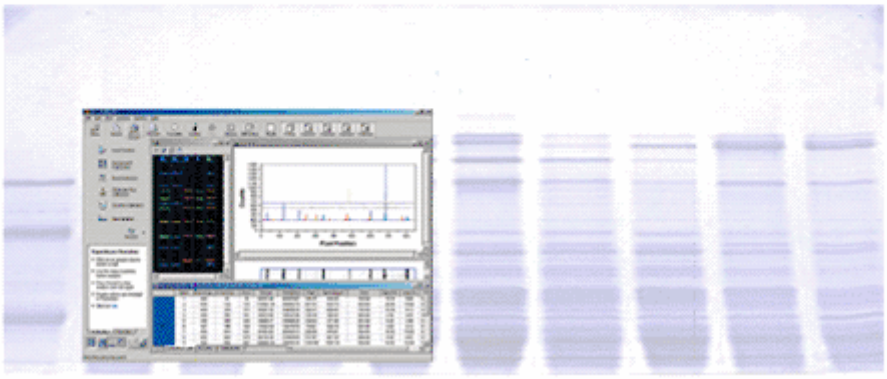
GE Healthcare

ImageQuant™-RT ECL

Version 1.0.1



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ENG 

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The following Gel Imaging Digital Systems,

Models: ImageQuant-100, ImageQuant-300, ImageQuant-400, ImageQuant-RT ECL

Are in conformity with the provisions of the following EC Directives, including all amendments, and national legislation implementing these directives:

Low Voltage Directive 73/23/EEC
EMC Directive 89/336/EEC

And that the following harmonized standards have been applied:

EN61010-1: 2001
EN61326-1: 1997+A1:1998+A2:2001 Class A
EN55011 Class A, EN61000-3-2, EN61000-3-3, EN61000-4-2,
EN61000-4-3, EN61000-4-4, EN61000-4-5, EN61000-4-6, EN61000-4-11

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Chapter 1: Introduction and Setup

1.1 ImageQuant Capture Imaging System

The ImageQuant Imaging System is a powerful digital imaging system, ideal for instant photography of a wide variety of samples. The CCD camera allows imaging of low-light samples in UV-illuminated, and fluorescent applications.

The instrument is controlled by ImageQuant™ Capture software, which is designed with ease-of-use in mind. ImageQuant Capture handles image acquisition and archiving, and can be used to prepare images for desktop publishing.

ImageQuant Capture includes tools to optimize the image display by adjusting contrast automatically or manually. Hard-to-see portions of the image can be clarified by converting the image from positive to negative, using digital filters, or applying a false color map. Notes, labels, arrows, lines and other drawing tools can be recorded directly on the image using the annotation functions available in ImageQuant Capture. Annotations are superimposed on the image when a hard copy is printed and can either be saved as a template file or as part of the image. All of these features are accessible via convenient on-screen buttons and menus controlled by the mouse.

ImageQuant Capture can also be used to transfer an acquired image to one of the ImageQuant TL modules for detailed analysis.

The ImageQuant System is a complete package, including all the hardware and software needed for image capture, enhancement and analysis. The system's computer need not be solely dedicated to ImageQuant Capture and can also be used to run other software such as word processing programs, spreadsheets, and desktop publishing software.

Images can be printed using a 256-level gray scale thermal printer or any printer with a Windows® driver. The low-cost, high-quality prints are ideal for record keeping in lab notebooks or for publication.

Images can be archived to the system’s hard disk, floppy disk, Zip or optional Jazz drive, or to a network drive if applicable.

In section 1.4, there is a condensed instruction sheet, which will be useful if you are familiar with the program but need to quickly refresh your memory. We suggest you photocopy the appropriate instruction sheet(s) and post them near the instrument for easy reference.

1.1.1 Mouse Functions

The mouse supplied with the ImageQuant System has two buttons. The left button is used to activate functions and otherwise make selections when using the software. In some cases, the right mouse button is used to recall or reactivate the function that was most recently assigned to the left button.

1.1.2 About This Manual

Throughout this manual, different fonts are used to indicate certain things:
This font indicates the name of a button, a menu, or a function found in a menu.
`This font` indicates an entry that is typed
Letters of words found between < > refer to keys on the keyboard.

NOTE:
Notes will be used throughout the manual to inform on interesting points and provide useful hints.

CAUTION:
Cautions will be used to inform the reader of action that may have the potential to either harm the instrumentation or affect the quality of the data.

WARNING:
Warnings are used to provide special notice of actions that have the potential to cause harm to the operator.

1.1.3 Starting ImageQuant Capture

To start ImageQuant Capture from Windows, double-click the ImageQuant RT ECL Capture icon on the windows desktop.



1.2 ImageQuant Imaging System Setup

1.2.1 System Components

The ImageQuant System includes:

- High-performance, high resolution, CCD camera with lens adaptor
- USB camera interface cable
- Camera power supply and power cord
- Zoom lens, fast lens, and interference filter
- Computer with keyboard, mouse, and SVGA monitor
- Windows® operating system (preinstalled)
- ImageQuant RT ECLimage processing and analysis software (preinstalled and calibrated with computer and hardware system)
- ImageQuant light cabinet with UV transilluminator and white light fold-down transilluminator
- (Optional) epi-illuminating UV lights
- (Optional) printer

Check the packing list included with the system to verify that all components have been received.

1.2.2 System Placement

As with all electrical instruments, the ImageQuant System should be located away from water, solvents, or corrosive materials, on a table or bench top that is dry and stable. Further, the system should be placed away from interfering electrical signals and magnetic fields. If possible, a dedicated electrical outlet should be used to eliminate electrical interference from other instrumentation in your laboratory.

1.2.3 Cable Connections

The cable ends and the ports into which they are inserted are keyed or unique for each connection to eliminate confusion. The connections are pictured and described in the next few sections.

WARNING
Make sure the power is OFF and all power cords are disconnected while connecting the cables and setting up the instrument.

The ImageQuant light cabinet weighs 37kg (81.4 lbs.). It is recommended that two people lift the cabinet from the shipping container to the desired location in the laboratory.

1.3 Computer, Camera and Peripheral Installation

In some instances all software, peripheral drivers and operating systems have been pre-installed at the factory. During the system installation all components should only need to be plugged into the correct ports (If the software is not installed, or the camera is not responding, see section 1.3.4 for software driver installation instructions.)

1.3.1 Setting up the Power Strip/Surge Protector

Turn the power switch on the power strip/surge protector off. Plug the power strip/surge protector into a wall outlet (preferably a dedicated circuit) and then turn the power on.

CAUTION:
Do not plug the light cabinet into the same power strip as the PC. Use a separate circuit if possible. The cabinet needs to be turned on after the operating system has been completely loaded in order for the software to function optimally.

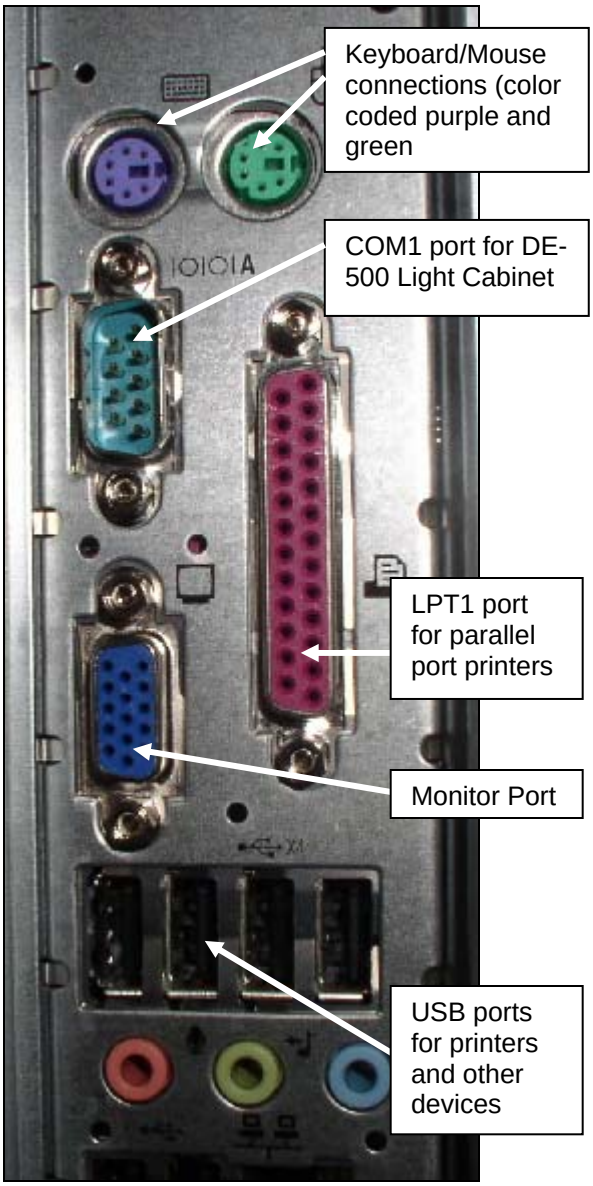
1.3.2 Setting up the Computer, Monitor, Mouse and Keyboard

The computer will need to have the monitor, mouse and keyboard connected to it in the correct ports (see the descriptions below). Also, a 3 prong standard power cable should be plugged into the back of the computer and the power strip.

Connect the monitor's video cable to the monitor port on the back of the computer. If after-market video cards were preinstalled into your computer system connect the monitor's video cable to the video card connector. Connect the monitor's power cable to the power strip.

The mouse and keyboard connectors are color-coded and icon identified. Attach the mouse and keyboard cables to the connectors on the back of the computer by matching the colors.

The computer is now ready to be turned on. Turn the computer on by pushing the power button on the front of the unit.



1.3.3 Cabinet Assembly

When you remove the light cabinet from its shipping carton, it is already partially assembled. The camera mounting assembly is packed separately in the same container. The UV transilluminator and cabinet top are both packed in separate boxes. Make sure you have received all the hardware before discarding the shipping carton. Start by placing the cabinet on a level flat surface, in a position where you can easily reach the top, front and back.

Attaching the Camera to the Cabinet

Take the following steps to attach the camera to the cabinet:

1. Remove the camera and lens from the packaging and attach the lens to the camera.

2. Attach the camera to the camera stand using the fastening knob. Make sure lens is fully extended and that the tip of the lens is level with the bottom of the stand.

3. Attach the cables to the camera.

4. Carefully lower the camera stand (with attached camera) through the top of the cabinet, placing it over the gasket and positioning the lens directly above the hole that leads to the filter wheel.

5. Fasten the camera post to the top shelf of the cabinet using the screws that are provided.

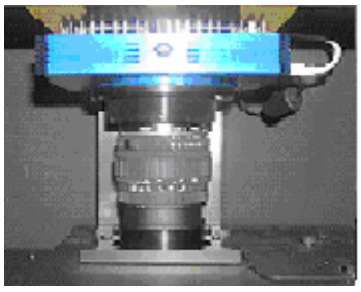
6. Adjust the location of the camera on the post so that the fully extended lens is slightly above the filter wheel. Make sure that the lens is fully extended when doing this to ensure that the lens will not come into contact with the filter wheel during cabinet operation.



Camera stand, camera and fastening knobs.



Completed camera/camera stand assembly.



Camera and stand being attached to the cabinet. Make sure that the fully extended lens is just above the filter wheel (but does not touch).

Installing the UV Transilluminator

Take the following steps to install the UV transilluminator (this is a box-shaped UV lighting apparatus that rests at the bottom of the cabinet):

1. At the bottom of the cabinet, there is an extendable metal tray that is held in place by a single screw (this is to prevent the tray from being damaged during shipping). Start by removing this screw and then pulling the tray out so that it is fully extended.

2. With the tray fully extended, re-insert the screw in its original position underneath the slide-out tray (This ensures that you will have the screw if you ever have to ship the unit and also prevent light coming through the threaded hole that seats the screw.)

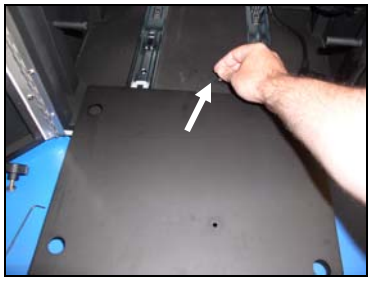
3. Note that the extended tray has four holes, which match the rubber legs on the transilluminator. Place the transilluminator on the tray so that its legs are firmly seated in these holes.

4. Plug the power cord at the bottom of the cabinet into the socket at the back of the UV transilluminator.

5. Push the tray back into the cabinet.



Close-up of the slide-out tray at the bottom of the cabinet. Note that there is a screw that holds the tray in place for shipping. Remove this screw to allow the tray to slide freely.



Once the tray is extended, replace the locking screw in its original position underneath the slide-out tray. This will prevent light from entering into the cabinet through the threaded hole.



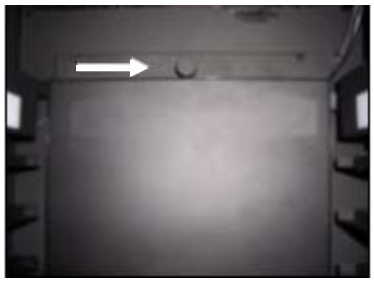
Place the UV transilluminator on the tray (making sure that its rubber “feet” are firmly seated in the holes on the tray) and then plug the cabinet’s power cord into the back of the transilluminator.

Unfastening the Fold-Down White Light Tray

The cabinet contains a fold-down light tray that is locked for shipping (see illustration below). Take the following steps to unfasten the light tray:

1. The light tray is held in place by a detachable knob that screws into place just above the light tray. Unscrew the knob until it is separated from the cabinet and the tray can move freely.

2. Note the bottom of the tray has a threaded hole. Insert the knob into this hole and turn it clockwise until it is firmly seated.



Close-up of the fold-down white light tray located at the back of the cabinet. This tray is secured by a detachable knob.



Release the white light tray by unscrewing the knob, which will allow the tray to swing down freely.



Once the tray is released, screw the knob back into the threaded hole underneath the light tray, where it will serve as a handle.

Attaching the Hood

Take the following steps to attach the cabinet hood and connect the hood fan:

1. Place the hood on top of the cabinet so the holes for screws are centered over the corresponding holes in the top of the cabinet.

2. From within the cabinet, attach the hood fan's power cord to the temperature board socket on the top-right side of the cabinet.

3. Fasten the hood to the cabinet using M4x16 socket head screws.



The cabinet hood is positioned over the top of the cabinet



Once the hood is positioned over the top of the cabinet, plug the fan cable into the socket on the top-right side of cabinet.



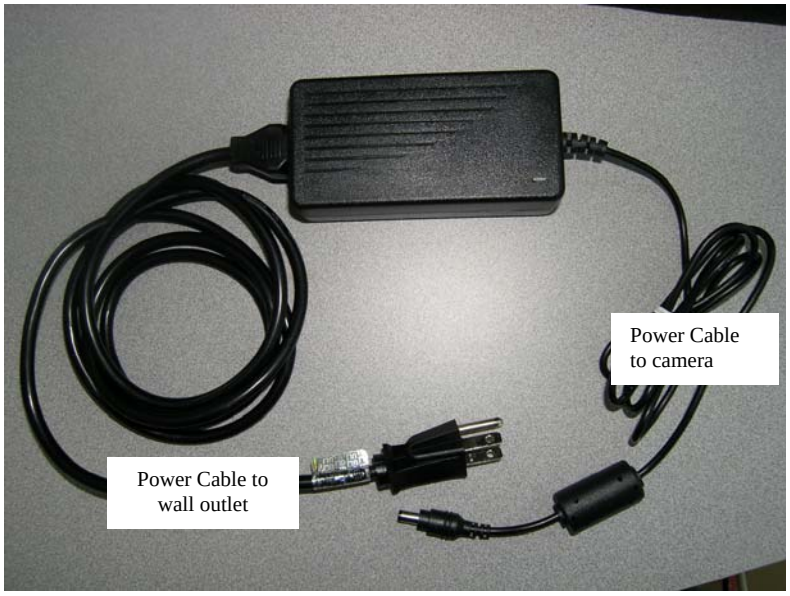
Close-up of socket.

1.3.4 Camera Installation

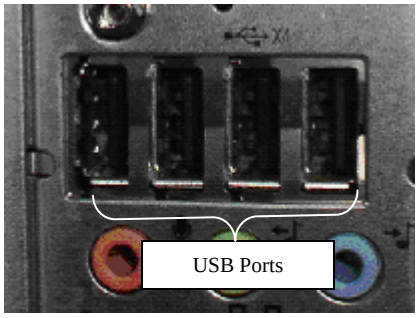
1) Connect the USB cable and power supply barrel connector to the back of the camera.



2) Connect the standard three-prong end to the power strip.



3) Complete the installation by connecting the USB cable into the back of the computer into one of the USB ports.



1.3.5 Software Driver Installation Instructions

The following instructions apply only if you have not purchased a pre-configured system and need to install the camera card manually. The camera driver installation will install two drivers, the USB device loader and the USB camera engine. Windows normally detects new hardware automatically. However, if that does not occur, it's also possible to install the driver software manually through the Windows device manager.

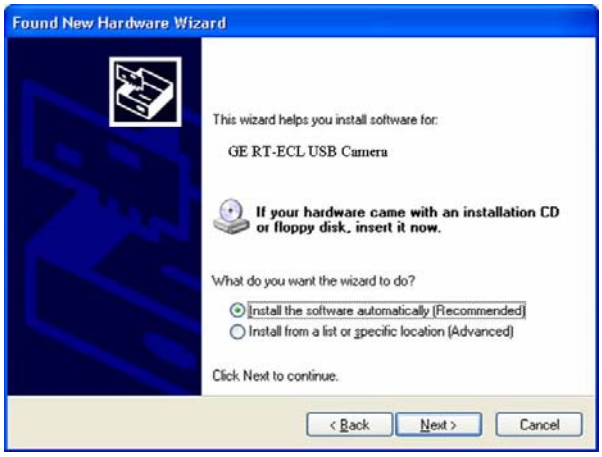
Run the application installer.

1. Turn on the camera and plug in the camera's USB connector. Windows will detect the device and launch an installation wizard:



2. Select the **No, not this time** radio button.

3. Press **Next**. the following screen will be displayed:



4. Select the **Install the software automatically** radio button and then press **Next**.

5. The following screen will be displayed:



6. After the SBIG Loader has been loaded the following screen will be displayed:



7. Press the **Finish** button to continue.

8. You are now ready to operate the software and the camera.

1.3.6 Connecting the Printer

The Mitsubishi P91DW (UB) printer connections are color coded for convenience (see figure on previous page). Plug the USB cable into the back of the printer and into the proper USB connector on the back of the computer. Plug in the standard 3 prong power cable to the back of the printer and to the power strip, then turn the power on.

Note: The Mitsubishi P91DW (UB) printer may be connected to any USB port on the computer; however, the driver may need to be manually reinstalled.

The Mitsubishi CP700 and CP770 printers connect via the on-board parallel (LPT1) port. Plug in the standard 3 prong power cable on the back of the printer and into the power

strip, then turn the power on. Set the printer up for color or black and white printing following the directions in the owner's manual supplied with the printer.

Power on the cabinet after the Windows operating system has fully loaded. The ImageQuant Imaging System is now ready for use.

1.3.7 Starting ImageQuant Capture

To start ImageQuant Capture from Windows, double-click the ImageQuant icon on the windows desktop.



1.4 ImageQuant System Quick Guide

Note: This is intended as a quick reference guide for acquisition. For more detailed information on the individual features reference section 1.6 of this manual.



1. Power on the system:
    a. Turn on the computer, monitor, and optional printer.
    b. After the computer has booted up completely to the Windows desktop, turn on the power to the cabinet.
    c. Activate ImageQuant Capture software by double clicking on the ImageQuant Capture icon.

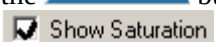


2. Positioning and Focusing on the Sample:
    a. In the 'Tool Bar', select the 'Acquire' icon to activate the image acquisition software features.
    b. In the Image Acquisition window, select the 

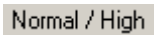
button.
    c. Open the door to the cabinet and position your sample on the preferred illumination source. Fluorescence samples that require epi or transillumination of UV energy should be placed on the purple UV filter glass. For colorimetric samples such as protein gels, film, or blots, use the fold-down the white light table for your sample. For chemiluminescence use the fold down white light table, or, the adjustable tray if used in conjunction with the fast lens.
    d. Open the aperture on the camera lens all the way open to the smallest number.
    e. With the door still open to allow light to enter into the cabinet, use the monitor real time readout display to position and focus your sample in the middle of the image acquisition window.
    f. Focus on the object (more details on how to focus on an image are provided in 1.6.1). For fluorescence and chemiluminescence if there is too much light to obtain a good focus setting, use the door to the cabinet to adjust the amount of light that enters into the cabinet. Do not adjust the aperture to do this. For colorimetric samples it is necessary to decrease the aperture to acquire the image.

