel Doc™ XR+ system and 10016999 for the ChemiDoc™ XRS+ system) 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:

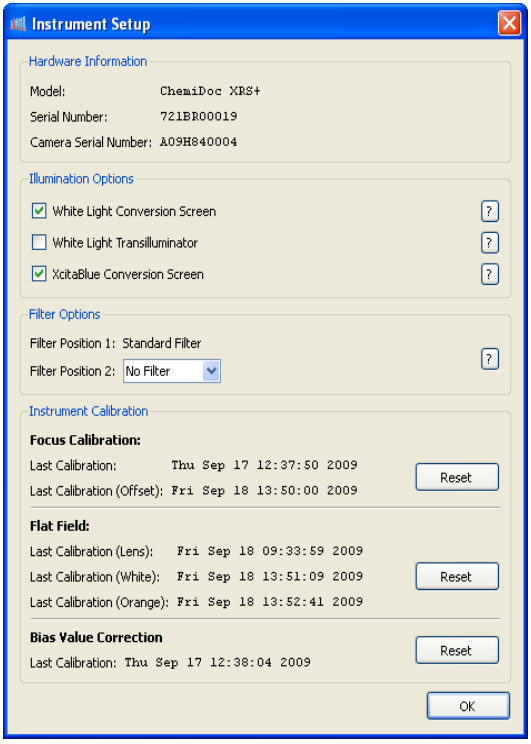
- **Focus Calibration**—this calibration allows automated focus settings at any zoom point, using a (patent pending) software algorithm. Therefore, your focus remains correct whether you view an entire sample or just an area of interest
- **Focus Calibration with Height Offset**—this calibration takes 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** (Gel Doc XR+ system only)—this calibration determines and corrects any background signal present in your imager
- **Bias Reference Image** (ChemiDoc XRS+ system only)—this calibration provides a bias value correction
- **UV Flat Field Calibration**—this calibration generates the flat field correction profiles needed for the UV light source. Because of this calibration, images have more accurate quantity reporting and 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 Gel Doc XR+ or ChemiDoc XRS+ 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 remain until you make further changes.

5 Image Lab™ Software Overview

Image Lab image acquisition and analysis software works with the Gel Doc™ XR+ and ChemiDoc™ XRS imaging systems 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
- 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.

Interface Overview

The following screen shows the Image Lab software main window. The accompanying paragraphs describe the main software elements.

Main Window

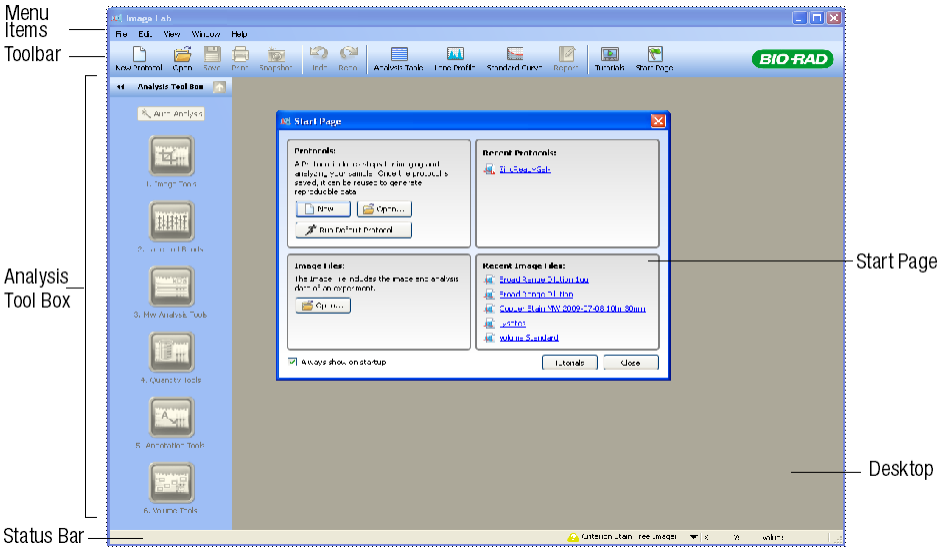


Image Lab software displays a single main window. All image and protocol dialog boxes that present choices open in the workspace, which is the gray area of the main window.

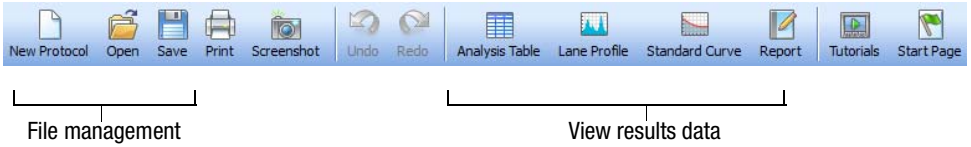
If many screens are open in the workspace, you can make one visible by clicking the title bar at the top of the selected screen. A list of open protocols and image files also appears in the Window menu; you can select one to bring it to the top.

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 Viewing Images on page 47.



Results Data

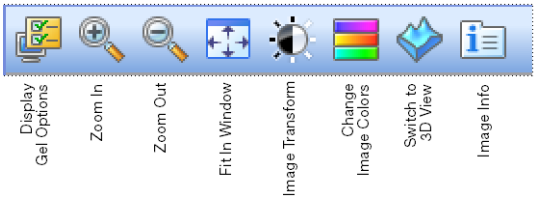
Results data associated with gel images can be viewed as an analysis table, a lane profile, a standard curve, or in a report. These views always display the analysis for the selected image.

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

See Displaying Data on page 54 for details.

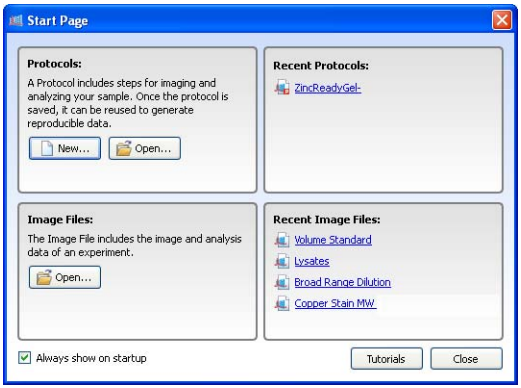
Display Toolbox

The display toolbox near the top of every image enables you to display images in the most useful ways. See Viewing Images on page 47 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 the analysis tools available.

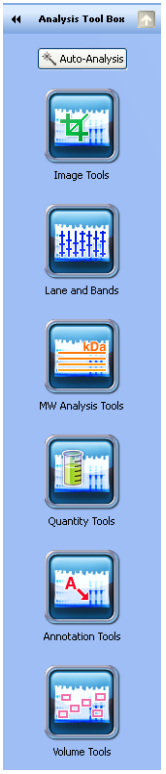


Image Tools enable you to flip, rotate, and crop images and to transform the image files.

Lane and Band Tools control the detection function, enabling you to resize, adjust, and bend lanes and 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.

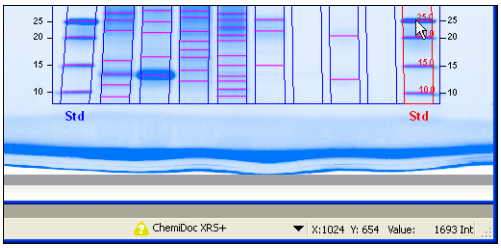
Annotation Tools are useful for drawing attention to any area of a gel.

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

These tools are described on page 61.

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 Gel Doc XR+ and ChemiDoc XRS+ Systems is 0–65,535, but the range varies depending on the values contained within each image.

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 edited and stored for reuse.

Open browses your 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.

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 88 for more information
- **Export for Analysis**—creates a TIFF formatted file that retains all of the 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 89 for more information
- **Export for PulseNet**—reduces the image to an 8-bit TIFF image file. Resolution is limited and file size is restri cted to 300 dpi
- **Lane and Band Table to Excel**—if Excel (or Numbers, on a Macintosh) 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 Macintosh) is installed on your computer, it will launch with your volume table displayed as a spreadsheet
- **Volume Table to File**—exports as a CSV (comma-separated values) file so your volume table can be opened in a database application. See Exporting Results on page 87 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 such as the exposure time and illumination source used, in three tabs (Image Details, Analysis Settings, and Notes). See Image Info on page 53 for more information.

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

- **Exit** closes Image Lab software (after prompting you to save changes to your protocols or images).

Edit Menu Commands

Undo undoes your last action.

Redo restores your 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. The image can be placed on the clipboard or saved in a file.

Default Imager enables you to switch between multiple imagers.

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

Report Settings enable 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 you to choose whether to include all, or just some lanes, with appropriate identifiers. Lane profiles can also be included
- The Volume Table tab enables you to choose appropriate identifiers for the Volume Table and provides the option of excluding the table from reports

Preferences displays 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, regardless of the color of the gels

View Menu Commands

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 into a small section of an image.

Image Transform opens a window showing a histogram, with which you can adjust the light and dark values of a gel image. This adjustment does not change your data; it changes only the way the data displays on your monitor.