    Hint: Business cards and other pieces of paper with small text often are the easiest objects to obtain optimal focus settings with.

    g. If using a zoom lens, adjust the zoom setting so that the area of interest on the sample takes up all of the image size on the screen.
3. Capturing a bright sample like fluorescently labeled gels, colorimetric samples and film:
    a. Close the cabinet door.
    b. Choose the appropriate optical filter for your sample type:
        a. Position #1 for colorimetric gels and film (no filter)
        b. Position #2 for ethidium bromide gels
        c. Positions #3-5 for other fluorescently labeled gels (optional filters).
    c. Turn on the illumination source (UV or white light) using the touch panel or 'virtual' software controls.
    d. Select the 

button.
    e. Select 

    f. Select 

(for details on how to adjust the auto expose settings refer to section 1.6.4 of the manual)
    g. Select the 

resolution setting (for details on the resolution settings refer to section 1.6.6 of the manual)

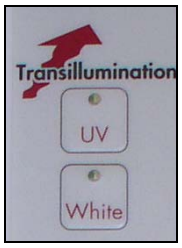
h. Once the preview mode image does not contain any saturation (red false color palette for white bands, green for dark bands) select Capture. The exposure bar will turn green when this is complete. If the exposure bar is pink in color, saturation is still present in the image. For really bright images, particularly in white light applications, it may be necessary to reduce the aperture setting until the saturation is removed from the image.

4. Capturing a low light image (like chemiluminescence)
   a. Close the cabinet door (there should not be any lights on in the cabinet).
   b. Choose Position #1 in the filter wheel for no filter.
   c. Select the Preview button.
   d. Select Show Saturation
   e. Select Chemi Display
   f. Select Auto Expose (for details on how to adjust the auto expose settings, refer to section 1.6.4 of this manual)
   g. Select the Super Sensitivity resolution setting (for details on the resolution settings refer to section 1.6.6)
   h. Wait for the exposure bar to indicate that the proper exposure time has been found and that there is no saturation in the image - the exposure bar will turn green when this is complete. If the exposure bar is pink in color, saturation is still present in the image.
   i. Select Medium / Medium resolution.
   j. Select Capture.

Note: The exposure time will vary depending on which resolution setting is selected in step 4.h. If the exposure time calculated by the auto expose setting is too long, it is possible to select the High / Low or High / Medium setting instead. Alternatively, if the exposure time is short enough, Normal / High may be selected for a full resolution (4 million pixel) chemiluminescent image.

5. Save the original image
   a. Click on the Save Image function in the FILE menu or click on the SAVE or SAVE AS icon in the Tool Bar.
   b. Enter a file name and select the directory to which it should be saved (the directory path should be less than 100 characters).
   c. Specify the file format (TIF, BMP, PCX, MAC, color TGA)
   d. Click OK to save the file
6. Enhance the display [optional]
   a. Adjust the black, white and gamma levels by moving the slider bars at the right of the image in the “Contrast Adjust” window, or select auto contrast.
   b. Apply digital filters, found in the Tool Box under the FILTERS (to stop a filter, hit any key on the keyboard; to reverse the effects of a filter, click UNDO).
   c. Add text, boxes, arrows, etc. to the image using the annotation tools in the Tool Box under the ANNOTATE tab.
7. Print the image using the large PRINT button in the Tool Bar or the pull-down FILE menu option.
8. Analyze the sample by using the analysis options in the toolbar (1D, Array, Colony and Toolbox). When one of these buttons is pressed, the image is transferred to the appropriate ImageQuant TL module for analysis.

1.4.1 Cabinet Controls



Transillumination Controls
These two buttons are used to toggle the UV transilluminator and the reflective white light table on and off.



Reflective Light Controls
These two buttons are used to toggle the reflective UV and white light sources on and off.



UV Override Button
Normally, if UV light is being used the cabinet automatically shuts off the UV light source when the cabinet is opened. This button can be used to override this behavior (the button should be held down 5-7 seconds before opening the cabinet).

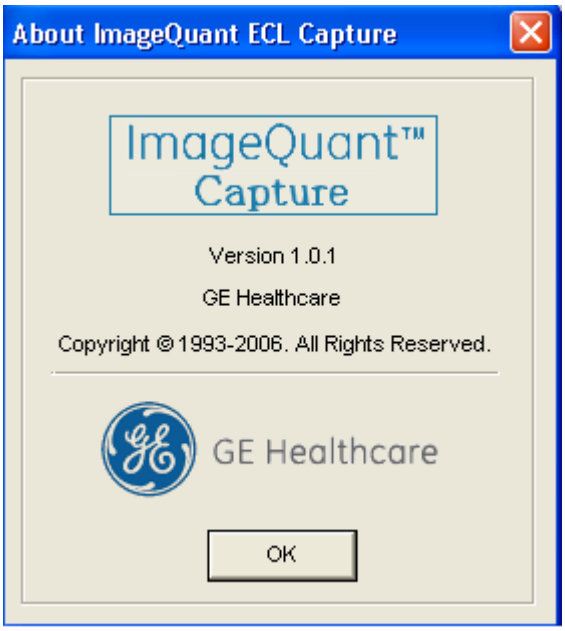


Filter Wheel Position
These controls are used to set the filter that will be utilized for acquisition. Use the arrow buttons to cycle through the available filter settings. The LED indicates the number of the filter that is currently being used:

1 No filter – use for colorimetric gels and film.
2 Ethidium Bromide
3-5 Optional filters

1.5 System Information

To display system information, select the About option in the Help menu. This button accesses a pop-up box.



ABOUT Pop-Up Dialog

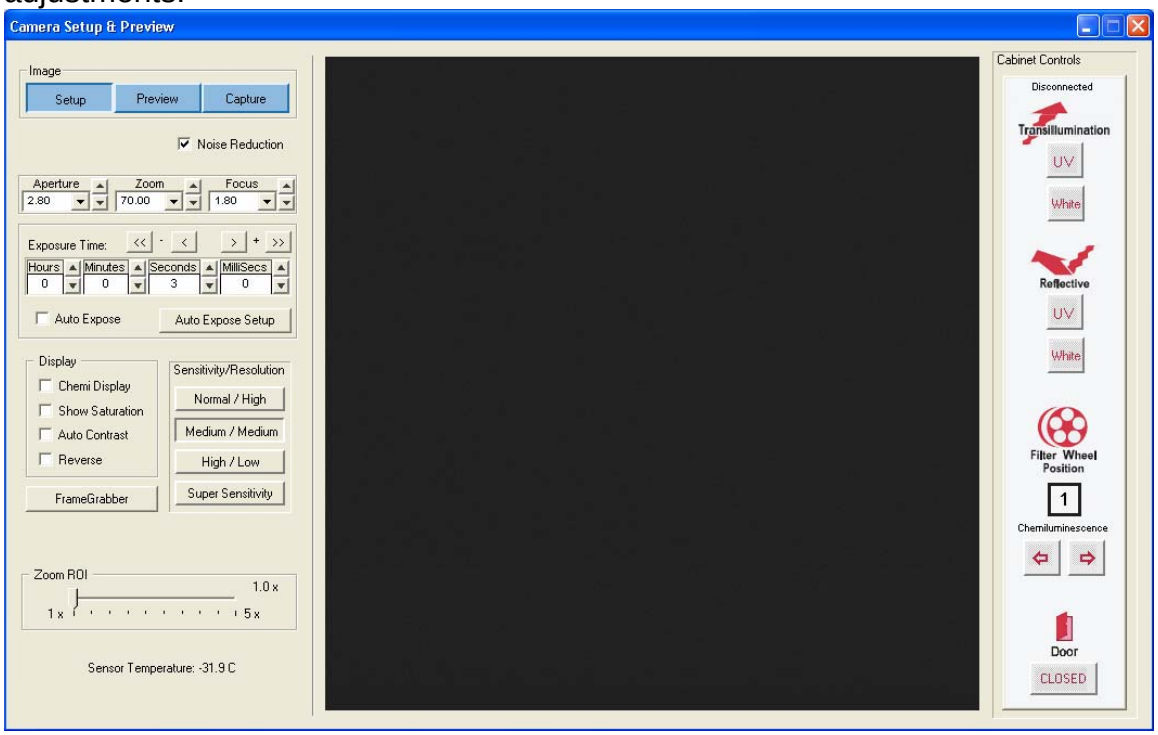
This box shows the instrument serial number (where appropriate) and the Software version number. Use the information specific to your instrument and software when calling for technical support, software upgrades, etc.

To close the dialog box, click on the OK button.

1.6 Acquiring an Image

In the TOOL BAR window, click on the Camera Acquire icon: 

Once the Camera Acquire Icon is pressed, the CAMERA SETUP AND PREVIEW window is activated to provide exposure control of the camera, sensitivity/resolution controls, all lighting and filter wheel position controls, contrast display options and cabinet door open/close indicator using a software 'virtual control' menu. Also, Movie Setup is controlled and activated from this window. This window will open in the Focus control mode activated (blue button) provide a near real time readout to allow for easy sample positioning and optics adjustments.



1.6.1 Focusing on an Image

Focusing on an image in the ImageQuant-RT ECL Capture software is easy with the real time display. Simply adjust the parameters on the manual lens, or, if the lens is controlled via the software, click on the appropriate software control button.

.



1) To open the aperture, turn the lens's aperture ring counter-clockwise for more light. Conversely, if the image is too bright, close the aperture by turning it clockwise until an image is visible on the monitor.

2) Then, zoom in on the image so that the area of interest on the sample takes up the entire field of view on the screen.

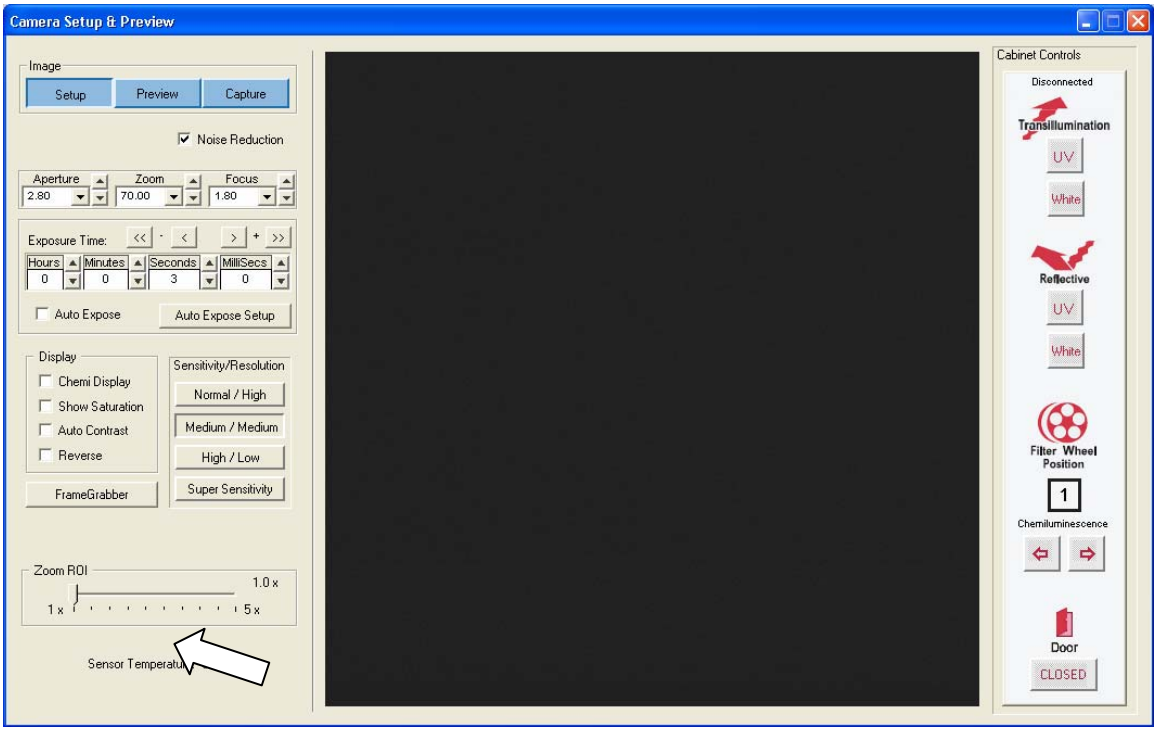
3) Last, if the image appears blurry, turn the focusing ring (lower ring on the lens) until the sharpest image is obtained.

Note: This protocol may vary slightly with different accessory lenses. For example, the fast lens may have an aperture setting of F1.8 and will not have a zoom ring.

1.6.2 Using the Zoom ROI Tool

Zoom ROI is a tool that is designed to be used with a fast or fixed focal length lens. These lenses typically have lower F stop numbers than zoom lenses but do not contain a zoom function. Fast lenses are most commonly used for chemiluminescence to decrease the exposure times, however, they can also be used for fluorescent gels and other applications as well. Since there is not a zoom ring on a fixed focal length lens, it is now possible to adjust the region of interest of the acquired image using the ImageQuant RT ECL software interface.

When the region of interest of the sample is smaller than the acquisition window, and the system is not equipped with a zoom lens, the zoom ROI tool is the ideal work around without having to switch lenses on the system. Focus on the image as explained in 1.4 and 1.6 of the manual and then slide the zoom ROI slider across the screen until the region of interest occupies the entire image area in the acquisition window. Use preview and capture to acquire the final image. This eliminates a post-acquisition step of cropping the image down to the appropriate size.



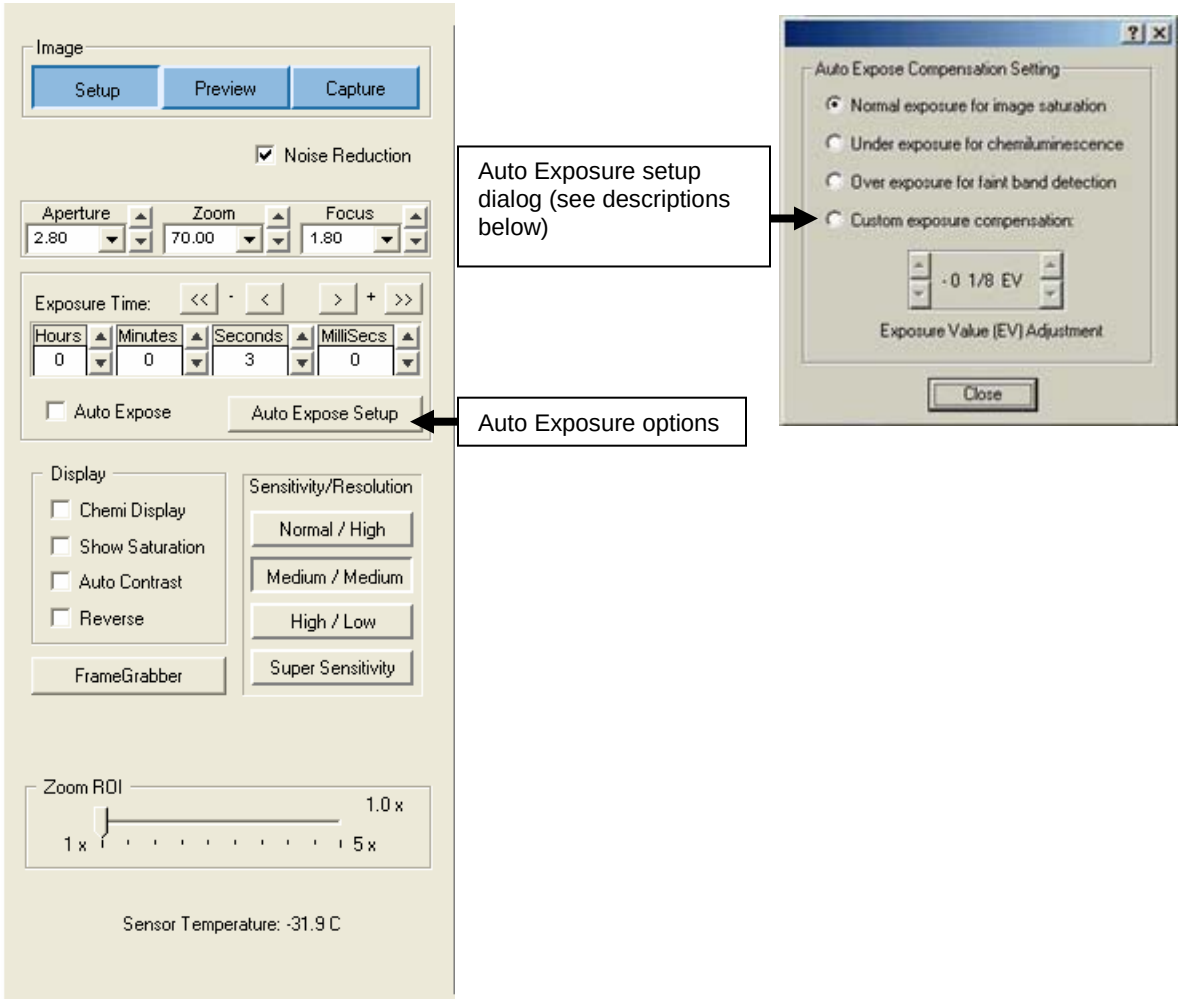
1.6.3 Acquire and Transfer the Image

Once the appropriate image is displayed on the screen, click on the **Capture** button to acquire and display the image. The acquired image will be automatically adjusted by a zoom factor based on the resolution setting to display the image optimally. From there, the image can be enhanced using ImageQuant Capture's enhancement tools and/or transferred to an ImageQuant TL module for analysis.

Once a satisfactory image has been captured, we suggest saving it as an original file. However, if you do not save before transferring the image to an ImageQuant TL module for analysis then you will be prompted to save as part of the transfer process.

1.6.4 Auto Exposure and Auto Exposure Setup

The acquisition window for ImageQuant Capture includes a check box and setup window for Auto Exposure.



Auto Exposure Setup (Auto Exposure Compensation Setting):
4 choices available

- Normal Exposure for image saturation: Use this choice for normal Colorimetric and Fluorescent imaging.
- Under Exposure for chemiluminescence
- Over Exposure for faint band detection.
- Custom Exposure Compensation: user definable Exposure Value (EV).

The up/down arrows allow the user to change the EV value by whole units (left up/down arrows) or by $1/8^{th}$ (right up/down arrows).

Auto Exposure works in both "Preview" and "Capture" modes. As the software calculates the correct exposure time you will see the status bar change from red to yellow and then to green. When it reaches green the software has achieved the correct exposure time. If the bar turns pink, this is an indication that there are saturated pixels in the image area at the exposure time calculated.

In Capture mode the image will be acquired when the bar turns green. While in Capture mode, the software will continue exposing over and over at that exposure time until a different mode is selected. If "Capture" is selected after the software has achieved the correct exposure time in Preview mode the software will begin acquiring at the last exposure time calculated during the preview—it does not start the calculation over from scratch. Similarly, if a different sensitivity (binning) mode is selected after the exposure calculation has been calculated, it will do a quick conversion and does not have to start the calculation from scratch.

1.6.5 Cabinet Controls - Activating the Light Source and Selecting a Filter

Use the mouse to click on the desired light source in the 'virtual' cabinet control interface or you can use the controls on the cabinet itself. Both mechanical and software controls are linked and communicate display setting selections.

Note: There is a slight delay when the button is depressed until the light source is fully activated.

Standard lighting choices include:

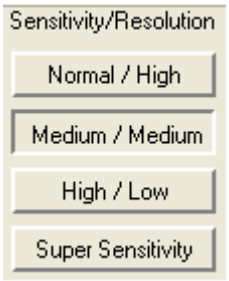
Transillumination White:	For protein gels, autorads, film, plates, flasks
Transillumination UV:	For fluorescent gels such EtBr, SYPRO® Red, etc.
Reflective White:	For colorimetric blots and membranes
Reflective UV (optional)	For SYBR® green, TLC plates, and Chemifluorescence
(No Light Selected)	For chemiluminescence

You can also select your FILTER to correspond with your sample staining. The options include:

Filter Position #1:	Chemiluminescence samples, no filter
Filter Position #2:	Ethidium Bromide, colorimetric stains, film, SYPRO® Orange (595nm)
Filter Position #3 (optional):	SYBR® green (557nm)
Filter Position #4 (optional):	Fluorescein, SYBR® Gold (520nm)
Filter Position #5 (optional):	SYPRO® Red, Texas Red (630nm)
(optional):	Hoechst Blue (460nm)

Note: Each Filter has an approximate bandwidth of +/- 40nm to allow for use with other fluorescence stains as they are developed. If you have a custom application, please feel free to contact us directly to explore having a custom filter designed for your application.

1.6.6 Sensitivity/Resolution Controls



Sensitivity/Resolution Settings

ImageQuant Capture allows you to acquire images depending on your critical needs. Are you looking for the shortest possible acquisition time, the highest resolution, or a combination of both? Acquisition modes:

Normal Sensitivity/High Resolution

Recommend for Fluorescence and Colorimetric Imaging. This mode captures the image using the CCD sensors full 2048x2048 pixel resolution generating high quality, vibrant images that are publication quality.

Medium Sensitivity/Medium Resolution

Recommend for Chemiluminescence. This mode captures the image performing a 2x2 pixel bin to decrease the exposure time of the image, yet still maintain a publication quality 1024x1024 pixel resolution image. Medium/Medium sensitivity selection will decrease the exposure time needed over a full resolution image by approximately a factor of 4X.

High Sensitivity / Low Resolution

Recommend for Chemiluminescence. This mode captures the image performing a 4x4 pixel bin increasing the sensitivity by a factor of 16X.

Super Sensitivity / Low Resolution

Recommend for Chemiluminescence. This mode captures the image performing a 8x8 pixel bin increasing the sensitivity by a factor of 64X.

1.6.7 Use Preview to Set Exposure Time

Once the sample has been positioned and the camera has been focused, close the door to the ImageQuant light cabinet and make sure that the appropriate illumination source is turned on. Also make sure the cabinet door indicator in the Cabinet Control software interface indicates **CLOSED.**

Click on the blue **PREVIEW** button and select the desired exposure time in the menu options to give the desired image intensity quality. Individual adjustments for 1/30 seconds, seconds, minutes, and hours are available.

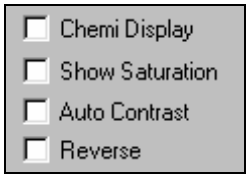
- For most white-light applications, 50 millisecond exposures are sufficient with final adjustments of the aperture for best image quality.

- For UV fluorescence applications, usually an 8 milliseconds to 4 seconds is sufficient and the aperture should be adjusted fully open for the standard zoom lens. The 'show saturation' option should be selected for these applications.

- For low-light applications, such as chemiluminescence, a longer exposure time may be appropriate and it is best to go to the **CAPTURE** button to directly acquire the image once the desired exposure time is set. Alternatively, if a good exposure time is unknown, try selecting Auto-Expose and using the super sensitivity selection. Auto-Expose will generate an image showing the signal intensity usually in less than 3 minutes. Afterwards, a different sensitivity (binning) mode can be selected to generate a better resolution image and the software will automatically calculate the new exposure time for you.

Note: When the system is switched to PREVIEW mode, the image may flash or change brightness because the camera collects photons from the image for a longer period of time before sending the image to the computer's display readout.

1.6.8 Optimizing the Gray Scale for Saturation and Contrast Displays



If an image will be analyzed, it is important that it not be over-saturated (too light) or under-exposed (too dark). Using Show Saturation, the user can see the areas of the image that are assigned to each end of the gray scale spectrum, and can adjust the imaging controls accordingly**.**

The Show Saturation checkbox (found below the camera control functions) allows the user to access the Saturation Palette during image acquisition. The Saturation Palette is a modified gray scale palette in which black (gray level 0) is replaced with green, and white (gray level 255, 4,095, or 65,535) is replaced with red. With this palette, over- and under-exposed areas of the image are shown as green or red, while areas within the linear range of the CCD chip are shown in gray scale.

During image acquisition, note regions of the image that appear red or green. Adjust the exposure time and/or the camera aperture to minimize the amount of red and green in the image area. If an image will be quantified, it is especially important that the actual sample area be neither red nor green. Once you are satisfied with the displayed image, you may click off the Show Saturation**.**

The other three selections Chemi Display, Auto Contrast, and Reverse are visualization tools designed to enhance contrast and provide flexibility in regards to how the user wishes to view the image. Auto Contrast will display the image with automatic black, white and gamma adjustments according to the image histogram information (black/white levels). Reverse will show the sample as a negative by switching the black and white values. Chemi Display utilizes both the auto contrast and reverse functions as well as adjusting for gamma. It is intended for use with chemiluminescent samples. These options are also available in the FrameGrabber portion of the acquisition software. It is important to note that these Contrast Display options are only visualization tools and are NOT changing the image data that is being acquired by the CCD camera.

1.6.9 Acquire and Transfer the Image

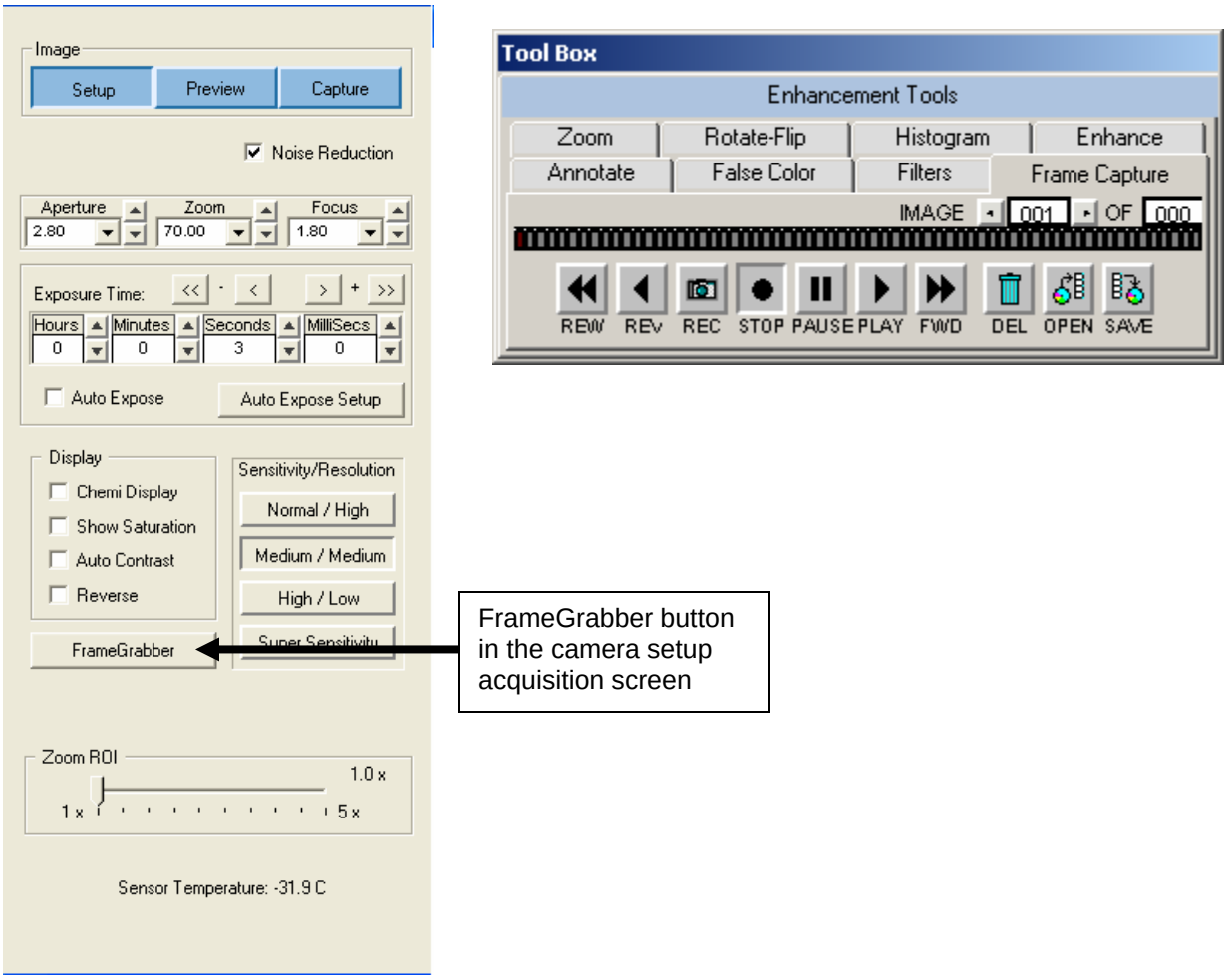
Once the appropriate image is displayed on the screen, click on the **Capture** button to acquire and display the image. The acquired image will be automatically adjusted by a zoom factor based on the resolution setting to display the image optimally. From there, the image can be enhanced using ImageQuant Capture's enhancement tools and/or transferred to an ImageQuant TL module for analysis.

Once a satisfactory image has been captured, we suggest saving it as an original file. However, if you do not save before transferring the image to an ImageQuant TL module for analysis then you will be prompted to save as part of the transfer process.

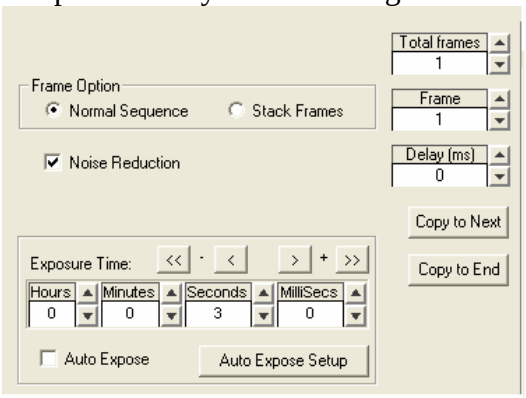
1.6.10 Capturing Images Using the FrameGrabber Option

If kinetic, multiplex, color, or chemiluminescence experiments are desired where you wish to have the system automatically capture several images at preset exposure times, preset time delay between images, preset lighting sources, and preset filter choices, the FRAMEGRABBER tab can be clicked in the Tool Box. (FrameGrabber is also accessible in camera setup acquisition screen. To access this screen, select the acquire button on the tool bar and then select FrameGrabber on the acquisition screen. ImageQuant Capture stand alone software does not include FrameGrabber acquisition tools)

FrameGrabber Setup in the Tool Box



Once **FRAMEGRABBER** is selected, a display box will appear for independent control of all lighting, filters, and exposure delay for each image frame.

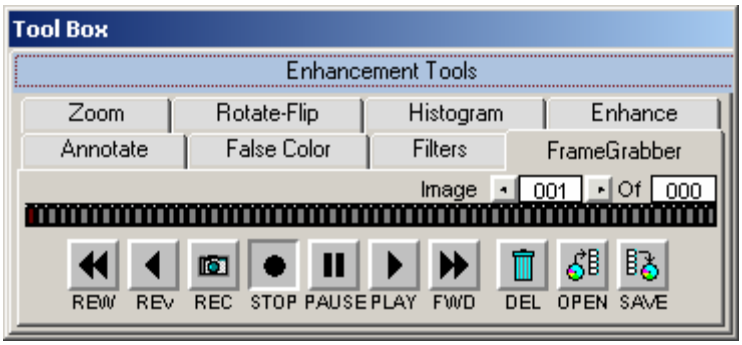


The **TOTAL FRAMES** setup provides you with the ability to determine how many individual frames (images) you want for the sequence of frames. There is a maximum of 50 frames (images) and a minimum of 1 frame that can be captured with each sequence.

The **FRAME** selection is used for setting up the conditions for each frame (image). For example, if three (3) images are to be captured, you would choose FRAME 1 and setup all of the desired lighting and filter requirements. You can then click on FRAME 2 and repeat the above. Or, you can click on **COPY TO NEXT** to help speed up the setup process. **COPY TO NEXT** copies all settings from the previous frame to the current frame. Usually, for chemiluminescence imaging, all lighting is off and the filter wheel is positioned for the chemiluminescence position for all frames. Thus, the only variable that is changing from one frame to the next is the exposure time. In this situation, **COPY TO NEXT** is a useful tool to save time in the setup process.

If you are performing kinetic experiments where you want to have a predetermined delay between captured images, then you can use **DELAY** to configure this function. The default **DELAY** is set for the shortest possible delay (19 milliseconds), but can be configured up to 50 minutes between each image. Also, if your exposure delay and/or exposure time and/or lighting options/filter position are consistent for the entire sequence, then once you set up the first frame, you can select the **COPY TO END** selection to automatically choose the first frame settings for the entire sequence of frames (images).

Once the frame sequence is set up to the desired configuration, click on the **GO** button. The software will then begin the image acquisition for each frame of the image. When it is complete, the Camera Setup and Preview window will disappear and the TOOL BOX will automatically display the FrameGrabber tab. This will allow you to play back the sequence, save or load the sequence, or record a new sequence.



Once all images have been captured, the FrameGrabber tab will be displayed in the Tool Box. The buttons on this tab perform the following tasks:

REC	Opens the Camera Setup and Preview screen to record a frame sequence.
PLAY	Display a continuous loop of all of the captured images.
STOP	Stop the sequence at the current frame display
PAUSE	Pause the playback of the sequence at a user defined image
REW (rewind)	Rewind the sequence to the first image
REV (reverse)	Play the sequence in a continuous loop in reverse
FWD (forward)	Forward the sequence to the last image
DEL (delete)	Delete the sequence on the display. THIS FUNCTION WILL NOT DELETE A SEQUENCE SAVED TO THE HARD DRIVE.
LOAD	Load a previously saved sequence. THIS FUNCTION WILL NOT LOAD INDIVIDUAL IMAGES PREVIOUSLY CAPTURED IN NORMAL CAPTURE MODE.
SAVE	Save a sequence of images.

1.6.11 Saving an Individual Image from a Sequence

After you load, play, and stop a sequence at the desired image, it is possible to save the individual image seen on the screen. Use the SAVE AS button located on the tool bar to save the image in the desired location and file format on the local or network drives.



Note: This will save the current image only. To save the entire sequence, select the 'save' icon on the FrameGrabber tab in the tool box.

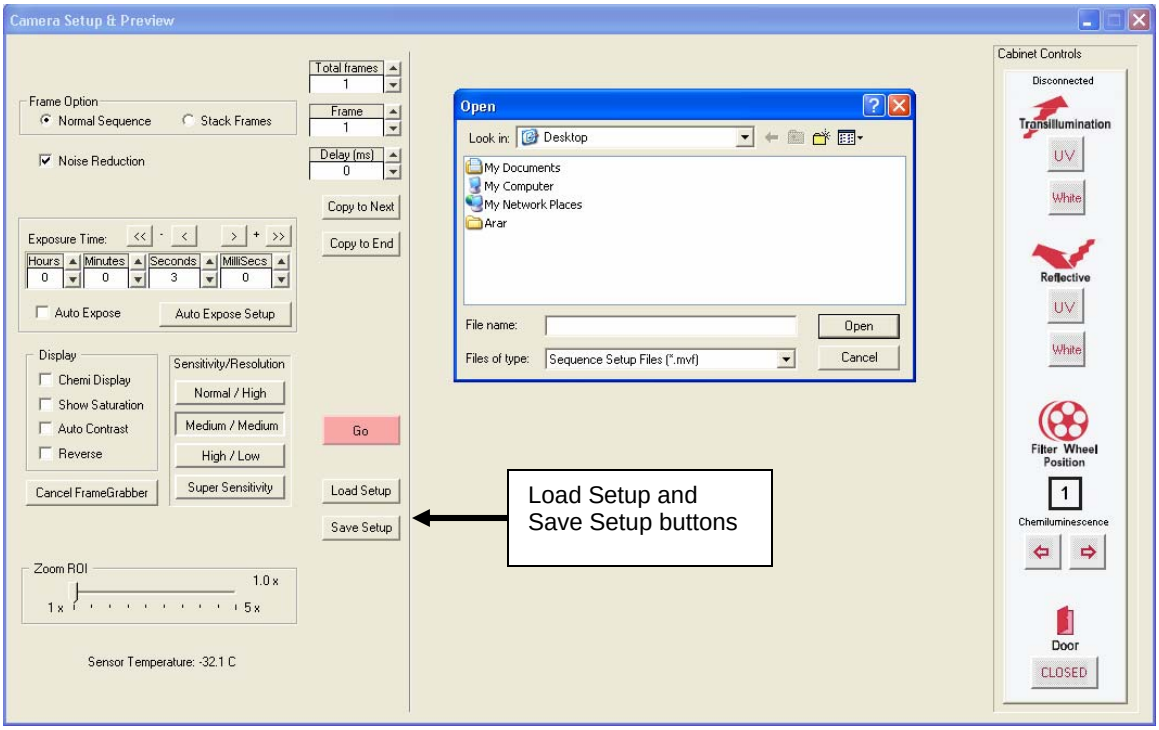
1.6.12 Loading/Saving an Entire Sequence



You can load or save an entire sequence by using the load and save icons in the FrameGrabber tab in the tool box. When a sequence is loaded the loaded frames will be seen as blue in the sequence reel. When a sequence is saved a dialog box will appear prompting you to select the location and name for the sequence that you would like to save the images as.

1.6.13 FrameGrabber: Save/Load FrameGrabber Setup Routines

Two buttons “Save Setup” and “Load Setup” allow you to save and load all FrameGrabber setup parameters. Files are saved as *.mvf files.



1.6.14 Frame Stacking

At the top of the FrameGrabber Setup window is an option for stacking frames. If this selection was chosen during acquisition of the image Stack Frames will use all previous exposure information to sequentially add images to one another. Normal Sequence will not perform this addition. Please note that stacking frames will increase the noise level in acquired images.

Sample case:

Capture 5 frames at 1-5 sec exposure for total time elapsed 15 sec

Display after summation of following frames:

Frame 1 (1 sec)

Frame 2 (1 + 2 sec)

Frame 3 (1 + 2 + 3 sec)

Frame 4 (1 + 2 + 3 + 4 sec)

Frame 5 (1 + 2 + 3 + 4 + 5 sec)

1.6.15 Capturing a Color Image Using the FrameGrabber Function

A color image can be generated by acquiring three images each taken with a red, green and blue emission filter. Once saved, these images are then combined in the Overlay pull down menu. Open the image captured with each of the three filters as instructed and a RGB (red, green, blue) true color image will be generated.

For color imaging, you will need to the following *optional* filters:

Red Filter	SYPRO® Red Filter (Catalog #: 63005664)
Blue Filter	Hoechst Blue Filter (Catalog #: 63005665)
Green Filter	SYBR® Green Filter (Catalog #: 63005661)

Chapter 2: Getting Started - Basic Imaging Functions

2.1 Contrast Adjustment

The Contrast Adjustment window allows for the best visualization possible of a sample utilizing the black, white, and gamma adjustments, as well as, image reverse and auto contrast.

The image on the screen is made up of picture elements (pixels) in an array. Each pixel is assigned a brightness (or a gray scale value) level between black and white. A very bright image has most of its pixels registering high gray level values and conversely, a very dark image has most pixels registering low gray level values (approaching zero).

The distribution of these gray values to the image is determined by the Contrast Adjustment Controls. These controls regulate the Black level, White level, and Gamma setting (brightness linearity), allowing adjustment of the display to obtain the best image possible.

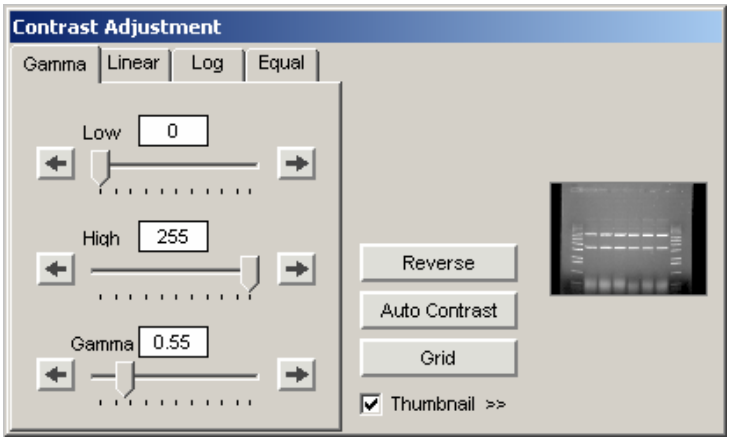
Note: These enhancement features modify the image display on the monitor only, and do not change the original quantitative data.

ImageQuant Capture can also import RGB color images. ImageQuant Capture automatically detects this process and the Contrast Adjustment tools are configured for color image adjustments.

An image can be enhanced using these tools and then saved as a modified file for publications. However, to preserve the original image information, it is recommended that the file be saved as a different file name when using the save modified.

2.1.1 Using the Contrast Adjustment Tools for Grayscale Images

There are three sliding scales found in the image control area to the right of the image. Below each scale is a box displaying a number that corresponds to the position of the slider. By adjusting these sliding scales, the image display can be optimized.