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

Window Menu Commands

The Window menu 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 Menu Commands

Image Lab Help displays the help system.

User Guide displays the instruction manual in .pdf form.

About displays Image Lab software version information and release date.

6 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.

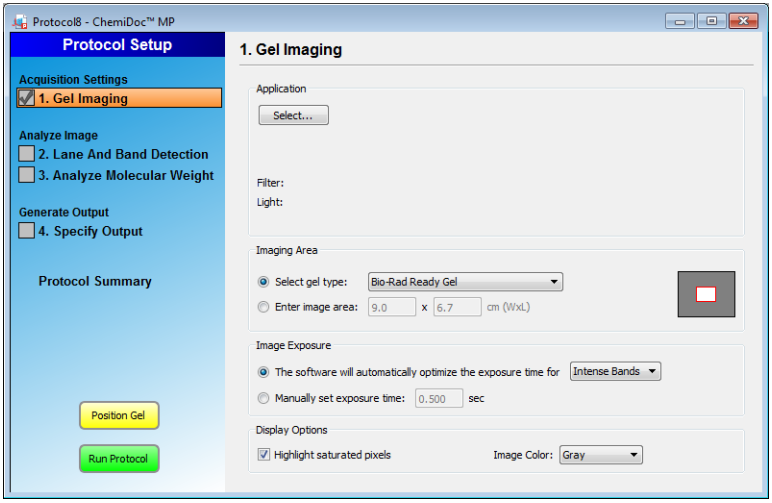
In Image Lab software, a protocol is any combination of settings for imaging, analyzing, and reporting that has been saved to run as a single workflow.

A protocol allows the choice of one application for acquisition of a single image from a sample, with the exception of single accumulation mode for chemiluminescence.

To access the Protocol Setup window:

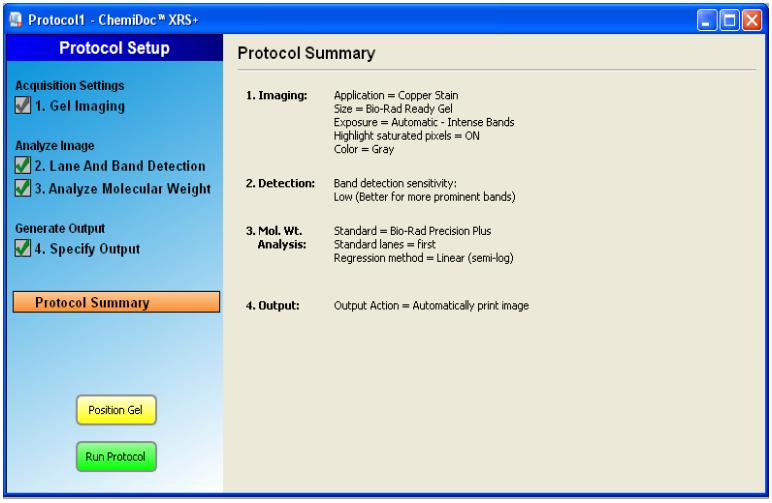
You start a new protocol by choosing the New Protocol box on the Start Page or by choosing New Protocol from the toolbar or menu bar.

The Protocol Setup Window



This is the Protocol Setup window. The left pane displays headings and, under the headings, 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.



- 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 Protocol

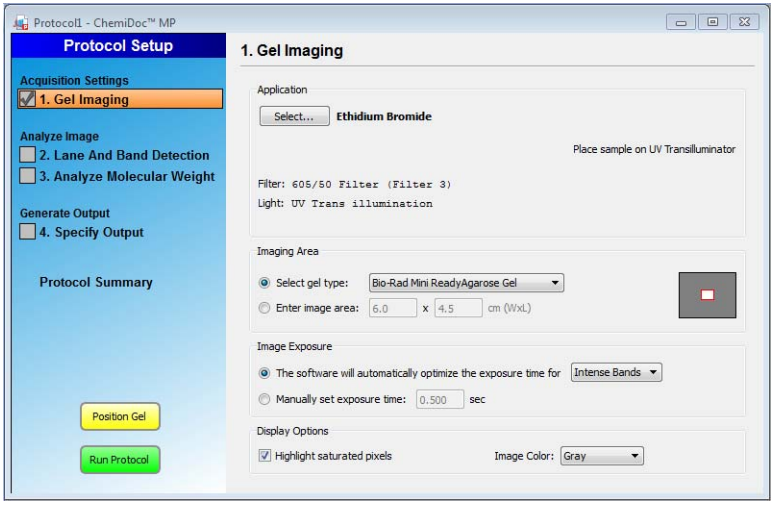
Setting up a protocol consists of three processes:

- Choosing acquisitions 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.

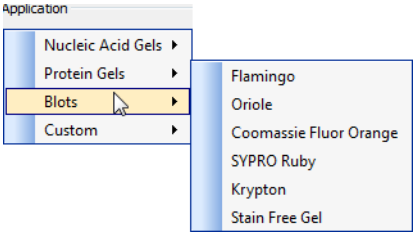
Acquisition Settings

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.



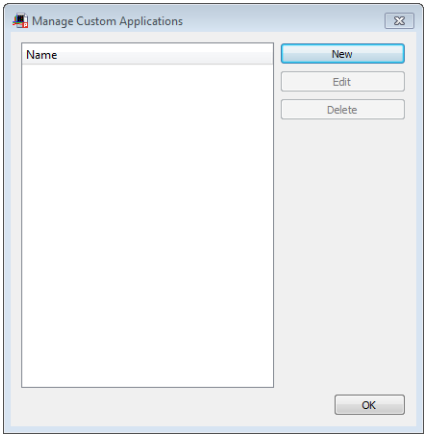
Note: For a list of applications with all required detection reagents, light sources, and any conversion screens or filters noted, see the Application Tables on page 45.

Custom Applications

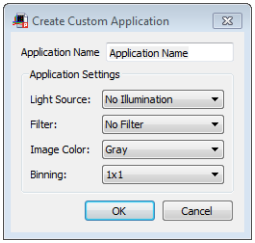
Choose Custom Applications to run an existing application with a new name or an application unlike existing applications. Selecting Custom on the applications menu displays the Manage Custom Applications dialog box. If you have stored Custom Applications, they display here.

To create a new custom application:

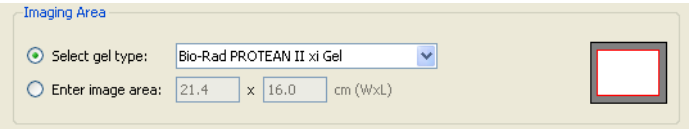
1. Select New in the Manage Custom Applications dialog box.



The Create Custom Applications dialog box appears.



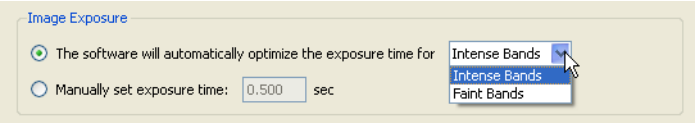
2. Choose a unique application name.
3. Select a light source, filter, and image color on the drop-down lists.
4. Select a binning setting. Choosing a higher binning setting combines pixels to increase the amount of signal without increasing noise. While a higher setting provides optimal sensitivity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



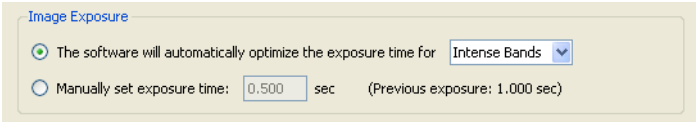
8. **Choose Image Exposure Time**—If you select the top radio button, Image Lab software acquires an image estimating an optimal exposure time. This option ensures the best use of the dynamic range.

- If you choose Intense Bands from the drop-down list, exposure is optimized for all bands
- Choosing Faint Bands from the drop-down menu results in a longer exposure where faint bands are more visible, but more prominent bands may be overexposed
- Select the manual exposure radio button to override automated imaging. This may cause saturation (overexposure) of more prominent bands. Exposure time can range from 0.001 to 99

seconds using the Gel Doc™ XR+ system and from 0.001 to 7200 seconds for the ChemiDoc™ XRS+ system.

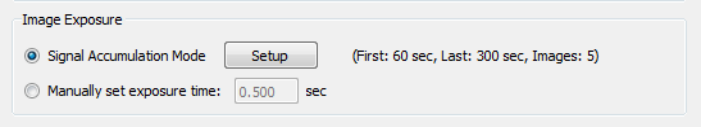


After imaging a gel with automatic exposure optimization, the used exposure time displays in the protocol (see below), so that you can manually adjust the exposure time if needed.



Note: You can also view the exposure time of the image later, in the Image Info dialog box (refer to Image Info on page 53).

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

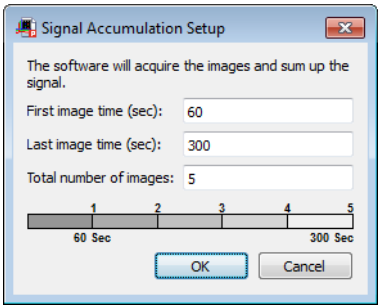


SAM is used to simplify capture of a good image of a chemiluminescent sample. This sample type often requires long integration times to obtain an image that represents the best range of signal.