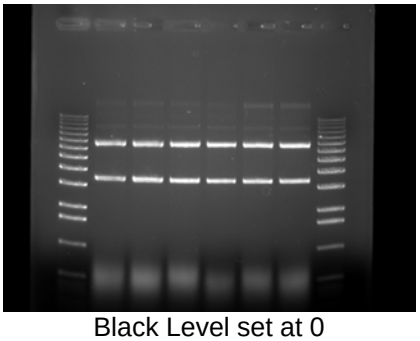


Imaging Display Tools: Black Level, White Level,
Gamma Setting with B/W/G, Linear, Log, and Equalize options

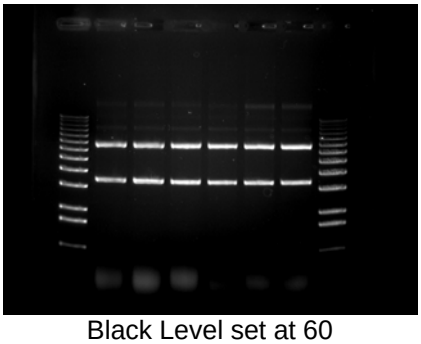
To adjust any of these settings, place the cursor on the slider. Click and hold down the left mouse button while dragging the slider to a new setting. As the slider is moved along the scale, the image display is updated, along with the change in numeric value. The arrows above and below the scale bars can also be clicked to change the settings in single unit increments, or, the user may type in a specific unit.

2.1.2 Black Level Adjustment

The number beneath the Black Level scale corresponds to a gray level. There can be 256, 4095 or 65,536 possible gray levels depending on the system type. For the example below, an 8 bit image will be used with 256 total gray scale values. When the Black slider is at the very top of the scale, the number is 0. As the slider is moved downwards along the scale, the number increases and the image becomes progressively darker. This is because all pixels at the specified gray level and lower are shown on the screen as black pixels. If the slider is set to 0, all the pixels whose gray levels are at 0 are shown as black. If the setting is then changed to 60, all the pixels between 0 and 60 are shown as black and the image appears darker.



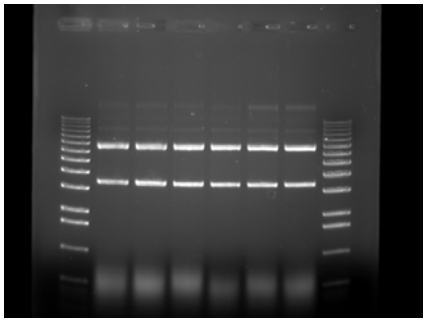
Black Level set at 0



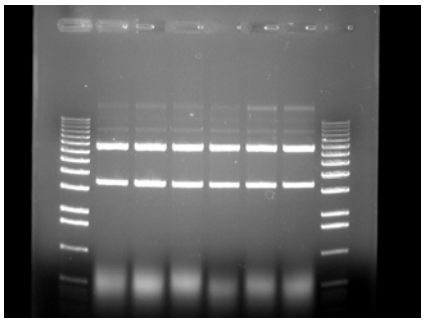
Black Level set at 60

2.1.3 White Level Adjustment

The number beneath the White Level scale also corresponds to a gray scale value. When the slider is at the very bottom of the scale, this number is 255. As the slider is moved upwards along the scale, the number decreases and the image becomes progressively lighter. This is because all pixels at the specified gray level value and above are shown on the screen as white pixels. For example, if the slider is set to 150, all the pixels between 150 and 255 are shown as white and the image appears lighter.



White Level set at 255

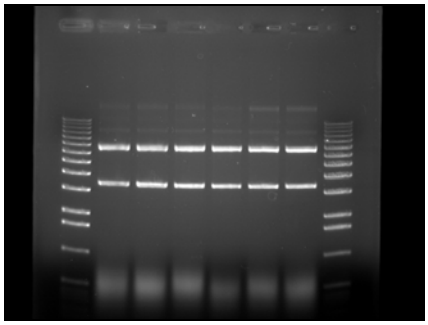


White Level set at 150

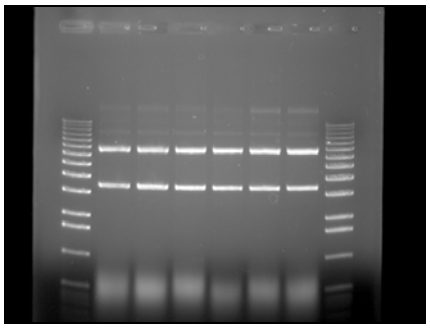
2.1.4 Gamma Setting Adjustment

Changing the Gamma setting affects the image brightness by adjusting the linearity of the image on the screen and printouts, but does not affect quantitative data.

The camera sees objects linearly while the human eye does not. When the Gamma setting is set to a value of 1, the image is displayed as the camera sees it. This, however, is different from what the human eye detects. By adjusting the Gamma setting, the user can make the image on the screen correspond to what is seen when he/she looks directly at the object. We recommend a Gamma setting of 0.55 for best visual representation.



Gamma set at 1.0



Gamma set at 0.55

2.1.5 The Auto Contrast Selection

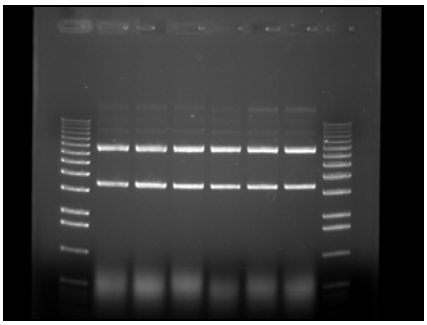


The Auto Contrast feature will automatically scale the black and white values of an image to more tightly fit the gray scale intensity profiles (histogram). This selection will use different black and white values for different images depending upon their unique histograms. A more dramatic visual change will take place for low light level images (such as chemiluminescence) where smaller portions of the histogram are used. This selection can be turned on or off and will adjust differently for each image.

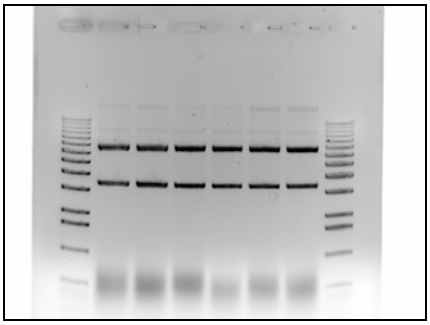
2.1.6 The Reverse Button



The Reverse button inverts the gray levels of the displayed image, converting a positive image to negative, or vice versa. For instance, an image with black bands on a white background is converted into an image with white bands on a black background by simply clicking the Reverse button. Clicking the button a second time returns the image to its original form.



Original Image



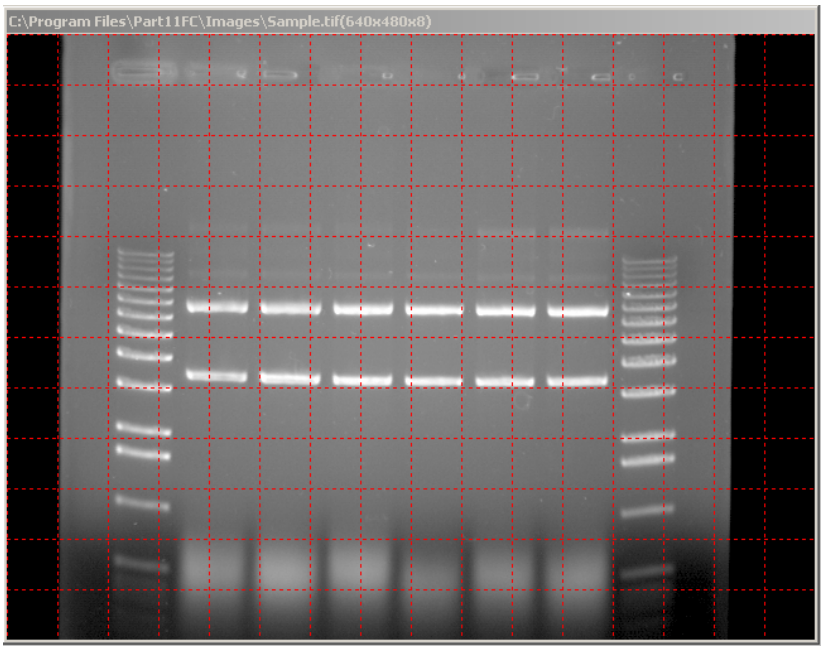
Reversed Image

Note: Reversing an image changes the way it is displayed on the screen, but does not change the quantitative data. For example, the bands in the above gel have the same density, regardless of whether the gel is displayed as white bands on a black background or black bands on a light background. For information on reversing pixel values, see Invert option under Setup|Preferences (see Chapter 4 for more info about this option.)

2.1.7 The Grid Button

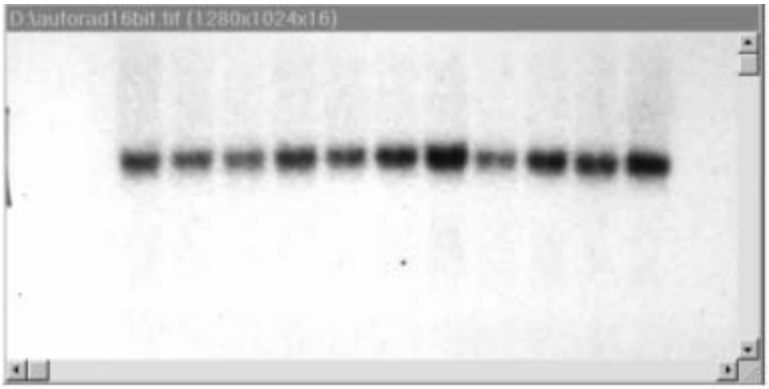


The Grid button provides an on-screen grid after image acquisition to check for proper sample alignment.



2.1.8 Making Linear, Log, or Equal Adjustments

Original image of film with default BWG settings



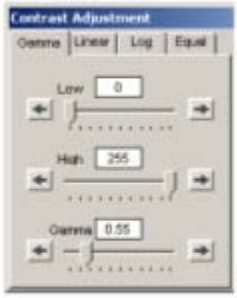
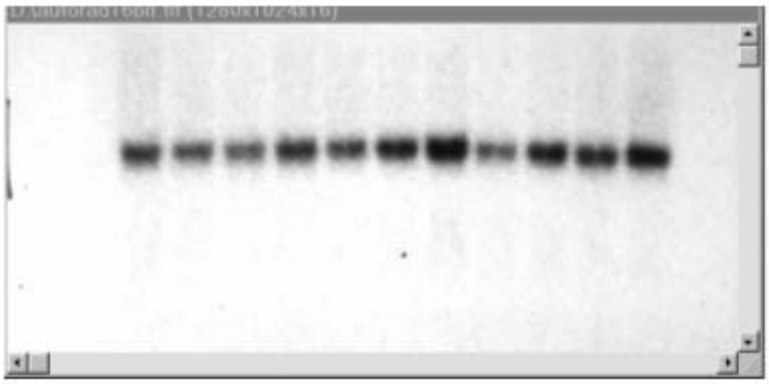


Image of film with linear Contrast Adjustments selected. Linear provides minimum and maximum adjustment tools from 0 to 100%. Linear stretches the grayscale range of the displayed image to the maximum system dynamic range of 0-65,535 grayscales.



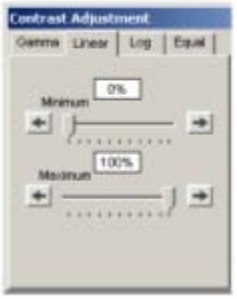
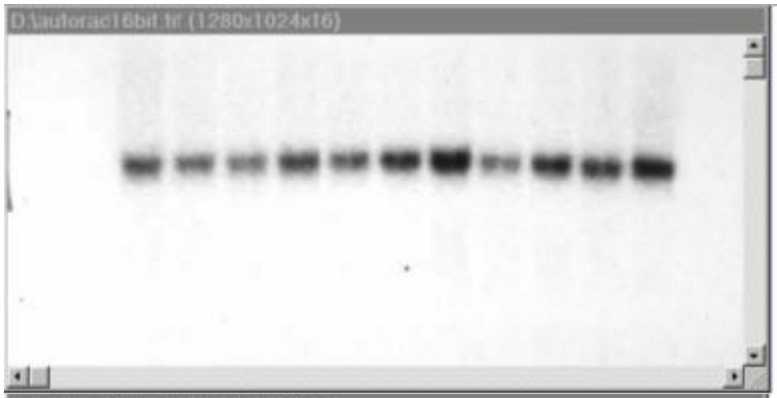


Image of film with log Contrast Adjustments selected

log provides minimum and maximum adjustment tools from 0 to 100%. Log performs a logarithmic adjustment to the grayscale range of the displayed image.



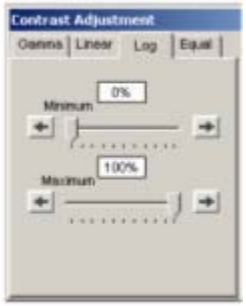
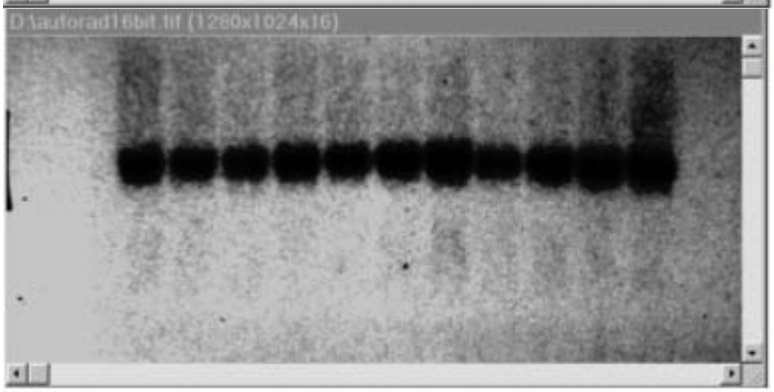


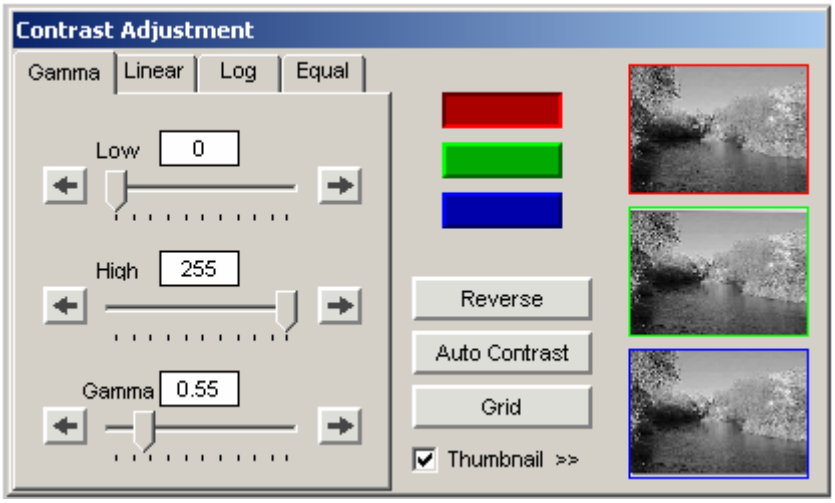
Image of film with equal Contrast Adjustment selected. Equal automatically adjusts the image display for maximum contrast which is beneficial for faint band detection.





2.1.9 Using the Contrast Adjustment Tools for Color Images

ImageQuant Capture can also import RGB color images. ImageQuant Capture automatically detects this process and the Contrast Adjustment tools are configured for color image adjustments.



Contrast Adjustment display with Show thumbnail clicked for color image

The Black, White, and Gamma bars can now be adjusted individually for each of the three RGB color channels by selecting on the Red, Green, or Blue button and making the appropriate B/W/G adjustments

Also, by clicking on the Show thumbnail option, a thumbnail display of each of the three color channels is displayed. These thumbnails also display any black, white, or gamma adjustments for each color channel.

2.2 Tool Bar

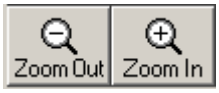
The Tool Bar window provides intuitive buttons for the most common functions in ImageQuant Capture.



Tool Bar Display Window



The Open button functions identically to the File Open function in the upper menu bar. This function is used to open previously saved images. Detailed instructions are available in Chapter 3.



The Zoom Out and Zoom In buttons provide easy zooming ability while you are active in image enhancement functions providing increased versatility. Detailed instructions are available in Chapter 4 as this function is also available in the Tool Box.

Note: The Status Bar always displays the image zoom setting in real time.



The Saturation button allows for a quick image display of saturation. Completely saturated black regions (gray scale 0) will turn green and saturated white regions (ie. gray scale 255, 4095, 65,535) will turn red.

This is a useful tool to check for linearity of an image before analysis occurs. Saturation is a feature that is most important during the acquisition stages and is thoroughly detailed in the acquisition features of the system manuals.

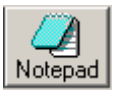


The Img Drag button is useful for to pan with a zoomed image. To activate this function, click on the button and move the mouse cursor to the image. The cursor will have changed to a small hand. Click the left mouse button and drag to move the image. When you are done, you can click the Img Drag button again to deactivate it.

Note: Img Drag is only active when the image is zoomed in beyond 1X (greater than 100%). The button is grayed out in other zoom modes.



The Print button allows for quick and easy image printing with the active default printer. Detailed instructions on image printing are available in Chapters 2 and 3 as printing can also be accomplished via traditional Windows File menu options.



The Notepad button opens up a dialog box to allow the user to quickly track experimental conditions, comments, and any other details to be saved as an electronic copy for future reference. Detailed instructions are available in Chapter 3 as this Notepad function is duplicated in the Utilities function in the upper header bar.

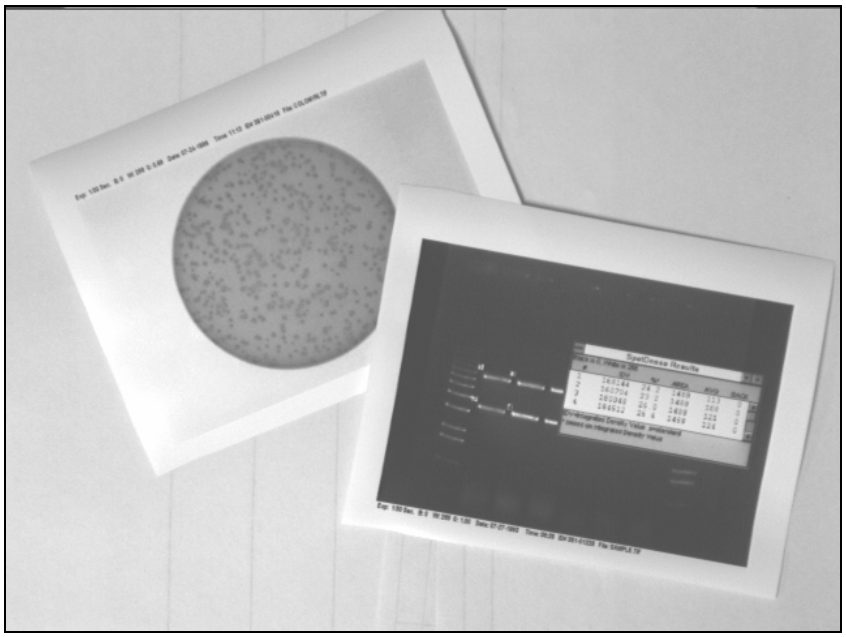
Clicking Reset returns the image to the system defaults as specified in the active default file. This is detailed later in Chapter 3.4 of the manual.



Clear removes any overlays currently displayed on the image. This function can be useful if annotations or other displays obscure parts of the image.



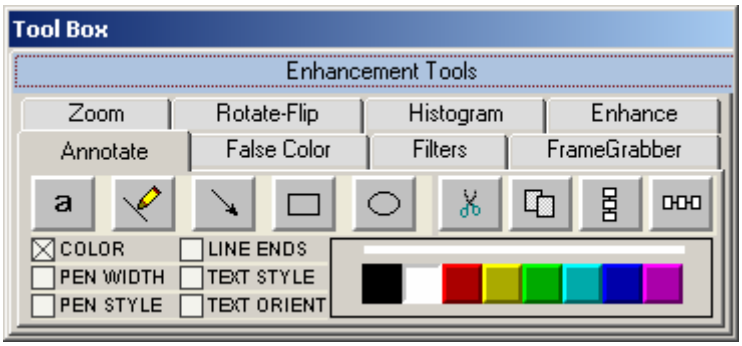
Once an image is displayed, it can be printed on the default printer by clicking the Print button in the Tool Bar window display. Most printers can be configured through the Windows operating system to be the default printer. Refer to your Windows operating manual for more information on installing a default printer.



Sample Printouts

2.3 Tool Box

The Tool Box contains an intuitive interface for performing all image enhancement functions.



Tool Box

The Tool Box contains the controls for enhancing and adjusting the image. This includes software filtering, false colors, zoom factors and other unique features. The Tool Box options are detailed in chapter 4 of this manual.