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 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, 3 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, starting at 1 min and ending at 5 min. The second one-minute image is added to the first one-minute image, resultng 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 does not result in data as accurate as 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.

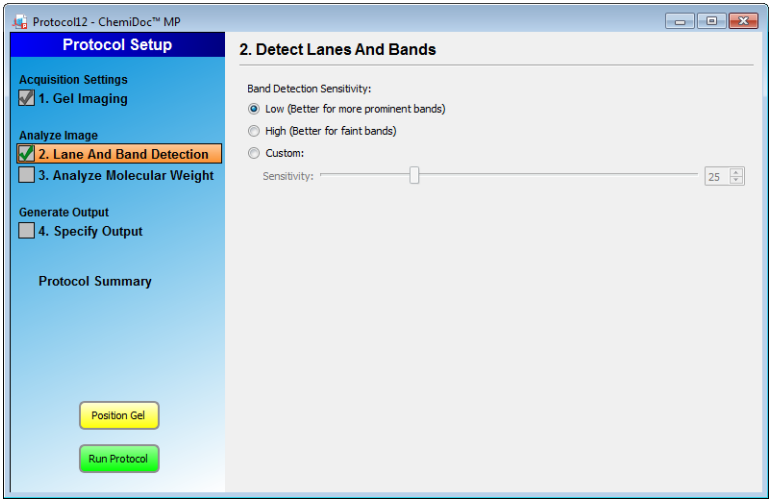
9. **Set the Display Options**

   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 51 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 automatically subtracted. Refer to Lane and Band Tools on page 66 for details. You customize band detection with the following options.

10. 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 Tool Box. See Lane and Band Tools on page 66.

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

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

STEP 3. ANALYZE MOLECULAR WEIGHT

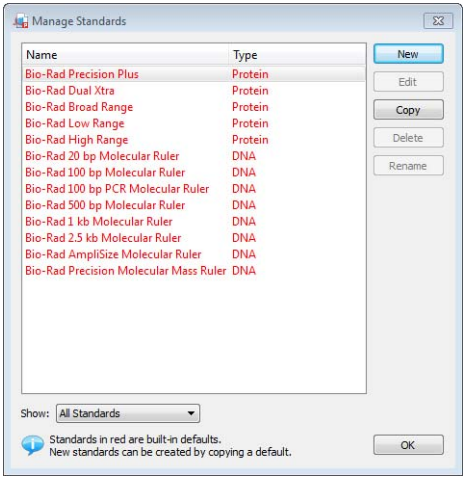
11. To analyze molecular weight automatically, select Analyze Molecular Weight in the left pane of the Protocol Setup window. When this checkbox is selected, the software calculates the molecular weight for each band based on the specified standard.

12. 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 the selection of the proper protein standards. Many protein standards are available from Bio-Rad. Many different DNA standards are also available. See Ordering Information on page 103 for all standards available from Bio-Rad and their catalog numbers.

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



STANDARD LANES

13. 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 four regression methods.

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.

14. 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 (for more information, see Standard Curve on page 58). 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 fits the standard curve.

The molecular weight of each band is displayed in the analysis table in the Mol. Wt./Base Pair column. Refer to Copy Analysis Table to the Clipboard on page 90 for more information about molecular weight.

For information about the calculations behind the regression methods, see Regression Calculation Methods on page 111.

Output Settings

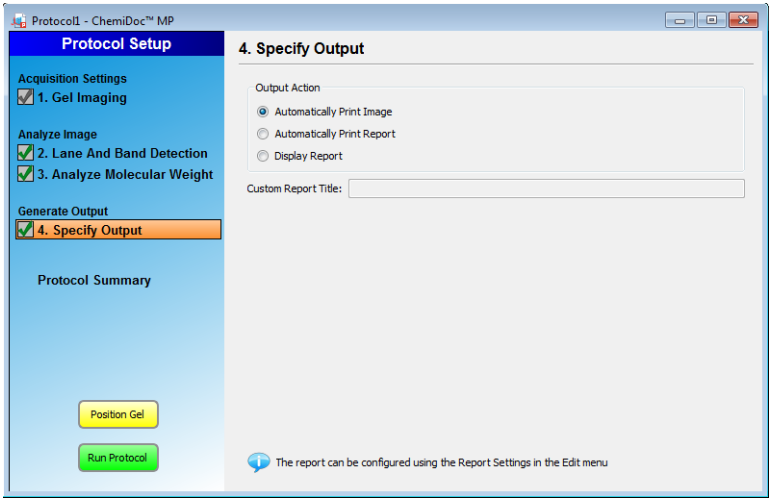
Image Lab software prints to the default printer unless you select otherwise.

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 Mitsubishi P93/P95 Thermal Printer Settings on page 107 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



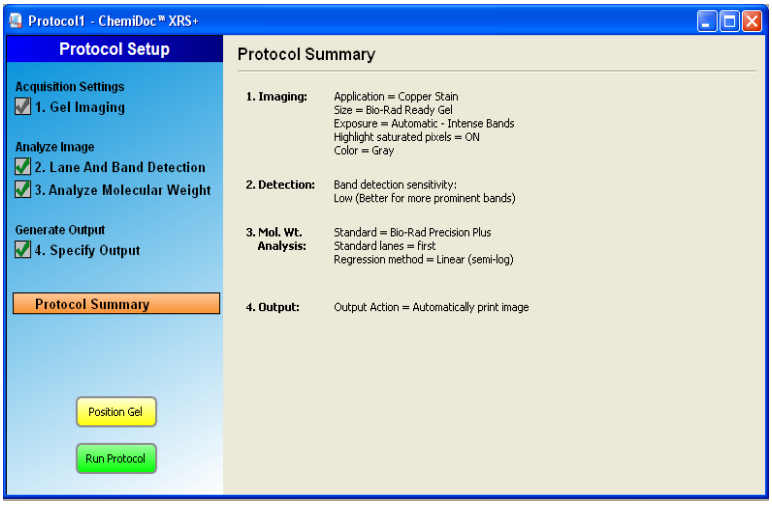
15. Select Specify Output in the left pane of the Protocol Setup window to display output options.
16. In the right pane, you can choose whether to automatically display or print an image or a report after a protocol runs.

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

    For information about reporting options, see Report on page 83.

Review Protocol Settings

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



Choosing a Protocol

To open a saved protocol:

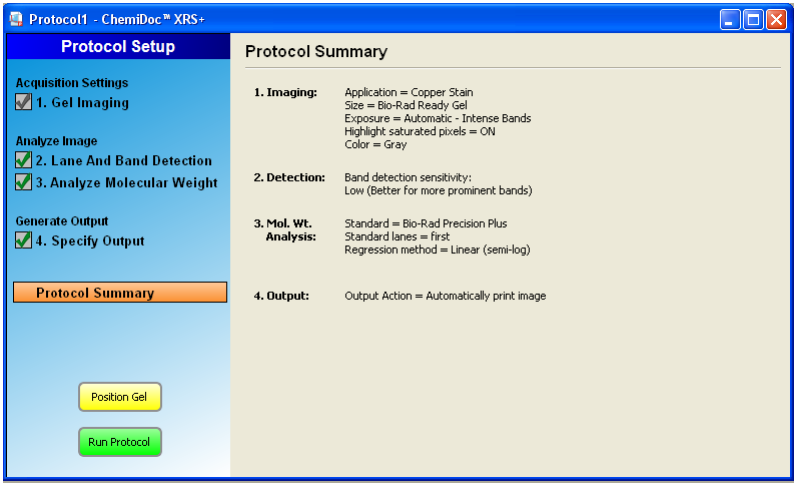
- Click Open on the Start Page or click File > Open on the menu bar.

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

Editing Protocols

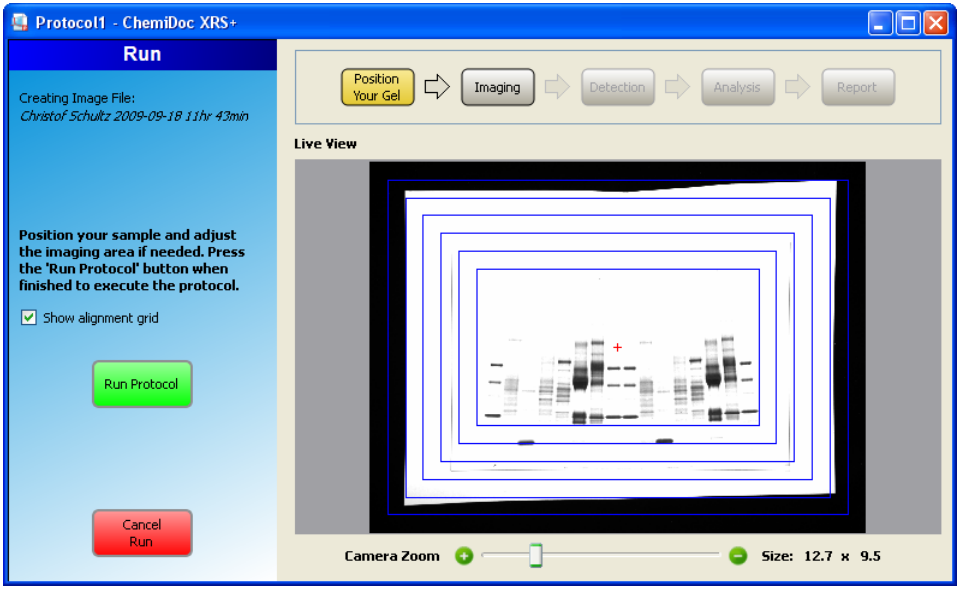
Existing protocols can be changed and/or renamed using the many tools in Image Lab software.