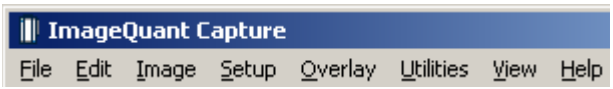
2.4 Status Bar

The Status Bar is located on the bottom of the monitor and provides a real time display of the mouse cursor x, y position, the image zoom factor, and the grayscale intensity at the mouse cursor x, y position.



Chapter 3: Pull-Down Menus

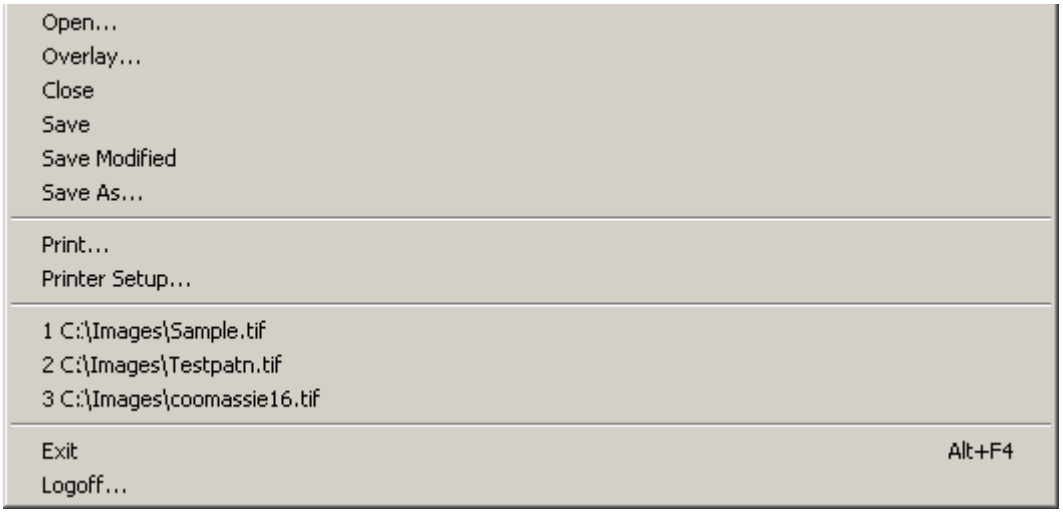
Across the top of the screen is a Windows menu bar containing several system operation functions. These include file saving and loading, edit, image, setup, overlay, file utilities, view and help functions.



ImageQuant Capture Menu Bar

3.1 The File Menu

Use this menu to save an image as a file, retrieve a previously saved image, select different printers, print an image to a parallel printer, overlay multiple images in RGB color channels, close an image, log-off of the system or exit the system.

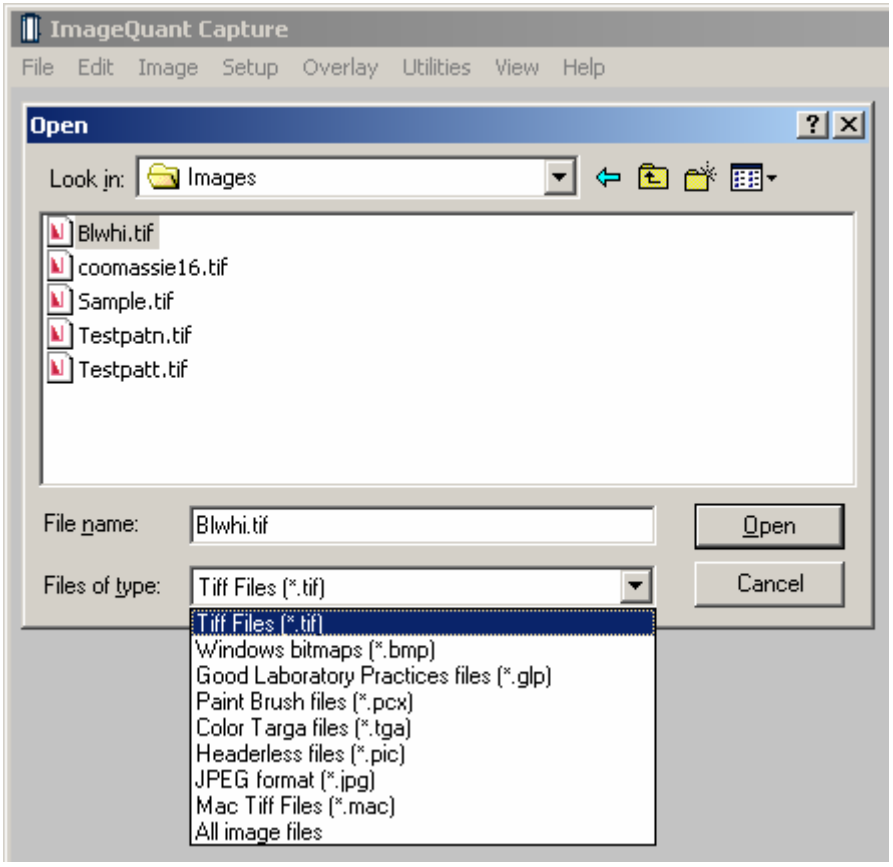


ImageQuant Capture File pull-down menu

3.1.1 File|Open



This function opens an image that has been previously saved as a TIF, GLP, BMP, PCX, TGA, PIC, JPG or Macintosh® TIFF (MAC) file.



File Open Dialog Box

Using the left mouse button, click on the name of the file to be loaded. That name is then highlighted in the list and appears in the text box below the File Name prompt.

Alternate disk drives can be accessed using the "Look In" dialog box.

Once the file has been selected, click on the Open button to load the file. (Alternatively, double-click on the file name.) The dialog box disappears and the selected image appears in the image window on the screen.

To dismiss the dialog box without loading an image, click on the Cancel button.

3.1.2 File|Overlay 

To superimpose images, use the OVERLAY function under the File menu. This function will display separate multiplexed images or a RGB color image as a compiled image with the appropriate color channel images added together. A simple way to acquire multiple images for this function is to use the FrameGrabber function in image acquisition and acquire a series of identical images.

The Overlay Images option allows you to overlay up to three different images with three different color channels. You can select the BROWSE button for each color channel and select the appropriate images to be used for generating a color image. For example, if you have a saved grayscale images of an identical gel taken with a SYPRO® red filter for the red stain and a SYBR® green filter for the green stain, you can choose these images in the appropriate Red and Green Channels to generate a composite image with the red and green colors mapped onto the compiled image.

Note: The images must be the same bit depth and resolution for the software to overlay the images.

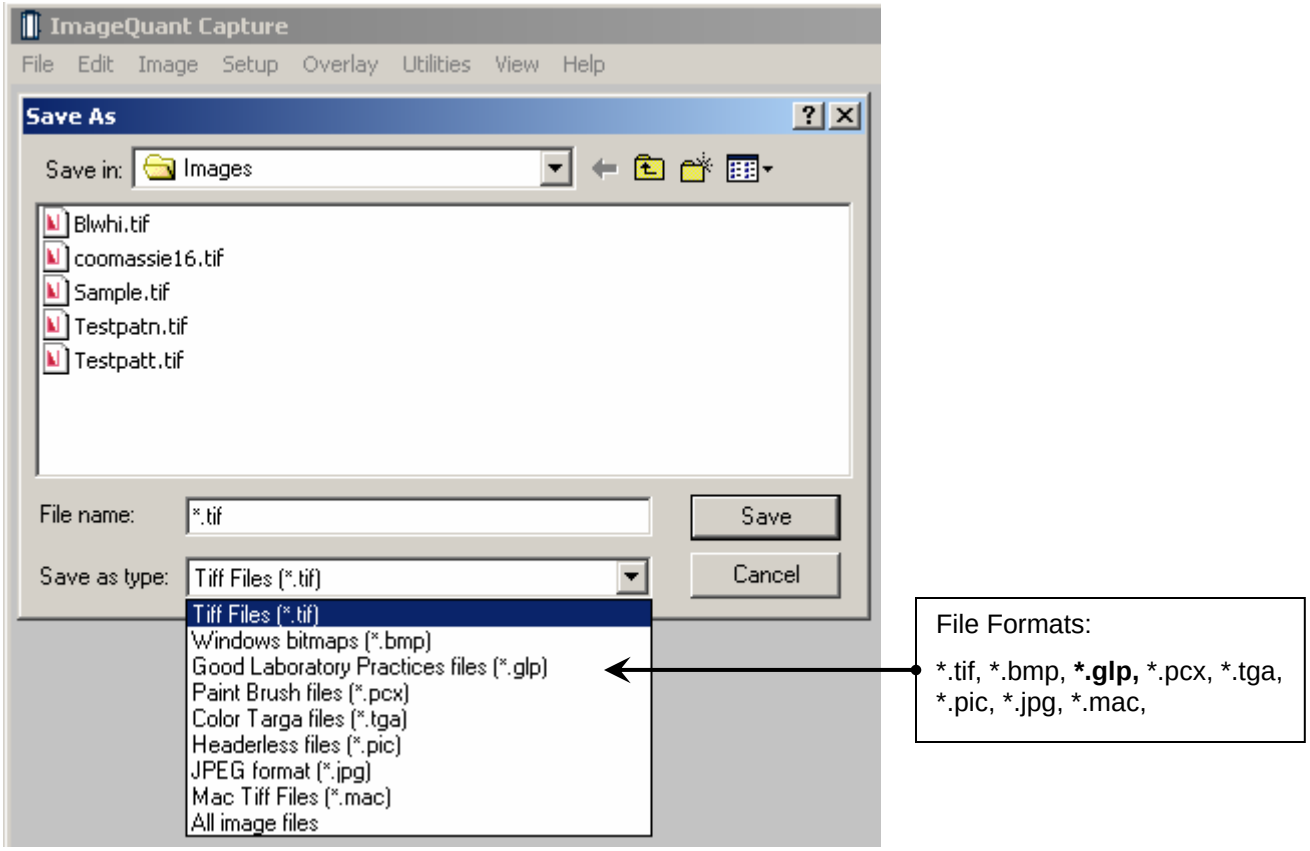
3.1.3 File|Close

This function closes the image currently displayed on the screen.

3.1.4 File|Save and Save As

Save allows original images to be saved in several different formats. Save As allows images that have previously been saved to be saved in a different location or as a different file type without affecting the original image.

ImageQuant Capture has the ability to save files in several formats, see the following figure:



Enter a new file name in the text box adjacent to the File Name prompt. Next, choose a file type from the Save As Type list.

ImageQuant Capture will automatically give the appropriate 3-character extension. ImageQuant Capture will also create a file with the same base name and an .STP extension. This setup file saves information specific to this file, such as Black Level, White Level, Gamma Setting and 1D-Multi template placement. If the file is accessed later, these settings will be recalled.

File Types

TIFF is the default file format for ImageQuant Capture files. TIFF is an acronym for "tagged image file format" and was developed as a flexible and machine-independent graphic file format. Saving as a TIFF file will allow users to double-click TIFF files from Windows Explorer and automatically launch the application on any machine that has ImageQuant Capture loaded on it. Users may customize this in the preferences section covered in section 3.4 of the manual if they wish to change the default file type.

Mac TIFF is the Apple Macintosh® version of the TIFF file format. Mac TIFF files have the extension .MAC so they can be easily distinguished from Windows TIFF files. Most software can distinguish between Mac and Windows TIFF formats and can accept either. ImageQuant Capture offers the option of both formats in the event that only one of the two is acceptable.

GLP is a proprietary file format that allows changes to only be made in ImageQuant Capture programs. It will accept 8 bit and 16 bit images and can not be opened in any other software program.

BMP, PCX, TGA, PIC, JPG, GLP are additional graphic file formats which may be useful when saving an image for desktop publishing. These file formats can be imported directly into many Macintosh® and PC programs. (See Appendix A for more information.) Do not use these formats to save images that will be analyzed later, since pixel data can be lost or altered when saving files in these formats.

Note: Not all of the file types listed above can be saved as a 16 bit file. Some may require you to convert the image to an 8 bit file first.

Original versus Modified Files

An Original image file is one in which the data is saved in an unaltered form. This option should be selected if the image will be analyzed later. If the Black level, White level, or Gamma settings have been adjusted, the new values are saved but the *pixel values are not altered*. When this file is opened at a later time, ImageQuant will display it with the values that were displayed when the image was saved, however, it is still possible to revert to the original raw image file by selecting Reset on the Tool Bar .

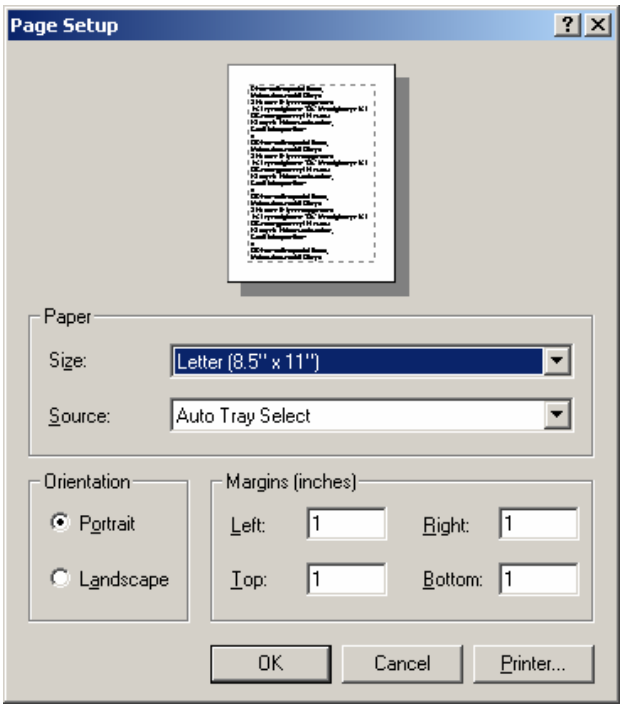
Annotation information cannot be saved with the Original image option. (It can, however, be saved as an Overlay. See section 3.5 for more information.)

An image that is saved as a Modified file permanently retains the changes to the image's Black level, White level, and Gamma setting. Annotations and any filtering performed are also saved with the image, replacing original image information with the new information.

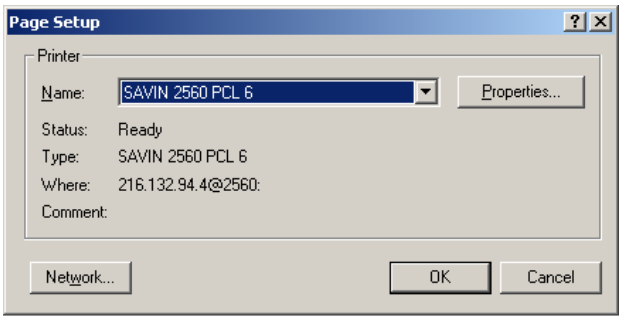
Note: If the image is saved as a Modified file it is converted to an 8-bit image.

3.1.5 Printer Setup

This function displays a dialog box in which the settings for the parallel printer are specified. When all the pertinent printing preferences have been specified, click on the OK button. If you purchased a printer with ImageQuant, this will be preset from the factory.



Printer Setup Dialog Box



Printer…. Dialog Box

For more information on using the Print menu, see the Windows manual.

3.1.6 Print



This function sends the image to the default printer specified in Print Setup.

3.1.7 Logoff

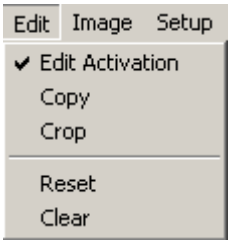
This function logs the current user out of ImageQuant Capture when security features are in use.

3.1.8 The Exit Function

The Exit function closes ImageQuant Capture. To restart ImageQuant Capture from Windows, double-click on the ImageQuant Capture icon on the desktop.

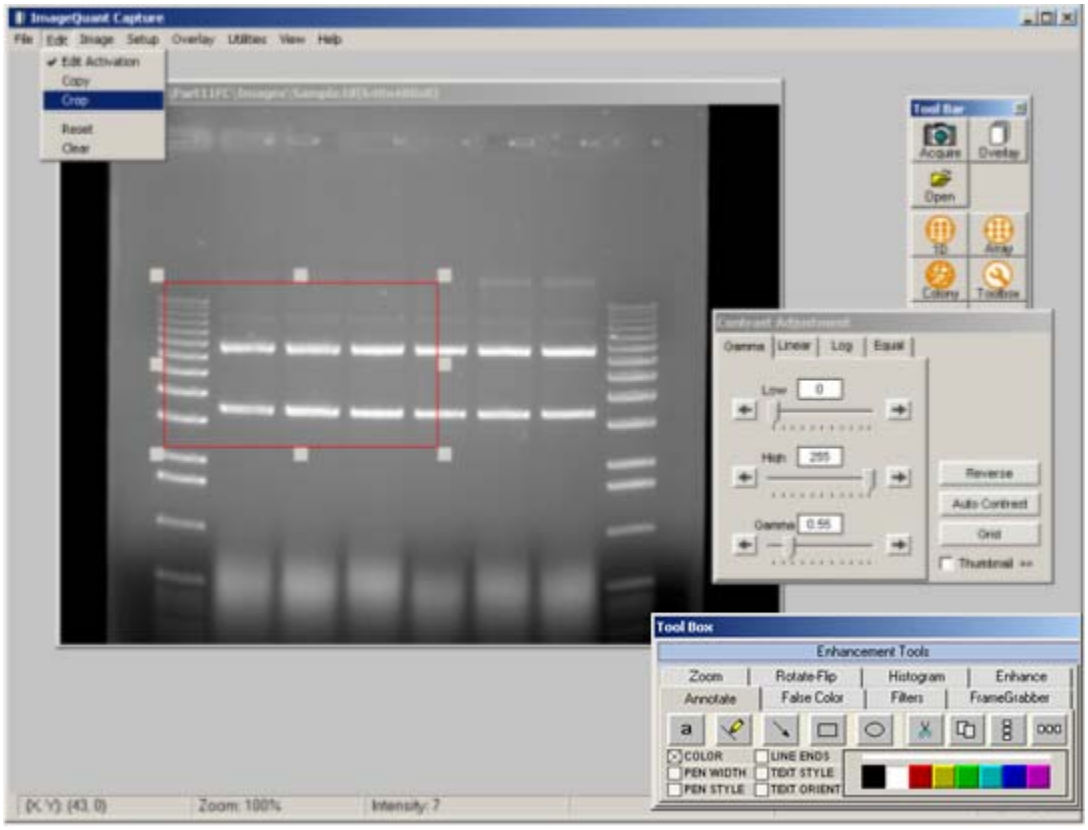
3.2 The Edit Menu

The Edit menu provides the ability to copy, crop and remove any annotations or filters that have been added to the original image.



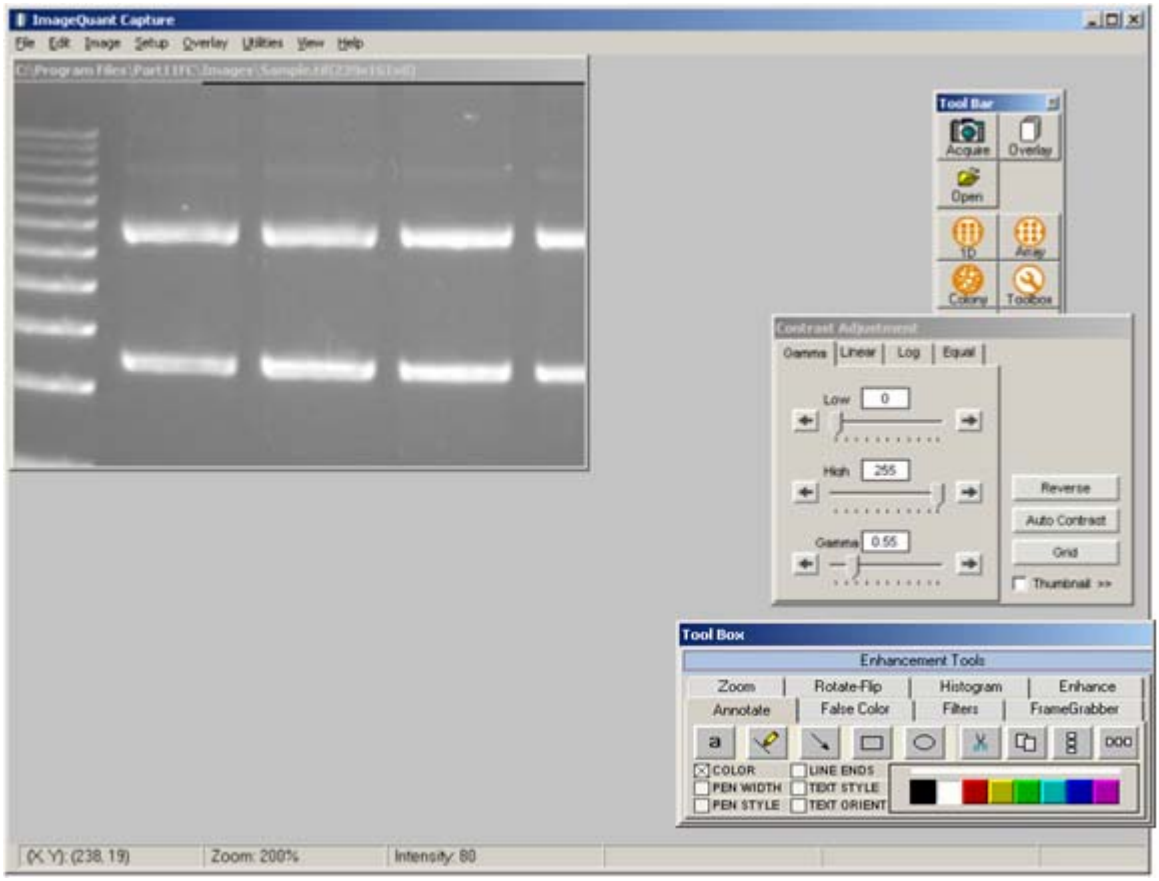
Edit pull-down menu

To activate the Copy and Crop functionality, place a check mark next to **EDIT ACTIVATION.** This will turn the mouse cursor into a + sign that will allow you to highlight the region of interest for the image. After Edit Activation is highlighted, the desired area of interest is drawn using the mouse.