Open any saved protocol. You are presented with the same set of menus and choices described in Creating a Protocol on page 31.



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.

Positioning the Gel



To position a gel:

1. Click the yellow Position Gel button in the Protocol Setup window shown on page 42.
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 and allows them to be centered quickly and easily. See Accessories on page 97 for more information.

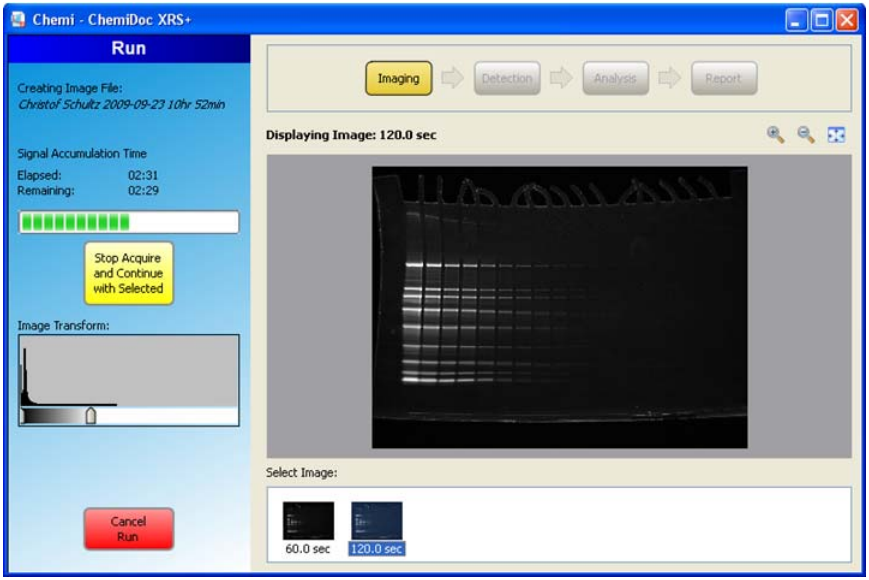
Running a Protocol

To execute a protocol:

- Click Run Protocol in the left pane

The software runs through the steps in imaging mode. 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.

Application Tables

The following tables contain all of the light sources, conversion screens, or filters you need for each application.

Table 1. Nucleic acid gels

Detection Reagent	Preferred Light Source	Alternate Light Source	Preferred Filter	Alternate Filter	Preferred Flat Field	Alternate Flat Field
Ethidium bromide	UV	None	Standard	630/30	Orange	Lens
SYBR® Green	XcitaBlue™	UV	560/50	Standard	Lens	None
SYBR® Safe	XcitaBlue	UV	560/50	Standard	Lens	None
SYBR® Gold	XcitaBlue	UV	560/50	Standard	Lens	None
GelGreen	XcitaBlue	UV	560/50	Standard	Orange	Lens
GelRed	UV	XcitaBlue	630/30	Standard	Lens	None
Fast Blast™ stain	White Trans	None	Standard	None	White Conv	Lens

Table 2. Protein gels

Detection Reagent	Preferred Light Source	Alternative Light Source	Preferred Filter	Alternative Filter	Preferred Flat Field	Alternate Flat Field
Coomassie Blue	White Trans	None	Standard	None	White Conv	Lens
Copper stain	White Trans	None	Standard	None	White Conv	Lens
Zinc stain	White Trans	None	Standard	None	White Conv	Lens
Flamingo™ stain	UV	None	Standard	Standard	Orange	Lens
Oriole™ stain	UV	None	Standard	None	Orange	Lens
Silver stain	White Trans	None	Standard	None	White Conv	Lens
Coomassie Fluor Orange	XcitaBlue	UV	560/50	None	Orange	Lens
SYPRO Ruby	UV	None	Standard	None	Orange	Lens
Krypton	XcitaBlue	UV	560/50	Standard	Orange	Lens

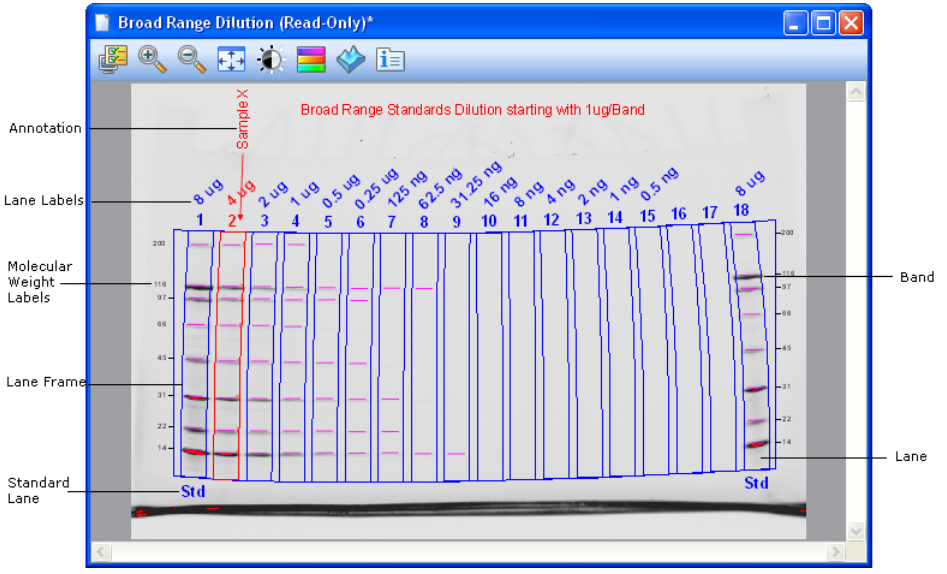
Table 3. Blots

Detection Reagent	Preferred Light Source	Alternative Light Source	Preferred Filter	Alternative Filter	Preferred Flat Field	Alternate Flat Field
Colorimetric	White Epi	None	Standard	None	Lens	None
Qdot 525	UV	None	520/30	Standard	Lens	None
Qdot 565	UV	None	560/50	Standard	Lens	None
Qdot 625	UV	None	630/30	Standard	Lens	None
Cy2	UV	None	520/30	Standard	Lens	None
Alexa Fluor 488	UV	UV	520/30	Standard	Lens	None
DyLight 488	UV	UV	520/30	Standard	Lens	None
Fluorescein	UV	None	520/30	Standard	Lens	None

7 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



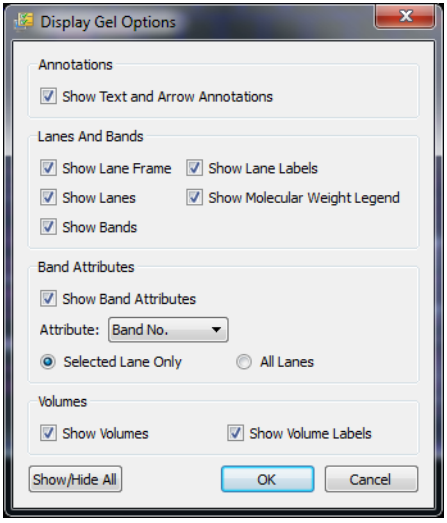
The screen shows a gel image with band and lane detection as well as annotations. The labels show overlays that you can show or hide.

Refer to Display Gel Options on page 48 for more information.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 as a report.

Displaying Gel Images

Toolbar buttons appear above the displayed gel image. Each of these tools is described in the following sections.

Display Gel Options 



Annotations

You can choose whether or not 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
- Abs. Quant.
- Rel. Quant.
- 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 minus sign to make the image smaller.

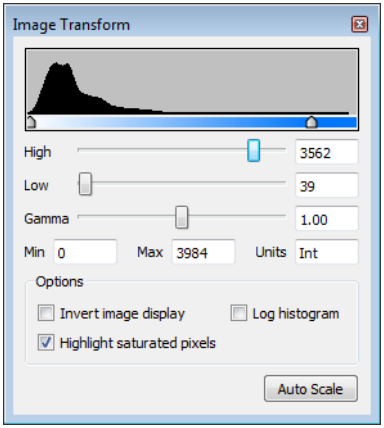
You can also zoom with the right mouse button. Click and drag to select an area you want to magnify. Right-click again to return to the original view.