Ready to Crop or Copy

Once this is completed, you can select either the **COPY** or **CROP** function in the EDIT menu options. **COPY** will copy the desired area of interest into the Windows Clipboard and allow you to paste into any desktop publishing package (i.e. Word, Excel, Adobe Photoshop, etc.). **CROP** will display just the region of interest as the active window in the ImageQuant Capture interface.



ImageQuant Capture interface after cropped region has been selected

3.2.1 Reset and Clear



The Reset option configures the Black, White, and Gamma settings to default settings. Clear removes any annotations that are present on the image.

3.3 The Image Menu

The Image menu option provides the ability to perform a variety of image processing functions.

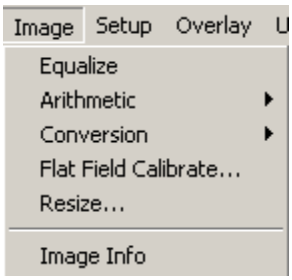


Image pull-down menu

3.3.1 Equalize

The equalize option performs a duplicate function to the EQUAL option in the Contrast Adjustment Window. This is a useful function for detecting faint bands on a sample.

3.3.2 Arithmetic

The Arithmetic function is used to add, subtract, average and divide several images together to generate a compiled image.

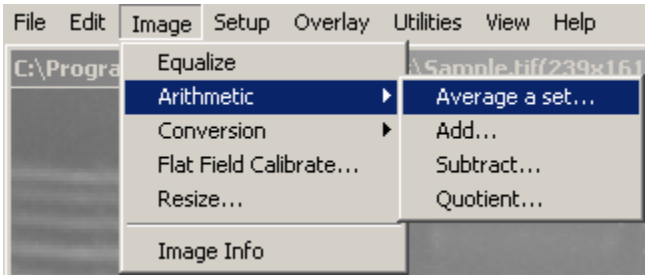


Image Arithmetic dialog box

To average a set of images together open one of the images in the set and then select 'Average a Set…' under the Image pull-down menu. A prompt will appear allowing the user to select all of the images that for the set. It is possible to browse the directories looking on the network drives and removable media if necessary. Once all of the images have been selected, click on the open button to finish the set. The resulting image is an average of all of the images together. This is a useful function for extending the dynamic range on a set of similar images by allowing bright spots and faint spots to be seen on the same image.

The other functions are adding, subtracting and dividing images together. Adding together images is frequently used for colorimetric markers run together with chemiluminescent samples. Subtracting images is often used to remove noise from a sample by running dark images first and subtracting them out of the final image. The

most common application for quotient is for those technical users who run their own flat field corrections. This can be done using the Flat Field Calibrate selection under the Image pull-down menu which will be described in detail later in this section.

All three of these arithmetic functions are performed by opening the main image that will be adjusted. Next select the appropriate arithmetic function under the image pull-down menu. Then select the image that is to be added, subtracted or divided from the original image and select open. The dialog box will disappear and the resultant image will appear.

Note: Images that have been arithmetically altered are ideal for publications and documentation, however, they are strongly not recommended for analysis as the pixel values have been adjusted.

3.3.3 Conversion

Since ImageQuant Capture can generate 16-bit files, the conversion option is useful when an image is to be imported into a program that only accepts 8-bit images. Choosing this option will convert a 16-bit image into an 8-bit image.

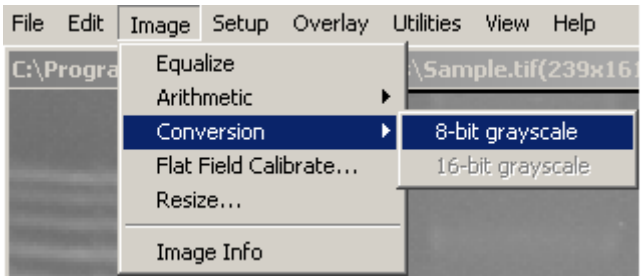


Image Conversion dialog box

3.3.4 Flat Field Calibrate

Flat Field Calibrate is a function that is used to 'flatten' the image so that the pixel data is even across the entire image area. This is a function that is useful for large gels and other applications that use the entire field of view for an image.

Creating flats can be art in itself; there are many documents on the internet that can help users interested in this arena to create the ideal flat for the application. However, some useful flats that have been created in the past involve very simple tools like a piece of 8.5 x 11 regular low quality copy paper (higher quality paper contains watermarks that will show up in the final image). It is essential that both the flat and the gel images be identical, including the aperture, zoom (if applicable) and focus settings on the lens.

Step-by-Step Flat Field Calibration:

1. Place the gel or other application in the cabinet or dark room.
2. Adjust the aperture, zoom (if applicable) and focus on the lens.
3. Use auto-expose set to the normal selection and acquire an image of the gel. (Alternatively, it is possible to select show saturation and then use preview mode and adjust the exposure time manually to just under saturation.)
4. Save the image of the gel.
5. Next, remove the gel from the UV transilluminator or white light tray and clean and/or dry off the surface if necessary using glass cleaner.
6. Place the white piece of paper onto the appropriate surface. (For example, if the UV transilluminator was used, place the paper onto the UV transilluminator; if the white light tray was used, place the piece of paper onto the white light tray.)
7. Turn on the appropriate light source used (white light, UV transilluminator, epi lights, etc.).
8. Without changing anything on the lens acquire another image of the 'Flat' image following step #3 again.
9. Save the Flat image.
10. Open the original gel or other application image.
11. Select Flat Field Calibrate from the Image pull-down menu.
12. Browse the directories for the 'Flat' image created.
13. Click on open. Make sure to save the flat field calibrated image for future use.

3.3.5 Image Resize

The image resize function can be used to resize an image to a specific dimension for use in graphical presentations. You have the option to 'Preserve aspect ratio' to avoid image dimensional distortion, or you can deactivate this function and configure the image resolution to the desired Width and Height dimensions.

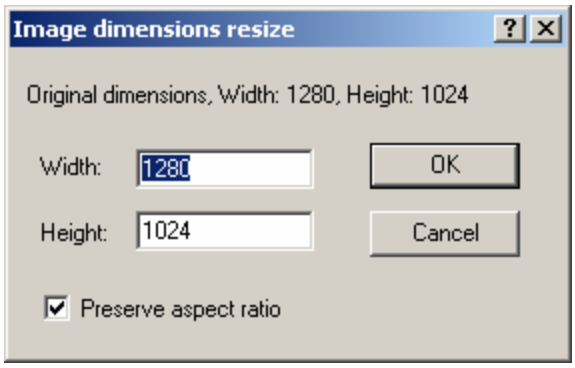


Image Resize dialog box

Note: It is recommended that you DO NOT perform quantitative analysis on resized images.

3.3.6 Image Info

The Image Info function provides a dialog box with all detailed image properties. An example is shown below. Click on the OK button to close this dialog box.

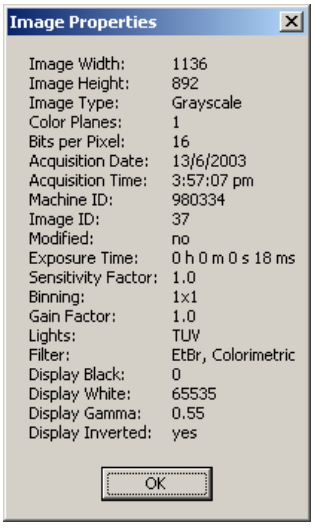
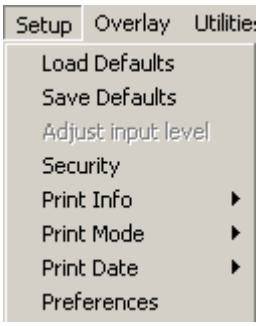


Image Info dialog box

3.4 The Setup Menu

This menu customizes the system settings by allowing users to save default parameter preferences and customize the software settings.



Setup pull-down menu

3.4.1 Default Parameters

Default parameter files eliminate the need to readjust the system settings each time the program is used, and can be especially useful if more than one person works with the program.

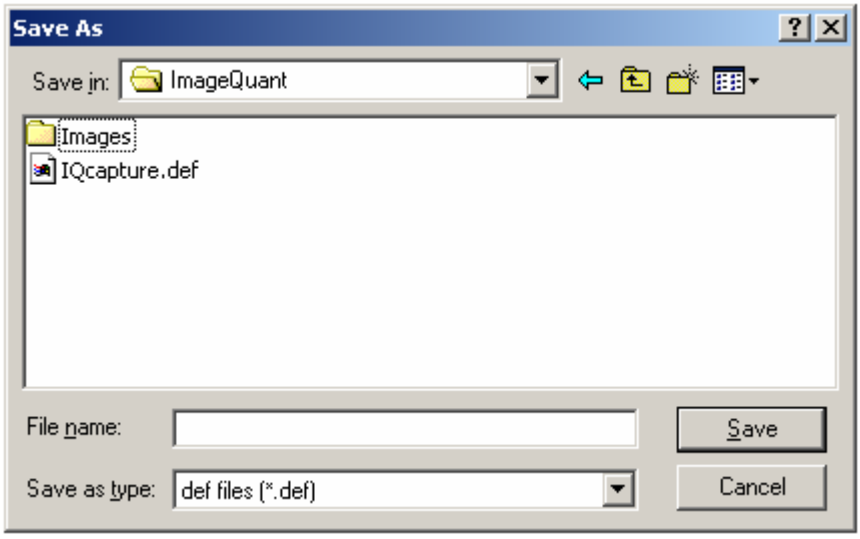
The default file used by ImageQuant Capture when it initially loads is called `IQcapture.def`. The system setting defaults are:

Exposure Time:	8/milli sec
Black Level:	0
White Level:	up to 255, 4095, or 65,535 - depending on the system type
Gamma Setting:	0.55
REVERSE:	off
ARRAY:	96 circles (12 x 8)
1D MULTI:	8 lanes

While these parameters can be changed, we do not recommend changing `IQcapture.def`. Instead, we recommend that each user set up an individual default file(s) reflecting their preferences.

3.4.2 Save Defaults

Every time a default file is saved, all of the settings for the parameters listed above are saved. These can be loaded later and applied to any image.



Save Defaults Dialog Box

Once any of the system settings have been changed, a new default file can be created. To save a default file, enter a file name by typing in the text box below the File Name prompt. If it is necessary to change the directory or drive to which the file will be saved, select a different directory under the Save In pull-down menu. ImageQuant Capture will automatically add the appropriate 3-character extension.

Click on the SAVE button. The Save As dialog box disappears and a new file is created.

To exit this function without loading a default file, click on the CANCEL button.

3.4.3 Load Defaults

This function retrieves system default settings from a saved file.

To open a default file, enter the name of the file by typing its name in the text box below the File Name prompt. If it is necessary to change the directory or drive to which the file will be saved, select a different directory under the Save In pull-down menu.

Once the file has been selected, click on the OK button to load the file. The Load Defaults dialog box disappears and the image controls are adjusted to reflect the settings in the file loaded.

To exit this function without loading a default file, click on the CANCEL button.

3.4.4 Security

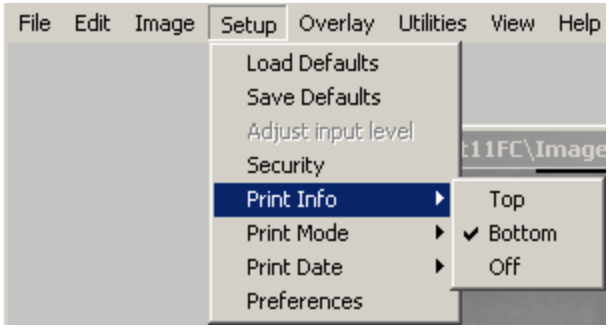
When the system is shared by a number of people or laboratories, a method of keeping track of its use may be helpful. The Security feature allows various levels of security and user log functions.

We suggest one user be designated as the supervisor of the system. This individual should refer to Appendix B, which describes the security features in detail.

Note: It is strongly recommend that you remove Appendix B from the manual to avoid unauthorized users changing the password and the security settings.

3.4.5 Print Info

When printing an image, basic image information is included on the print. This includes the exposure time, the Black level, White level, and Gamma setting, the date and time the image file was generated, an image ID number, and the name of the file to which the image is stored.



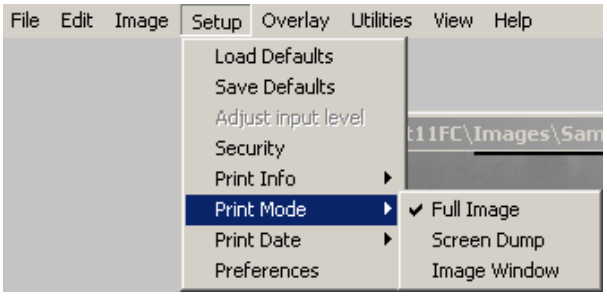
Setup Print Image Info Dialog Box

To print this information at the top of the print, choose Top from this menu. To print at the bottom of the print, choose Bottom.

Note: Printing image information at the top or bottom of a print may obscure a small portion of the image. To print the image with no information on it, choose Off.

3.4.6 Print Mode

ImageQuant Capture provides custom printing options.



Setup Print Image Info Dialog Box

Printing can be achieved using three different methods:

Full Image:	Prints the original image. Does not print zoomed images or images overlaid with data screens.
Screen Dump:	Prints the imaging area. Well suited for printing images overlaid with data screens and/or graphs, zoomed images, etc.
Image Window:	Prints the highlighted window.

3.4.7 Print Date

Under the “Setup” menu there is a selection labeled “Print Info”. This allows the user to change the format in which the date is printed. The choices are MM/DD/YYYY and DD/MM/YYYY.

3.4.8 Preferences

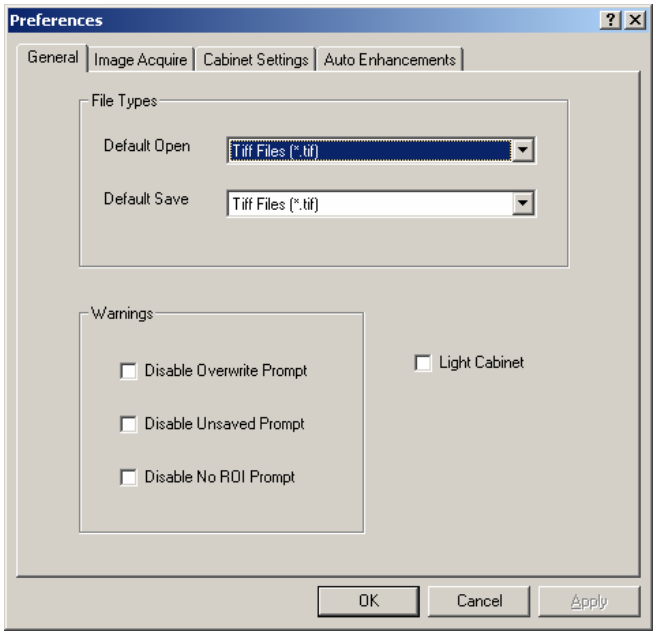
In order to change the preferences of the system, you will need to find the administrator of the ImageQuant Capture software program to log in. If you do not have an administrator for ImageQuant Capture, see Appendix B in this manual.



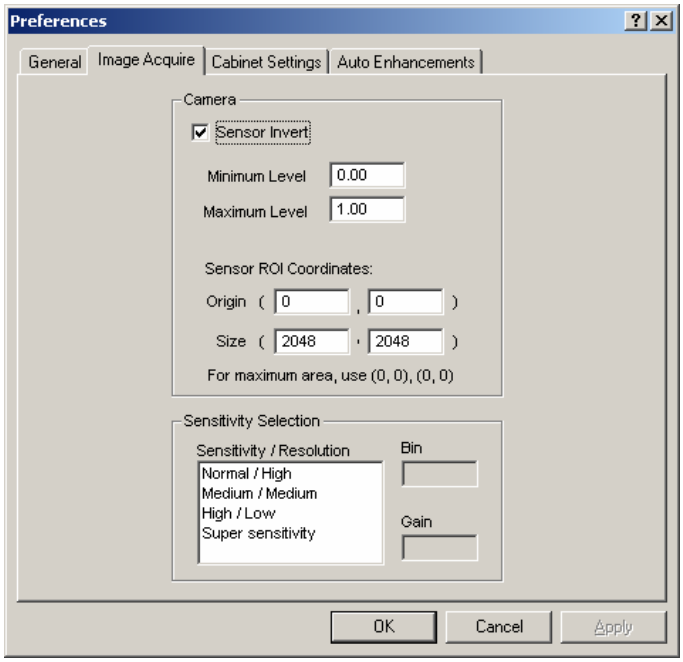
Login Dialog box for Preferences

There are four tabs in the Preferences menu (ImageQuant Capture standalone software does not contain the Image Acquire or Cabinet Settings tabs):

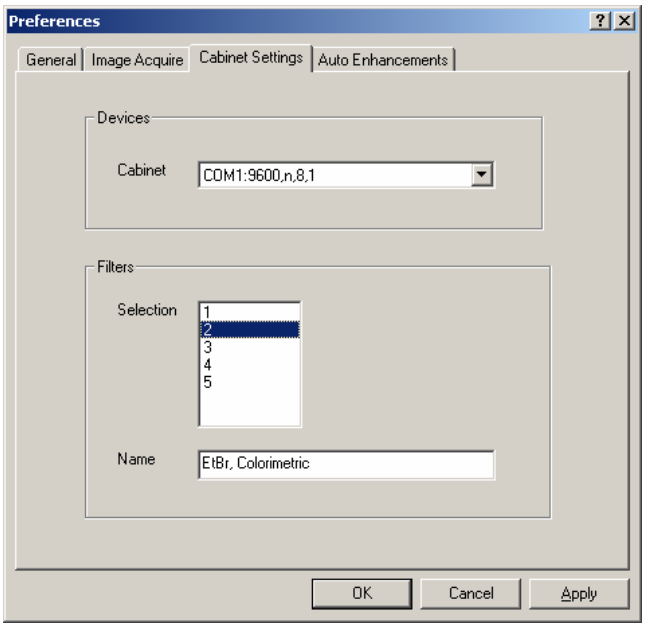
1. General – Configure prompts and file saving/opening formats.



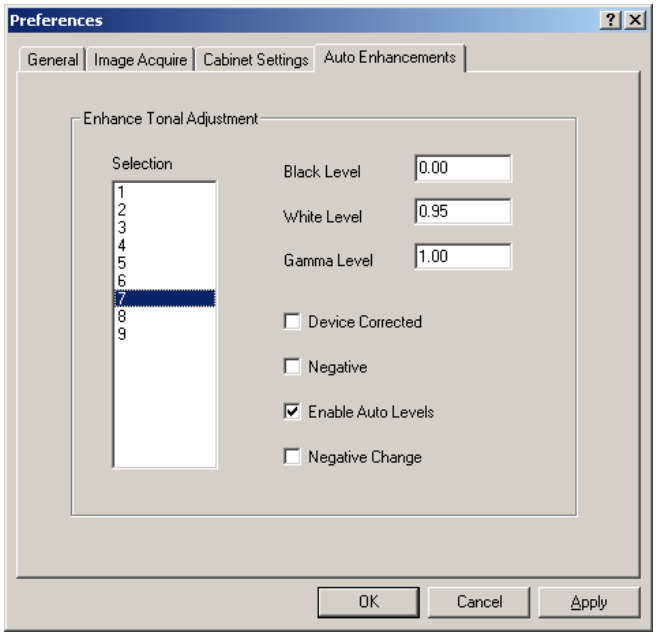
2. Image Acquire – Inverts the image seen by the camera and adjusts the ROI values.



3. Cabinet Settings – Used for adjusting the port settings and customizing filter positions on the cabinet.



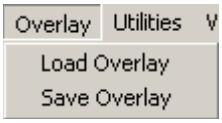
4. Auto Enhancements – Used to customize the Auto Enhance Levels located in the tool box.



To make changes to the preferences, select your change by typing in a new value or select/de-select the appropriate box with a check mark and then select apply. Some settings may require that the software be restarted before the change will take effect.

3.5 The Overlay Menu

The Overlay menu provides a means of saving and retrieving annotation overlays. This is especially useful when a standard gel format is run repeatedly. Lane numbers, molecular weight marker sizes, and other pertinent information can be stored as an Overlay file and retrieved at a later date. This eliminates the need to re-enter the information each time a new image is captured.

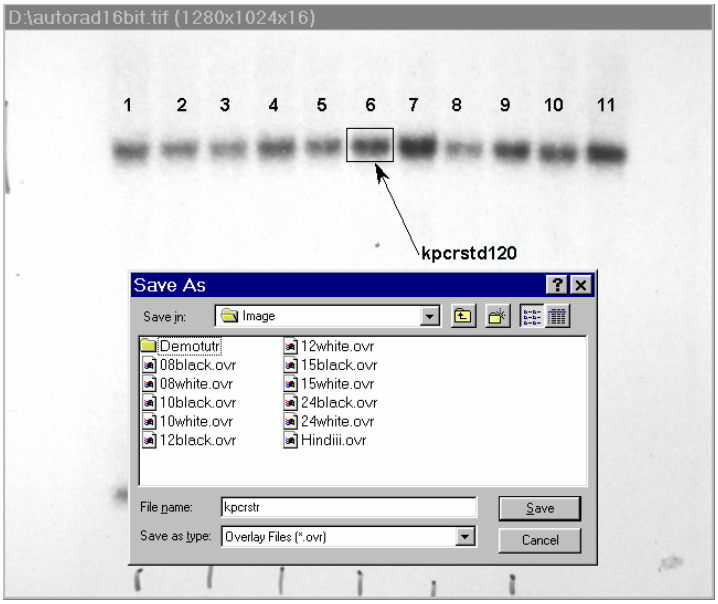


Overlay pull-down menu

An overlay is any set of annotations (text, boxes, arrows, etc.) that have been drawn on the image. They can be saved as a group and opened later. If repetitive samples are being imaged, an overlay eliminates the need to re-enter the same information (such as lane numbers, standard sizes, etc.) continually.

3.5.1 Saving an Overlay

Once annotations have been made, select Save Overlay from the Overlay menu.



Save Overlay Dialog Box

Enter a new file name in the text box below the File Name prompt. ImageQuant Capture will automatically give the appropriate 3-character extension.

The current directory is the one in which the new overlay file will be saved. If necessary, change the directory or drive as described in Section 3.1.

Once a name has been entered and the appropriate directory has been accessed, click the SAVE button to save the overlay file.

3.5.2 Loading an Overlay

The Load Overlay function allows Overlay files to be retrieved and applied to the image currently displayed.

Opening an Overlay after an image has been captured places the annotations on top of the image. They can be stored as part of the image by saving the file as a modified file. (See Save Image As in Section 3.1 for instructions.)

Select the name of the file to be loaded. (If necessary, change the directory or drive.) The file name is then highlighted in the list and appears in the text box below the Filename prompt.

Once the file has been selected, click on the OK button to load the file. (Alternatively, double-click on the file name.) The dialog box disappears and the annotations in the selected file appear on the image.

To dismiss the dialog box without loading annotations, click on the CANCEL button.

Overlay Libraries

ImageQuant Capture contains a library of overlays that can be accessed through the Load Overlay function described above. This library of overlays is stored in the Image folder located in the ImageQuant Capture directory:

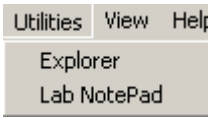
08WHITE.OVR / 08BLACK.OVR	8 lane labels in white/black
10WHITE.OVR / 10BLACK.OVR	10 lane labels in white/black
12WHITE.OVR / 12BLACK.OVR	12 lane labels in white/black
15WHITE.OVR / 15BLACK.OVR	15 lane labels in white/black
24WHITE.OVR / 24BLACK.OVR	24 lane labels in white/black
HINDIII.OVR	"λ*Hin*dIII" label

The objects in these overlays can be repositioned, resized, re-colored, copied or deleted as needed.

Note: Overlays are specific to the resolution of the image that they were created on. Therefore, if an overlay was created on an image with a different resolution than the image that the overlay is being loaded onto, the overlay may not match the original image.

3.6 The Utilities Menu

A number of functions are now handled by Windows programs. To access many of these programs while in ImageQuant Capture, open the Utilities menu and select the program of choice.

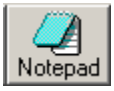


Utilities pull-down menu

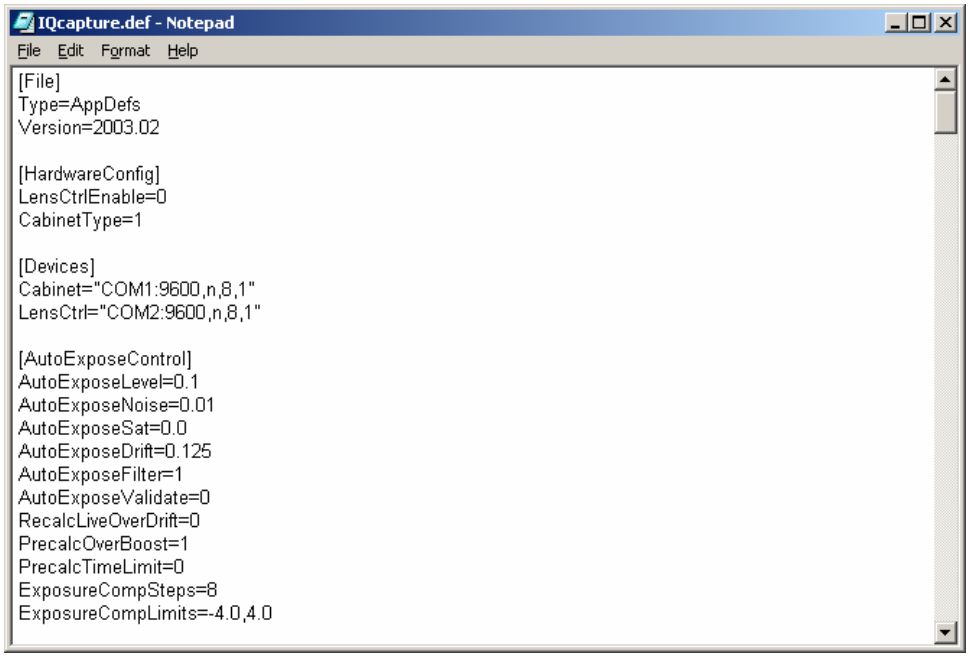
3.6.1 Explorer

This option can be used as a shortcut to Windows Explorer, which allows access to files and other information saved on the local machine or the network.

3.6.2 Notepad



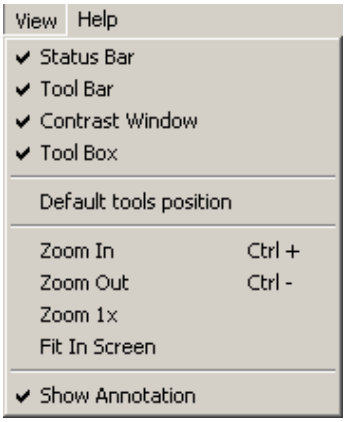
The Notepad is a blank screen that allows the user to make notes about the experiment and save them as an ASCII file. The Notepad is useful for saving any imaging comments or experimental conditions with the saved image for future reference.



Notepad Display Window

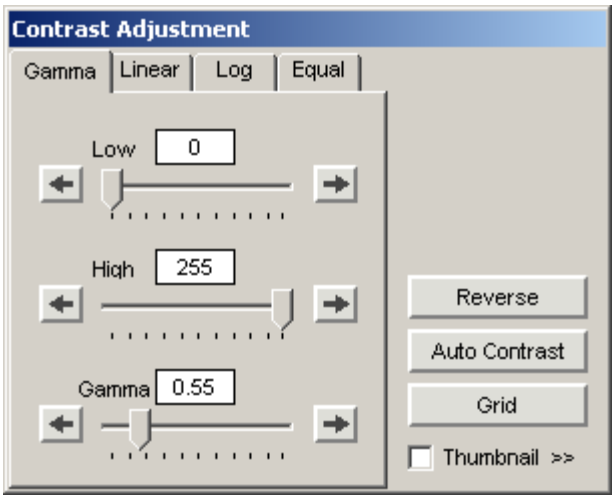
3.7 The View Menu

The View function provides the ability to control the display of the on-screen control tools as well as provide image enhancement abilities.

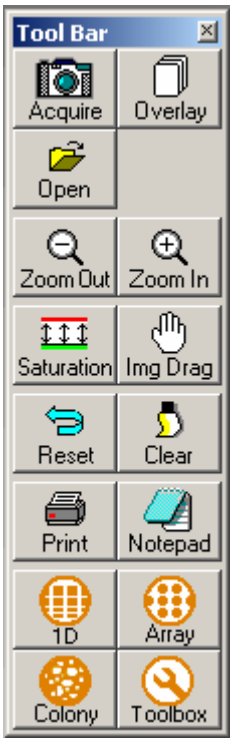


View pull-down menu

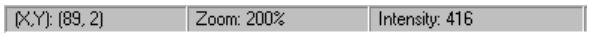
Four main tools exist within ImageQuant Capture: Contrast Adjustment, Tool Bar, Status Bar, and the Tool Box:



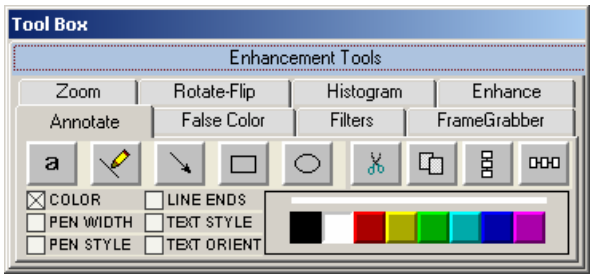
Contrast Adjustment Window



Tool Bar Window



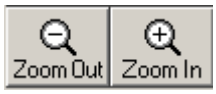
Status Bar



Tool Box

These tools are automatically displayed when ImageQuant Capture is launched for additional ease of use and to generate a common 'look and feel'. However, if you would like to hide any of these tools they can be turned off in the **View** menu by just deactivating the check mark next to the item that you would like to remove from the screen. Also, since these items are 'floating' tools, you can click on **Default Tools Position** to move all tools to the default locations for more intuitive operation. Lastly, except for the status bar, it is possible to select and move any of the other tools to a custom location.

3.7.1 Zoom Functions



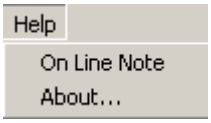
Additional options provide the ability to **Zoom In** and **Zoom Out** on the image**, Zoom to 1X** and to **Fit to Screen**.

Note: Zoom In and Zoom Out are duplicate functions for the Zoom In and Zoom Out icons in the Tool Bar and the Zoom options in the Tool Box.

3.7.2 Show Annotations

There is also an option to display/not display any annotations associated with the current image. To display the annotations, place a check mark next to the 'Show Annotations' option in the View menu. Otherwise, remove the check mark to remove the annotations from the image viewing area (the annotations are not deleted by selecting this option).

3.8 The Help Menu



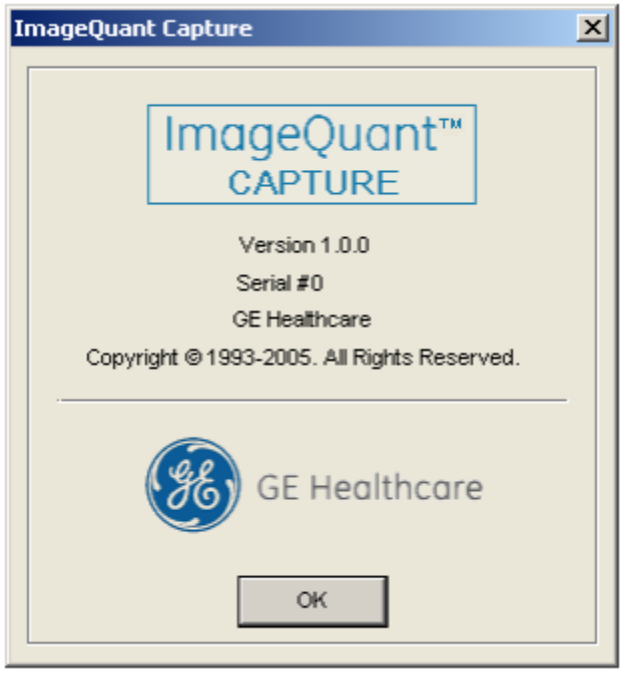
Help pull-down menu

3.8.1 On-Line Note

On-line help is available from the ON-LINE NOTE section of the help menu.

3.8.2 About

To display system information, select the About option in the Help menu. This button accesses a pop-up box. This box shows the system serial number and software version number. Use this information when contacting technical support, upgrading software, etc.



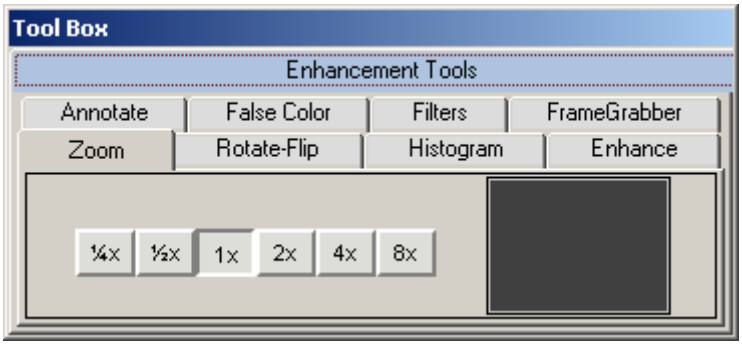
ImageQuant Capture About Help Dialog Box

To close the box, click on the OK button.

Chapter 4: The Tool Box

Image enhancement tools are contained within the Tool Box as indicated. This tool set allows the user to zoom the image, rotate-flip the image, show the image histogram, perform automatic image enhancement, annotate on the image, display false colors, apply software filters, and activate the FrameGrabber function.

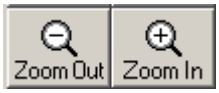
4.1 The Zoom Tool



The Zoom Tools

The Zoom tool is found in the Tool Box. This function magnifies an image, making details easier to see, and allows movement around the magnified image. An image can be displayed ¼x, ½s, 1x, 2x, 4x, or 8x larger than the original display by clicking on the appropriate buttons. To return to the original magnification, click on the 1X button. When an image is magnified, only part of it can be displayed on the screen at any one time. To see different parts of the magnified image, use the Pan Control box within the thumbnail. The outer box shows a thumbnail of the entire image while the small, inner box represents the portion currently displayed on the screen.

To view different regions of a magnified image, move the cursor into the inner box. Click and hold down the left mouse button. The cursor changes to a hand; use it to drag the box within the thumbnail until the desired region of the image appears on the screen. Alternatively, use the scroll bars in the main image window to move the image up/down, and left/right. On-screen Zoom tools are also available from the tool bar. These buttons duplicate the Zoom tool in Tool Box, and also allows for Img Drag to easily pan zoomed images.



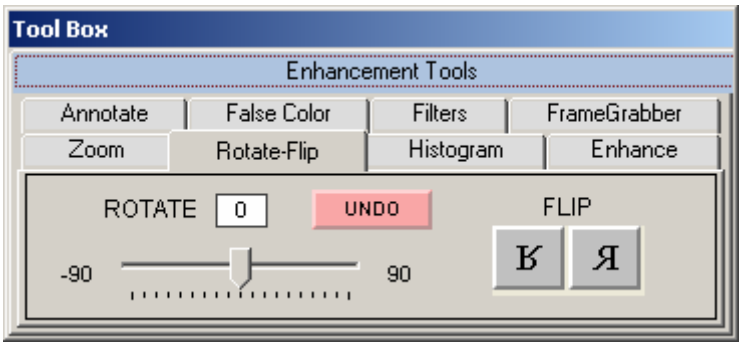
Zoom icons in Tool Bar



Img Drag Icon in Tool Bar

4.2 The Rotate / Flip Tool

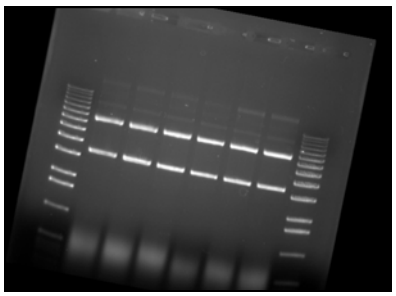
The Rotate / Flip tools are found in the Tool Box. This function rotates the image in a clockwise or counterclockwise direction by 1 degree increments up to a maximum of 90 degrees in either direction. This is a useful tool if the image is not aligned properly during the capturing process.



The Rotate / Flip Tool

To rotate an image, click and hold down on the center sliding bar with the left mouse button and move it left or right until the desired angle of rotation appears in the rotate box. Release the left mouse button and image will rotate to the desired angle. To undo a rotation, just click on the Undo button. Also, a Flip option allows for the image to be rotated 180 degrees in a vertical or horizontal fashion.

The Reset button on the main software interface will also remove any rotations or image flips and return to the display to the original image.



Rotated 12 degrees clockwise

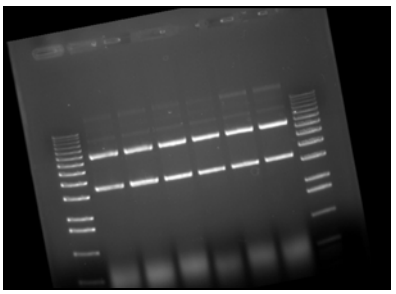


Image rotated -12 degrees (Counterclockwise)

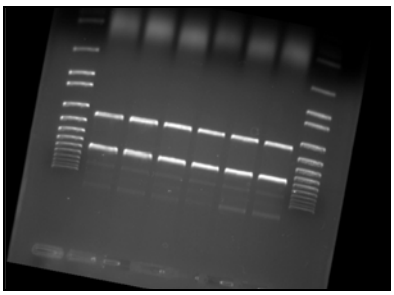
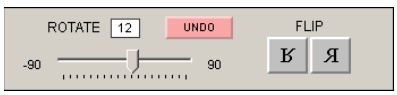
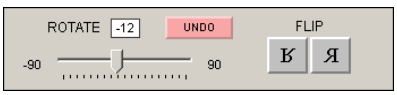


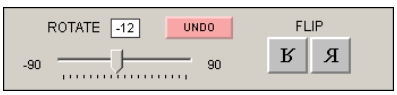
Image rotated 12 degrees and flipped vertically



Rotate / Flip box at 12 degrees



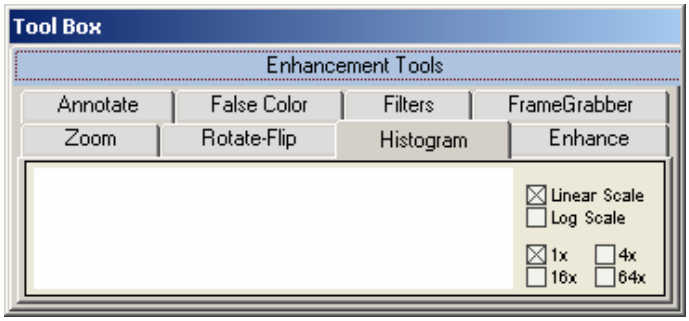
Rotate / Flip box at –12 degrees



Rotate / Flip box at –12 degrees and vertical flip button pressed

4.3 Histogram

The histogram is a graphical display of the proportion of pixels assigned to each of the image's gray levels. This tool is found in the Tool Box.

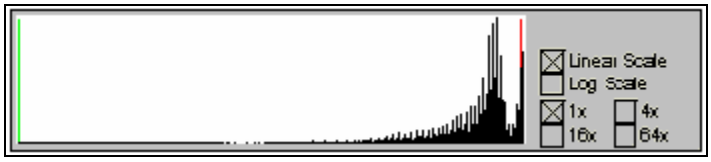


Histogram display in the Tool Box

The image is made up of picture elements (pixels) having brightness levels ranging from black to white. A very bright image will have most of its pixels registering high gray levels and conversely, a very dark image will have most pixels with low gray levels (approaching zero).