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

Fit in Window 

When you zoom in on an area of an image, clicking this button brings the entire image 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 grayscale (or other color) in the gel image
- The Low progress indicator determines which intensity value is shown at the minimum grayscale (or other color) in the gel image
- The Gamma progress indicator changes the grayscale curve. A value of 1 is linear. A value < 1 redistributes a greater proportion of the grayscale to the first half of the intensity values. A value > 1 redistributes a greater proportion of the grayscale to the second half of the intensity values

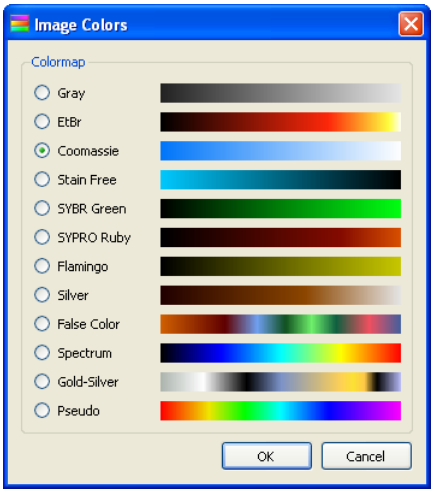
You can also type numerical values in the text boxes next to the progress indicators. Clicking anywhere on the progress indicator bars moves the progress indicator incrementally.

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

Image Colors

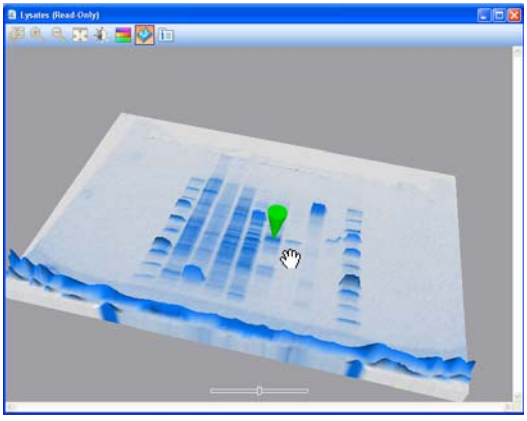


You can choose a colormap for an image results file. Viewing the image with a different color scheme can make it easier to see all of the elements in the image, but it does not change the data.

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 3-D 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.

To view the intensity of various bands:

1. Select 3-D 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 a 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 about the image.

Image Details

Acquisition and image information appear in this tab.

Analysis Settings

The settings that were used when the gel was analyzed are displayed here, for example, Band Detection and Molecular Weight Analysis, 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 Data

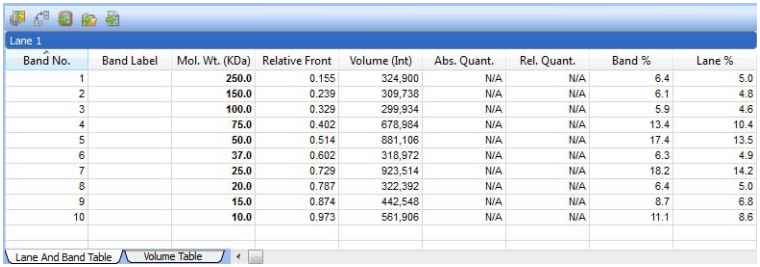
The results from analyzed data associated with the gel images can be visualized using an analysis table, a lane profile, a standard curve, or a report.

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 

Numerical data associated with an analysis can be viewed in the analysis table. Data from the Lane and Band analysis can be viewed in the Lane and Band tab. If volume analysis was performed, this 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 displayed by clicking the arrow buttons to move them from the Displayed to the Not Displayed field.

Default display settings—move selected lane to top is on or off, depending on whether the checkbox is selected.

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

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

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

Export delimiter—this field offers three delimiter options for the exported file.

- CSV (comma-separated values)
- Tab delimited
- Other user-defined delimiter

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 (comma-separated values) 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 the data. If you have Excel (or Numbers, on a Macintosh) 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 0–1 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—Unknown, B—Background, S—Standard) 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.

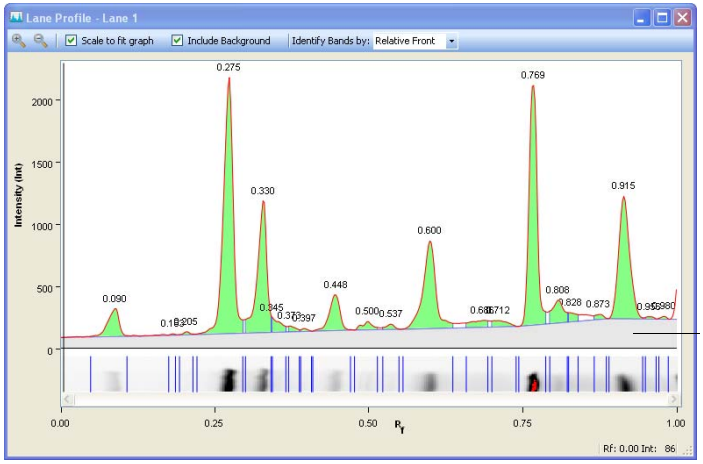
Maximum Value—intensity of the pixel with the maximum intensity inside the volume.

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 degrees. 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

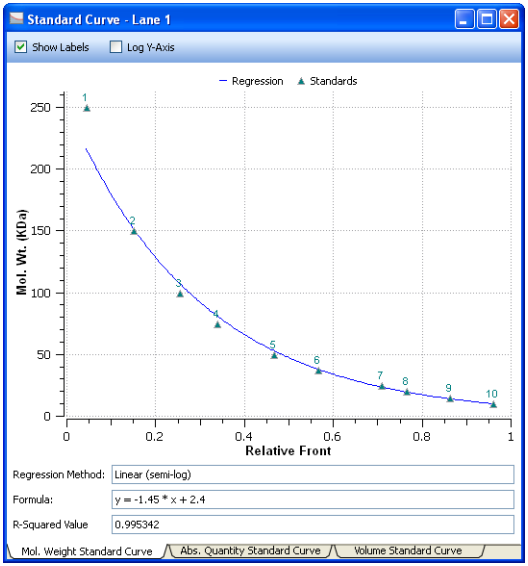
When you clear the Include Background checkbox, the area of the lane profile that represents the background of the image does not display.

IDENTIFY BANDS BY

You can display call outs on the Lane Profile, which indicate these attributes:

- Band Number
- Band Label
- Molecular Weight
- Relative Front
- Volume
- Abs. Quant.
- Rel. Quant.
- 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 display the standard curves for three different analyses.

Standards appear in green; unknown bands appear in red. You can change 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 display the Mol. Weight Standard Curve, the Abs. Quantity Standard Curve, or a Volume Standard Curve.

Report 

See Generating Reports on page 83 for information about reports.

8 Analyzing Images

Analysis Tool Box 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 Macintosh, 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.

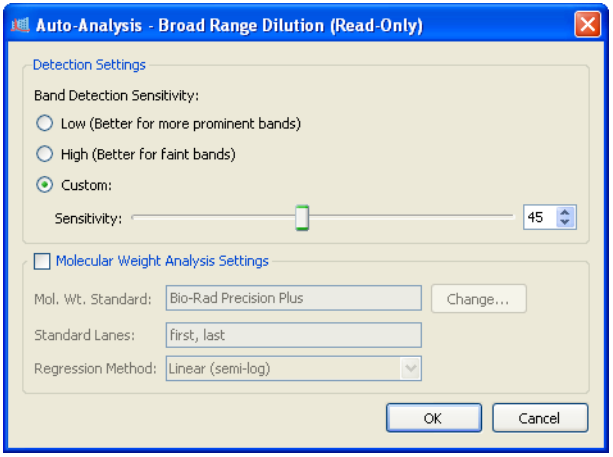
Auto Analysis Settings

Clicking Auto Analysis in the Analysis Tool Box enables you to do the following:

- Analyze images that were 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 the image file with a different name.

DETECTION SETTINGS



Band detection options are as follows:

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

High Band Detection Sensitivity—this 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 66.

Custom—you can set a numerical value between 1 and 100 to select the best detection sensitivity for your sample.

When Low 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 37.

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 39.

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.

> **Note:** Some tools delete the existing analysis.

To access a tool:

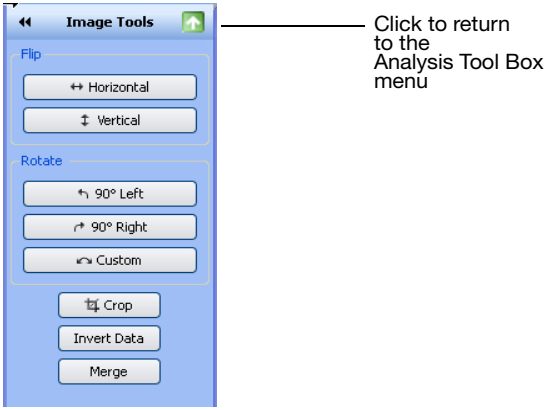
- Click any of the tool box buttons

To return to the Analysis Tool Box 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 gel image horizontally or vertically.

Rotate

You can rotate gel image 90° to the left or right by clicking the Rotate buttons.

To correct a slanted gel:

1. Click Custom.
2. Rotate the red arrows that appear over the gel to any degree between 0–360° by dragging them.
3. Right-click the gel image and choose Rotate to set the 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 to use 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 the 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 anchors 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 1–99 mm) that removes background levels along the length of the lane. The size of the disk determines how much background is subtracted.

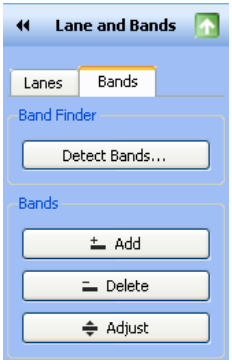
A large disk follows the profile trace less closely, touching fewer points along the trace and removing less background. A smaller disk more closely follows the profile trace, removing more background.

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. See Lane Background Subtraction on page 67.

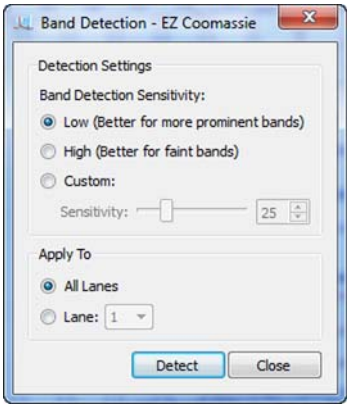
Apply to selected Lane—When you select this box, the level of background subtraction specified with the previously described buttons is applied only to the selected lane. This option enables you to set different background subtraction levels for each lane.

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 50.

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 the 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:

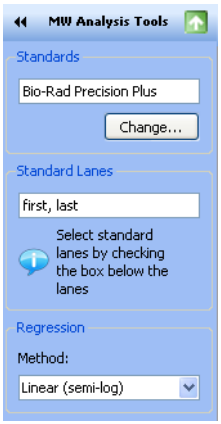
Lane 1 - Precision Plus

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

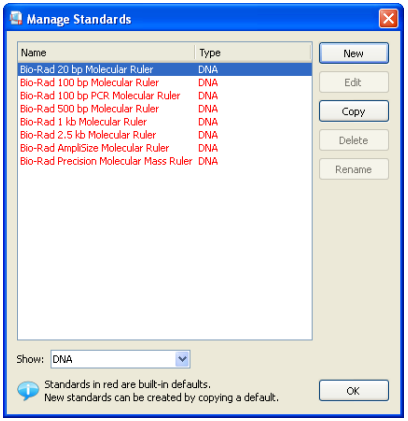
Lane And Band Table | Volume Table

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

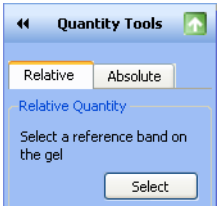
There are four regression methods. See Regression Methods on page 39.

Quantity Tools

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

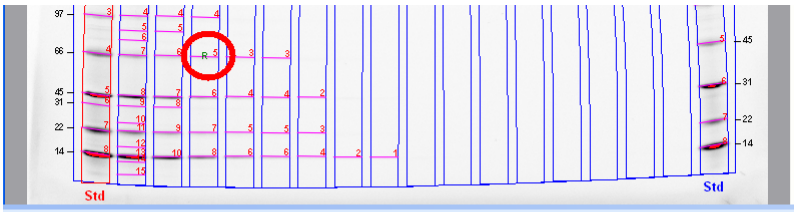
Relative Quantity Tab

To compare the relative quantities of bands, first select Quantity Tools (Relative tab) as shown.



To select a reference band from an image and quantitate all other bands relative to the reference band:

1. Click the Select button.
2. Click any band you want to use as a reference. A small R for Reference appears near the band you selected (see the red circle in the upper left).



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

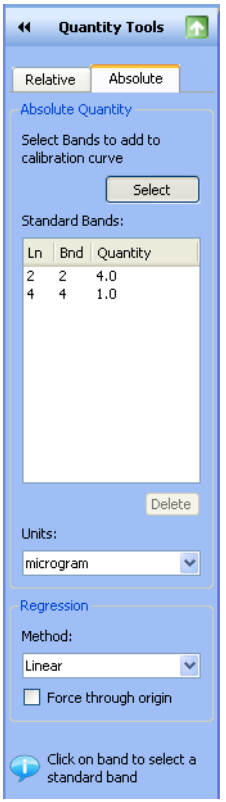
To review the relative band quantities:

- 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 Quantity Tools (Absolute tab) as shown:



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.

   Note: Any standard bands selections 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 ten choices listed in the Units drop-down list.
4. Select a regression method from the three listed in the drop-down list.

   Keep the following guidelines in mind while making your choice.

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

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

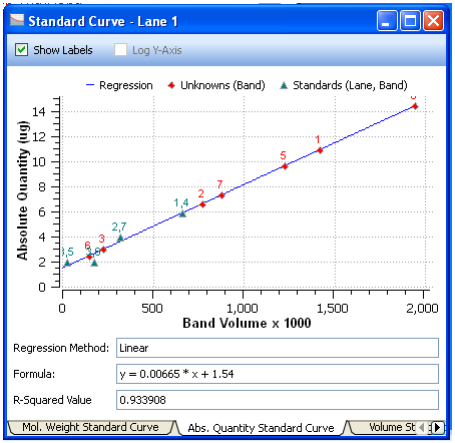
   Cubic spline—this method generates a smooth curve that connects each data point. At least four standard points are required to use this method of least-squares polynomial fits.

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. All standards are

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

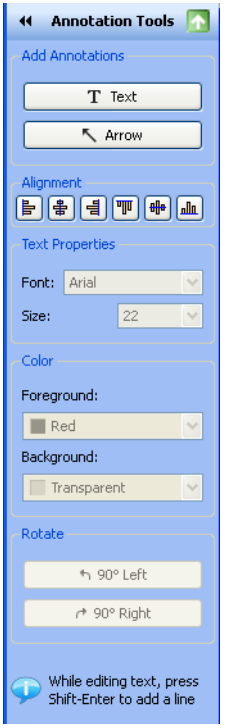
Note: Selecting the Force Through Origin checkbox always starts the standard curve graph at 0,0, regardless of the best curve fit.



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.

Alignment

Alignment Icons—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 and CTRL+V to paste the annotations.

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 list to show all fonts installed on your system. Click one of the font names to change the style of the text annotation.

Size—first click the text box you want to resize. Click the up and down Size arrows 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 use the drop-down list to choose a different color.

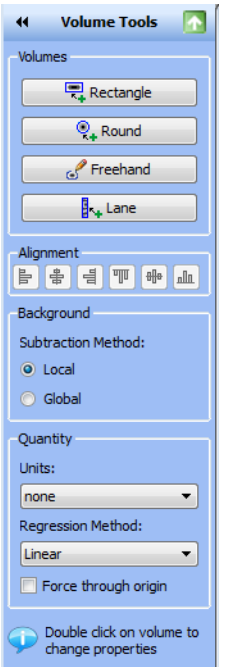
Background—click a text annotation. This also activates the Background field, so you can use the drop-down list to choose a background color.

Rotate

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

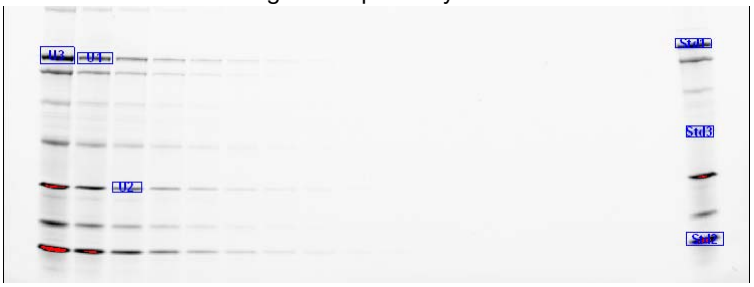
Volume Tools

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.



You can use volume tools to quantitate the signal intensity of bands, spots, arrays, and other image data. You define an area of interest by surrounding it with a shape. You can choose a rectangle, circle, freehand shape, or lane by clicking the buttons 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 56.

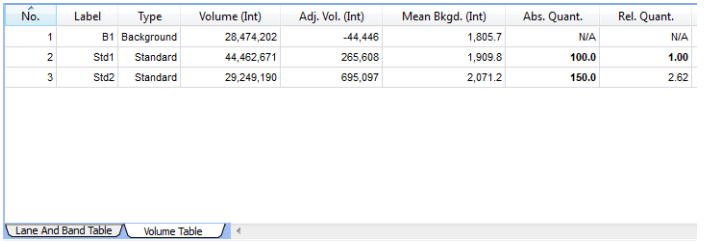
VOLUME TYPES

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 81.

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 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 non-data 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. Create a volume using one of the volume tools in a representative background region of your image (that is, a non-data 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 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

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 Regression Calculation Methods on page 111 to learn how each of these methods is calculated.

The data for volume standards is 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 and then CTRL+V to paste.

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

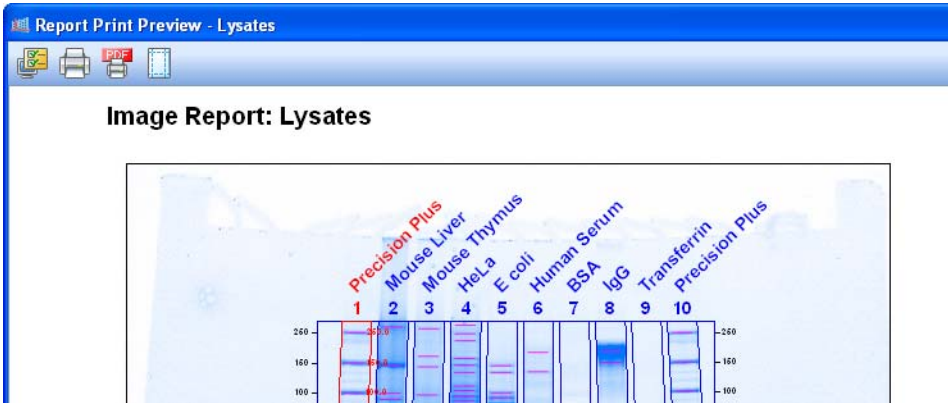
9 Generating Reports

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.

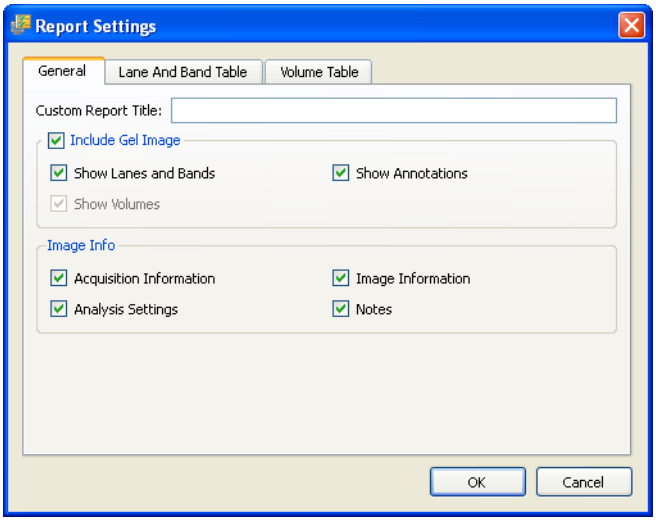


Clicking Print prints all of the above information to a printer.

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

Display Report General Tab

The default setting is for all information to be included. These choices may have been made at other stages of the workflow, but you can customize reports here.



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

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

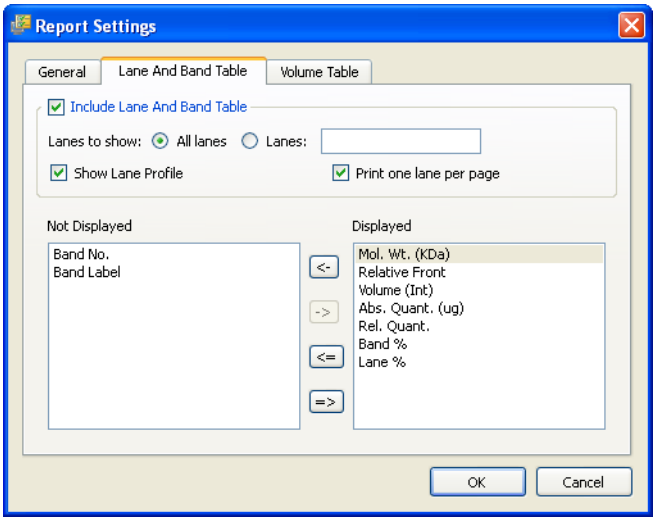
Include Gel Image—The following options determine which overlays display on the gel image.

- 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

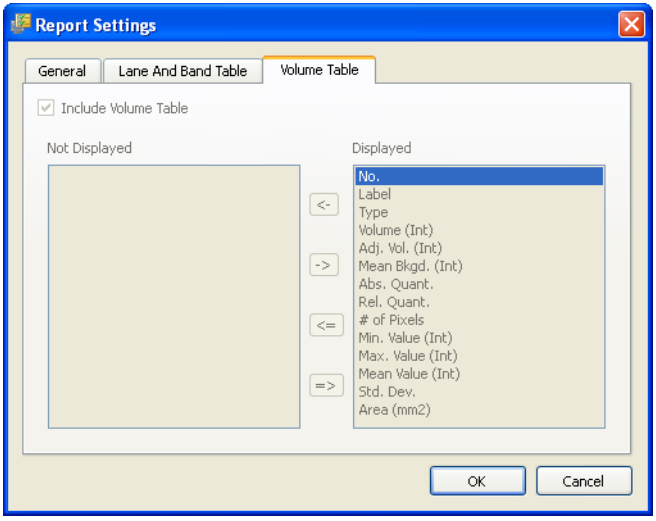
Lanes to show:

- All lanes or just 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.

Move labels to the left—if you do not want these columns to be included in the report.

Volume Table Tab



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

Move volume labels to the left—to exclude their columns from the report.

Print Report

Click Print Report to print a report.

Print Report to .pdf File

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

Adjust the Printer Settings

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

10 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.

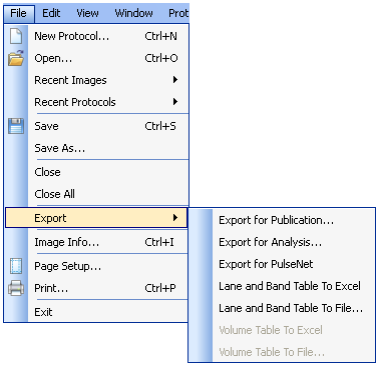
Exporting Gel Images

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.

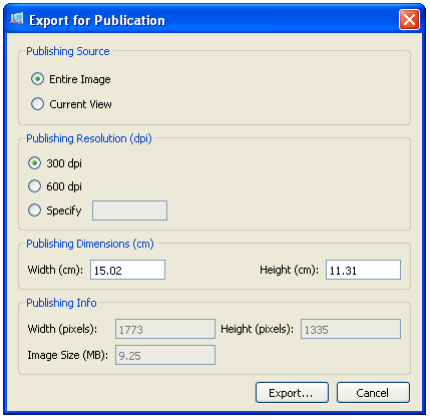
The available export options are described in the following sections.



Exporting Gel Images for Publication

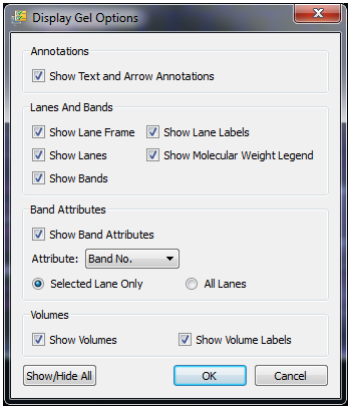
Use this format only to export visual information to presentation or word processing software, such as PowerPoint or Word.

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 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.

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.

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 Excel. 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 (comma-separated values) 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 to 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 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 Macintosh) installed on your computer, the data open in Excel.

11 Maintenance

This chapterincludes 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.

There are three types of bulbs available. The catalog numbers are listed in Ordering Information on page 95. The standard bulb is 302 nm. Optionally, the 254 nm bulb is used for cross-linking of proteins and nucleic acids, 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 the starter 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.

Epi-Illumination Lamp Replacement

The lamps are located behind two cover panels on the left and right sides of the universal hood's interior.

To replace the lamps:

1. Turn system power off and remove the power cord.
2. Open the enclosure door.
3. Locate the epi-white light housing inside the hood and the screw on the center of the cover.
4. Use a socket wrench to remove the screw, and the plastic cover that it holds in place.
5. Pull the cover to remove it from the housing, and the lamp will become visible.
6. To remove the lamp, hold it at the plastic receptacle and then pull it from the receptacle.
7. Insert the new lamp into the lamp receptacle and then push it into the receptacle.
8. Reassemble the epi-white light cover.

Fuse Replacement

Always unplug the instrument before changing or checking the fuses.

This unit is protected by 2 fuses (5 x 20 mm, 2A Slo-Blo). The fuses are located in the left side rear of the universal hood, in a fuse holder housed in the power entry module. See picture above.

To replace the fuses:

1. Unplug the main power cable from the power outlet.
2. Use a screwdriver as a lever on the fuse holder to extract the fuse.
3. Remove the blown fuses and replace them with two new ones (catalog #900-8935).
4. Slide the fuse holder into the power entry module until it snaps in place.

A Troubleshooting

Follow these suggestions to troubleshoot your Gel Doc™ XR+ or ChemiDoc™ XRS system.

Problem	Possible Cause	Solution
Gel Doc XR+ and ChemiDoc XRS+ Systems		
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 chemiluminescent, 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
Gel Doc XR+ System Only		
Hot (very bright) pixels are seen in the image	• Corrupted dark image correction	• In Edit > Instrument Setup, recalibrate the dark image correction file
ChemiDoc XRS+ System Only		
Images are grainy	• Low signal-to-noise	• For chemiluminescent applications, make sure no filter is used • For applications other than chemiluminescent, make sure the filter is in line with the lens • Use sample that generates more signal • Use longer exposure times

Appendix B 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 which arrives with your system, either 10016995 for the Gel Doc™ XR+ system or 10016999 for the ChemiDoc™ XRS+ system. If you acquire new conversion screens, light sources, or filters for a Gel Doc XR+ or ChemiDoc XRS+ imaging system after your original system installation, you will have to recalibrate your system to use them.

Refer to Chapter 4 for instructions on how to calibrate newly-acquired accessories.

Installing Optional Accessories

UV/White Light Conversion Screen

This optional white light conversion screen 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 the system:

- Go to the Edit > Instrument Setup dialog box and select the appropriate checkbox. 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.

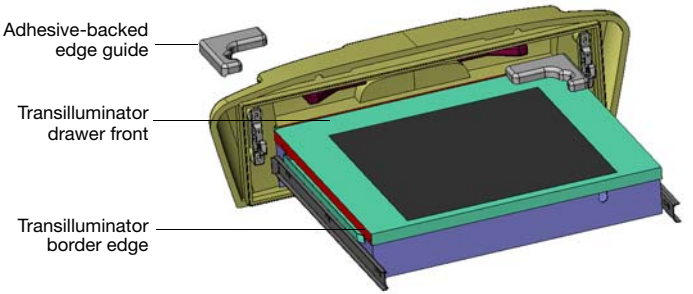
3. Image the gel using your preferred application.

XcitaBlue™ Conversion Screen

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

The XcitaBlue conversion screen is held in place by adhesive-backed edge guides. After installing the edge guides, 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. 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. When you need 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. See the Gel Alignment Template Kit for help centering 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, as well as ensuring 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 can be used with the XcitaBlue conversion screen kit (catalog #170-8182). The templates fit exactly into the XcitaBlue conversion screen frame.

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.

White Light Transilluminator

The white light transilluminator provides a white light that is independent of the UV bulbs. Between uses, this transilluminator can be stored in the rear of the universal hood.

To install the white light transilluminator:

1. Open the enclosure door and pull the drawer toward you.
2. Locate the power cable positioned on the inside left side of the enclosure, behind the drawer slide.
3. Remove the black rubber boot that covers the connector plug and insert it into the white light transilluminator. Make sure that the main power switch on the transilluminator is in the ON position.

   Note: Power to the white light transilluminator is controlled from either the software or the membrane touch pad on the universal hood.

4. Place the white light transilluminator on top of the UV transilluminator.

Your system must be calibrated to use the white light transilluminator.

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

Orange Fluorescence Reference Plate

The 0range fluorescence reference plate allows you to apply UV flat fielding corrections to your Gel Doc XR+ and ChemiDoc XRS+ imagers. Corrections are made for all UV illumination sources, filters, and the camera lens.

The 0range fluorescence reference plate can be used to correct for image non-uniformities for red and orange gels, including:

- Ethidium bromide
- GelRed
- Flamingo stain
- Coomassie Fluor Orange
- SYPRO Ruby
- Krypton
- Qdot 625

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

- Gel Doc XR+ 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 Your System to Use the Orange Fluorescence Reference Plate

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

To calibrate the system:

- 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 Gel Doc XR+ or ChemiDoc XRS+ systems, plus all optional accessories and replacement parts. For complete information, see the Bio-Rad catalog.

Catalog #	Description
Molecular Imager System (includes universal hood, camera, cables, and accessories)	
170-8195	Gel Doc XR+ with Image Lab system for PC/Mac
170-8265	ChemiDoc XRS+ with Image Lab system for PC/Mac
Installation Kits	
170-8199	Gel Doc XR+ installation kit
170-8299	ChemiDoc XRS+ installation kit
Universal Hood	
170-8180	Universal hood
Imaging Cameras	
170-8181	Gel Doc XR+ camera with motorized zoom lens
170-8255	ChemiDoc XRS+ camera with motorized zoom lens
Image Lab Software	
170-9690	Image Lab software, Windows/Mac
Optional Filters	
170-8074	Filter, 520DF30, GFP
170-8075	Filter, 560DF50, Cy3/Rhodamine
170-8076	Filter, 630BP30, SYPRO Ruby
Optional Accessories	
170-8001	UV/White light conversion screen (UV to white light)

Catalog #	Description
170-7950	White light transilluminator (Universal hood plug-in)
170-8182	XcitaBlue (UV to blue light) conversion screen kit, without standard detection filter
170-8183	XcitaBlue (UV to blue light) conversion screen kit, with standard detection filter
170-8008	Orange fluorescence reference plate
170-8089	Mitsubishi thermal 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	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)
1001-4106	Thumbscrew for camera
170-8081	Standard emission filter replacement, 62 mm
100-1370	UV bulb starter, quantity 3
930-2242	Multicolor target
	Lamps
100-2827	Lamp, epi-illumination, 5 W
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
100-2785	Universal hood right shield epi-illumination (lid complete)
100-1948	Universal hood opal filter epi-white illumination (without metal frame)
100-2786	Universal hood left shield epi-white illumination (lid complete)
100-2787	Universal hood feet, 4
170-8068	UV shield for Universal hood

Catalog #	Description
	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 software
170-9300	FPQuest™ software
170-9310	InfoQuest™FP software

Protein Standards	
161-0363	Precision Plus Protein™ Unstained Standards
161-0373	Precision Plus Protein™ All Blue Standards
161-0374	Precision Plus Protein™ Dual Color Standards
161-0375	Precision Plus Protein™ Kaleidoscope™ Standards
161-0385	Precision Plus Protein™ WesternC™ pack
161-0318	Prestained SDS-PAGE standards, broad range
161-0317	Unstained SDS-PAGE standards, broad range
Nucleic Acid Standards	
170-8351	EZ Load™ 20 base pairs molecular ruler
170-8352	EZ Load 100 base pairs molecular ruler
170-8353	EZ Load 100 base pairs PCR molecular ruler
170-8354	EZ Load 500 base pairs molecular ruler
170-8355	EZ Load 1 kb molecular ruler
170-8205	2.5 kb molecular ruler
170-8200	AmpliSize® molecular ruler
170-8356	EZ Load precision molecular mass ruler (base pairs/ng of sample)
Pulsed Field Standards and Markers	
170-3624	CHEF DNA size standard, 5 kb ladder
170-3707	CHEF DNA size standard, 8-48 kb
170-3635	CHEF DNA size standard, lambda ladder
170-3605	CHEF DNA size marker, 0.2-2.2 Mb
170-3667	CHEF DNA size marker, 1-3.1 Mb
170-3633	CHEF DNA size marker, 3.5-5.7 Mb

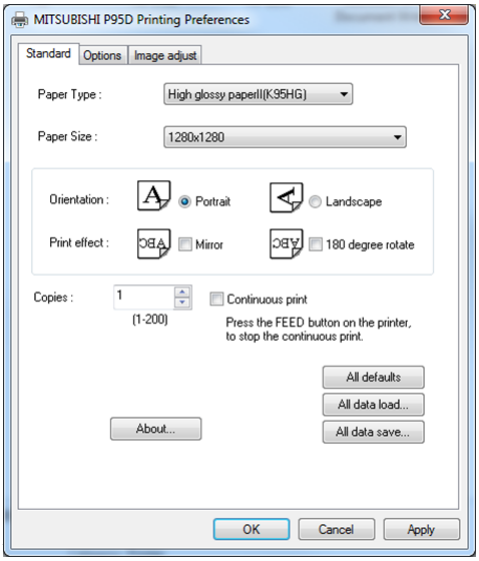
Appendix C Mitsubishi P93/ P95 Thermal Printer Settings

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. Select OK to apply your changes.

Macintosh

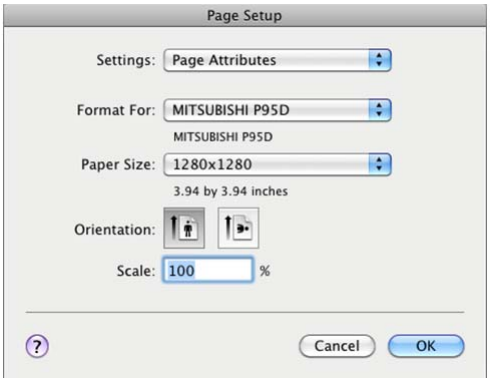
The printer driver can be found on the Image Lab software installation CD in the Misc directory.

To set up a thermal printer on a Macintosh 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 D 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 Gel Doc™ XR+ 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 which compensates for non-uniformities 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 0–1, and indicates the relative movement of the band from top to bottom.

Quantitative imaging: Determines the quantity of a sample's components (nucleic acids and proteins), 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

D

E

F

G

H

I

L

M

N

O

P

Q

R

S

T

U

V

W

X

Z

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