The histogram is displayed in the lower left corner of the screen, below the image window. The horizontal axis represents the gray scale range: black at the left end and white at the right end, with levels of gray in between. The number of pixels registering a particular gray level determines the height of each bar along the axis.

A Coomassie blue-stained protein gel visualized with a white light box has a histogram reflecting mostly bright pixels:



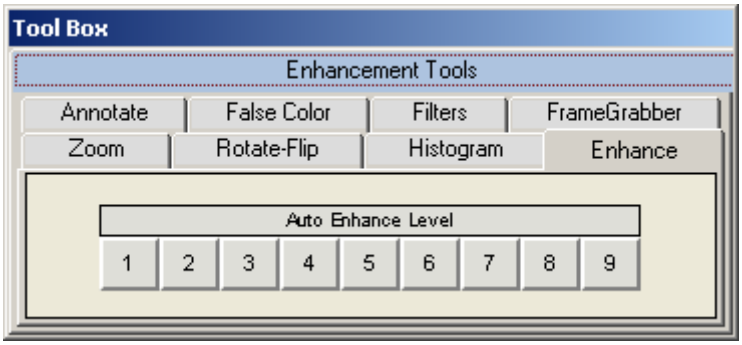
Histogram of a typical Coomassie gel

Most of the pixels are found in the light portion of this histogram. The dark bands represent a small number of pixels and include a variety of gray values, and therefore do not show up as a single peak.

The histogram function is particularly useful to verify that an image spans the maximum range of gray levels. When an image is to be used for analysis, it is especially important that the gray level range be as large as possible. If an image does not include most of the gray levels, we recommend repeating the image capturing process.

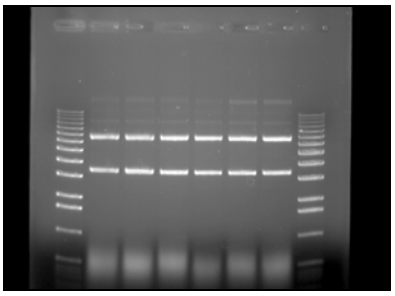
4.4 Automatic Enhancement

The Enhance tool is found in Tool Box. This function is ideal for new or inexperienced users of the system since it offers 9 levels of automatic image enhancement of the black, white, and gamma levels simultaneously. For an inexperienced user, it can be difficult to adjust each black, white, and gamma buttons to their respective optimal positions. By clicking on one of the nine Auto Enhance Level buttons, the image is optimized according to a unique level. Button 1 will make the image 'darker'. Each increasing button click will 'lighten' up the image until button 9 is pressed which will make the image the 'lightest' possible.

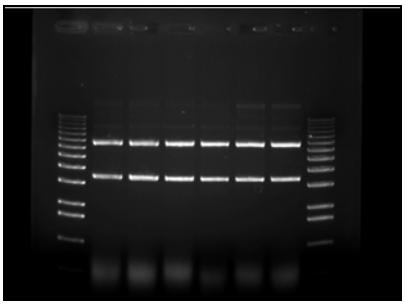


The Enhance Tools

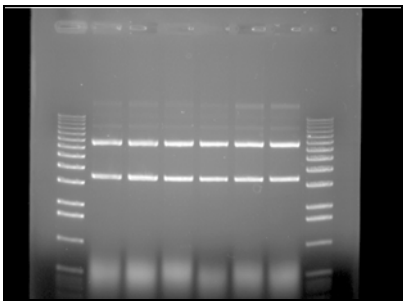
To undo any Auto Enhance Levels, just press on the Reset button on the main interface.



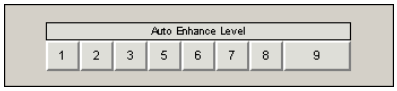
Original Image



Auto Enhance Level 2 Image



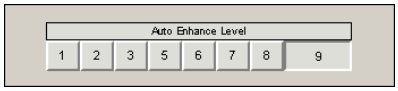
Auto Enhance Level 9 Image



Original Auto Enhance settings



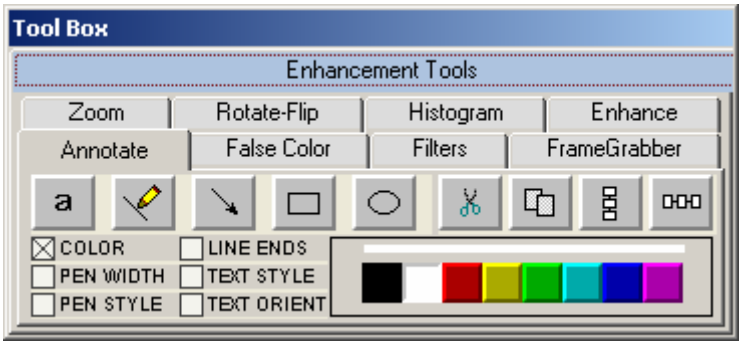
Auto Enhance with level 2



Auto Enhance with level 9

4.5 Annotations

The annotation tools, found in the Tool Box, include a number of different options for adding text (including Greek symbols), drawing arrows and otherwise marking an image. Note that these tools are for annotation only.



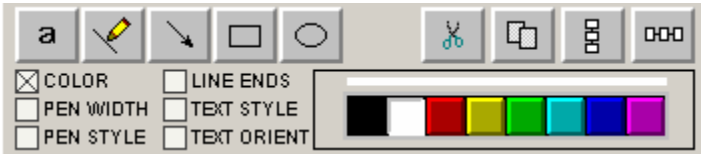
Annotations Tools

4.5.1 Object Attributes

Use the COLOR, PEN WIDTH, PEN STYLE, LINE ENDS, TEXT STYLE and/or TEXT ORIENT menus to specify object attributes. Attributes can be assigned to the cursor before drawing or typing. Alternatively, they can be assigned to an object while it is in "edit" mode (see the following pages for more details).

4.5.2 Annotation Colors

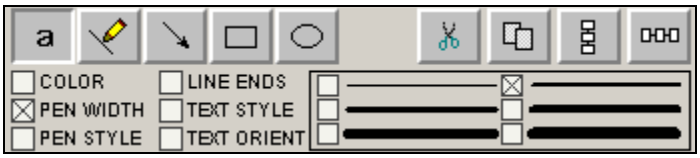
Annotations can be displayed in a variety of colors. The color options are displayed by clicking the COLOR checkbox. To select a color, simply click the cursor on the button labeled with the desired color. The color button appears depressed, indicating that it is selected. Any annotations subsequently entered will appear in that color. It should be noted, however, that annotations are printed in gray scale on the video printer. Further, when an image is saved as a modified image, the annotations are saved in gray-scale, not color.



Pen Color Selection Tools

4.5.3 Line Thickness

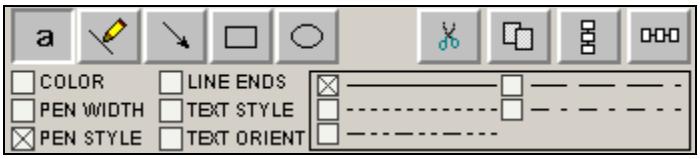
The PEN WIDTH menu specifies the thickness of lines when using the freehand, lines, box and circle drawing tools. Click on the appropriate checkbox for the desired width. All annotations subsequently entered will appear at that width.



Pen Width Selection Tools

4.5.4 Line Types

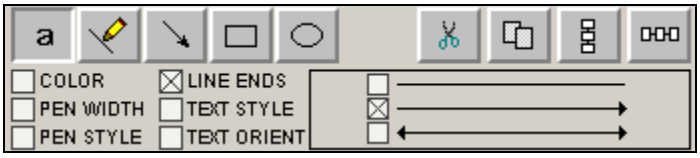
The PEN STYLE menu specifies the style of lines when using the freehand, lines, box and circle drawing tools. Click on the appropriate checkbox for the desired style. All annotations subsequently entered will appear in that style. Note: these pen styles only work with a thin line (see ***Line Thickness*** above).



Pen Style Selection Tools

4.5.5 Arrows and Straight Lines

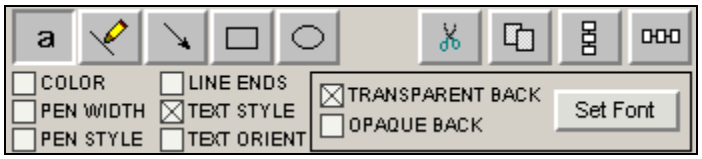
The LINE ENDS menu specifies the style of the ends of straight lines (no arrow, single arrow or double arrow). Click on the appropriate checkbox for the desired style. Note: these line ends work with any line thickness.



Line Ends Selection Tools

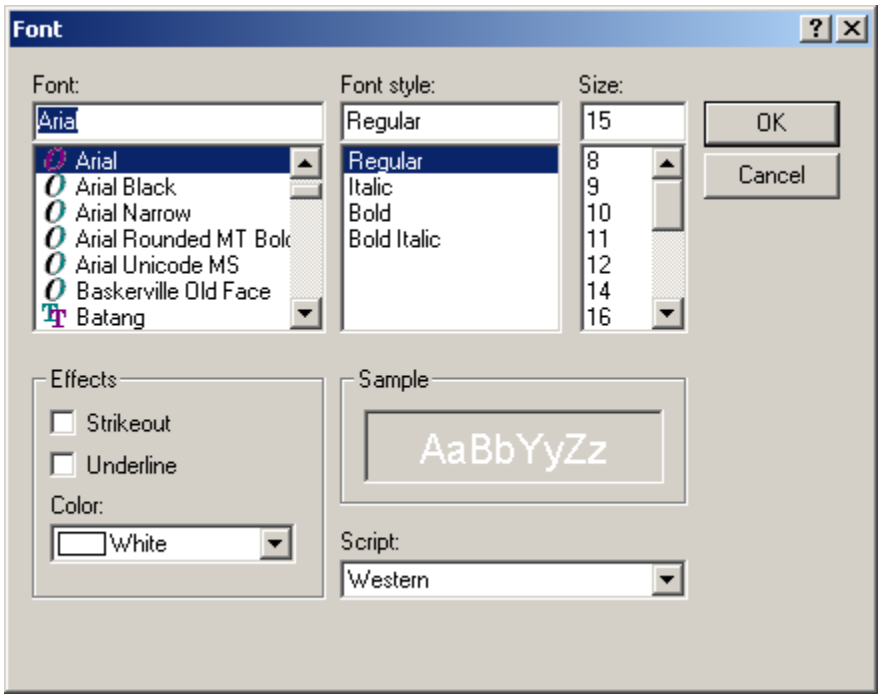
4.5.6 Text Background and Font

The TEXT STYLE menu specifies the style of text. Click on the appropriate checkbox to show text with or without a background. An opaque background is useful if annotations will be made on an image that has wide variations in gray scale. By using an opaque background, text will not be "lost" in the background of the image.



Text Style Selection Tools

This is also the window in which specific font is chosen. When the Set Font button is depressed, a selection box appears, from which text style can be chosen.



Font Selection Window

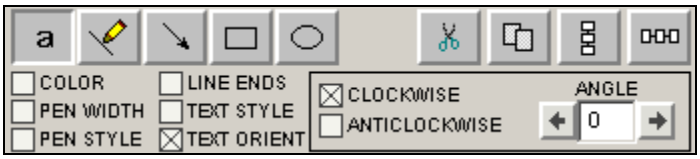
Note:
To choose Greek symbols (such as α, β, λ, π, and θ) choose the Symbol font:

a b c d e f g h i j k l m n o p q r s t u v w x y z

α β χ δ ε φ γ η ι φ κ λ μ ν ο π θ ρ σ τ υ ϖ ω ξ ψ ζ

4.5.7 Text Angles

In the TEXT ORIENT window, select whether text should be oriented vertically, horizontally, or at an angle (in 15° increments).



Text Orient Selection Tools

Note: only rotate fonts that are True Type (indicated by TT in front of the name); other fonts (such as Courier and Fixedsys) do not re-scale properly, giving unpredictable results.

4.5.8 The Drawing Tools

Once the object attributes have been defined, click the cursor on any of the drawing tool buttons to assign the function associated with that button to the mouse. The cursor will change from an arrow to a cross, indicating that ImageQuant Capture is in “drawing” mode.

After selecting a drawing tool, move the cursor to the correct position on the image to begin drawing. Press and hold the left mouse button and move the mouse to the end point of the object to be drawn. Release the left button, and the object should now appear.

Boxes will appear at the corners of the new object and the cursor will revert to an arrow, indicating that ImageQuant Capture is now in “edit” mode. At this point, the object can be resized or repositioned. The color, pen thickness, line type, etc. can also be changed, simply by clicking on the desired choice (as described in ***Object Attributes*** above).

To draw another object, click the right mouse button to return to “draw” mode, or click on one of the drawing tool buttons.



The button labeled with an "A" adds text to the image. Place the cursor at the location on the image where the left edge of the text should appear. Click the left mouse button and begin typing. To place another piece of text, click where it should be placed. Once all text is entered, click on the right mouse button. To edit text, double-click on it. An edit window will appear, in which changes can be made. To change fonts, see ***Text Background and Font*** above.



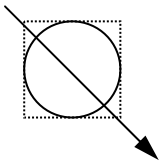
The button labeled with a pencil icon allows the user to draw lines freehand. After clicking on the pencil, move the cursor to the correct position on the image to begin drawing. Press and hold the left mouse button. Using the mouse, move the cursor as if it were a pencil. When finished drawing, release the mouse button.

 The button labeled with a diagonal arrow draws arrows and straight lines. After clicking on the button, move the cursor to the position on the image where the line should begin. Press and hold the left mouse button. Using the mouse, move the cursor to the other end point of the line then release the mouse button. The arrow can be adjusted by clicking on one of the boxes at the end (the other will serve as an anchor point) or by clicking in the middle to drag the entire arrow.

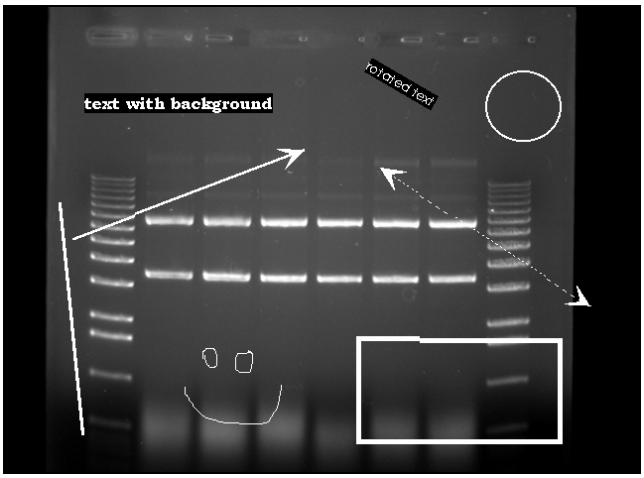
 The button labeled with a rectangle draws a rectangle or square of any size on the image. After clicking on the button, move the cursor to the position that should correspond to one of the corners of the rectangle. Press and hold the left mouse button. Using the mouse, move the cursor to enlarge the rectangle. When it reaches the desired size, release the left mouse button.

 The button labeled with an oval draws a circle of any size. After clicking on the button, move the cursor to the position on the image where the circle should be started. Press and hold the left mouse button. Using the mouse, move the cursor to enlarge the circle. When the circle reaches the desired size, release the left mouse button.

Hint: to draw a perfect circle around a portion of an image, first visualize a square surrounding the area of interest. Position the mouse in the upper left hand corner of the square. Click and drag the mouse down across the area of interest at a 45° angle until the circle encloses the area of interest.



Sample Annotations



Annotated image showing: freehand drawings, lines with various characteristics, circles, squares, text with various characteristics

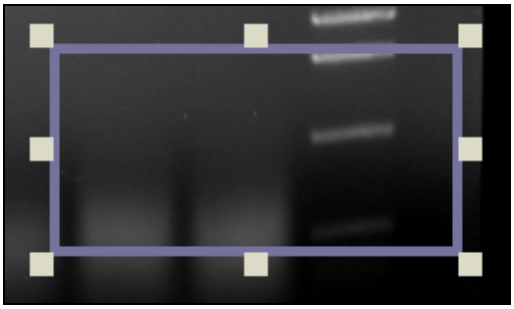
4.5.9 The Editing Tools

When the cursor is in "edit" mode, it can be clicked on an object to select it. (Note: the cursor can be toggled between "edit" and "drawing" modes by clicking the right mouse button.)

When an object is selected, small square boxes appear at the corners. Selected objects can be resized, copied, deleted or moved:

- To resize an object, click on one of the gray boxes at the corners of its perimeter and drag the box until the object reaches the desired size.
- To copy an object, use the Copy tool.
- To delete an object, use the Cut tool or the Eraser.
- To move an object, click within its boundary and drag it into the desired location.

To select more than one object, outline them with the mouse; any objects that fall completely within the outline drawn will be selected. (Note: The entire object must be enclosed by the cursor's movement in order to be selected.)

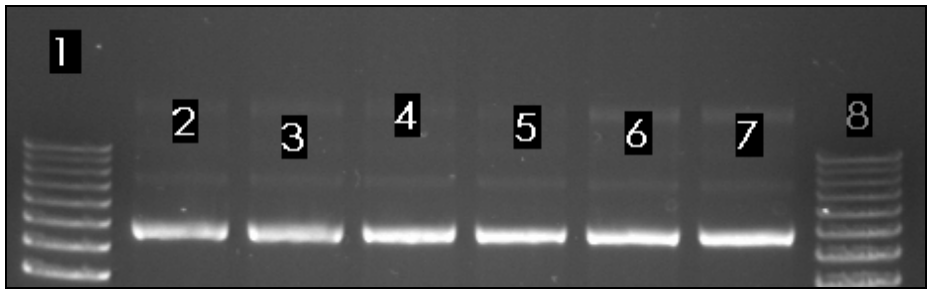


A Selected Object

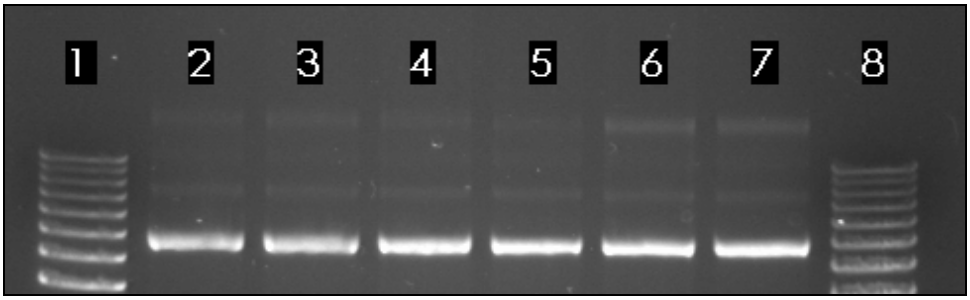
 Once an object or group of objects has been selected, clicking on the Cut tool deletes it from the image.

 The Copy tool makes an exact copy of the selected object. The new object becomes the selected object, and can be repositioned by placing the cursor within the object's boundary and moving it to the desired location.

 The Horizontal Alignment tool aligns annotations in a straight horizontal line. This is especially useful for labeling lanes, etc. To use this tool, draw text on the image, select it, click the Horizontal Alignment tool, then deselect the text. The text will now be aligned in a straight line across the image.



Text Prior to Horizontal Alignment

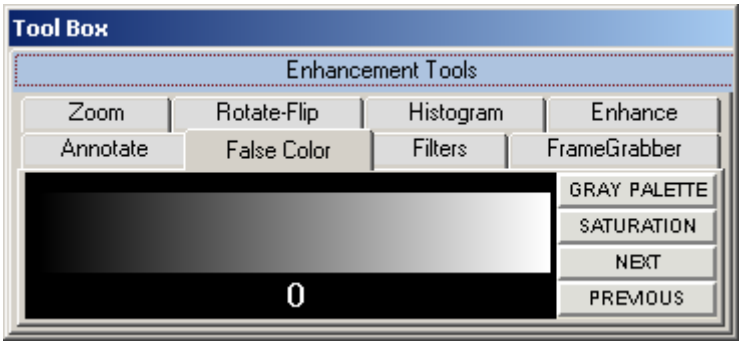


Text after Horizontal Alignment

 The Vertical Alignment tool aligns annotations in a straight vertical line. This is especially useful for labeling markers, etc. To use this tool, draw text on the image, select it, click the Vertical Alignment tool, then deselect the text. The text will now be aligned in a straight line down the image.

4.6 False Color

These tools consist of eleven pre-defined color palettes that can be applied to an image. To select a palette, simply click on one of the four buttons labeled GRAY PALETTE, SATURATION, NEXT or PREVIOUS.



False Color Selection Box

When a palette is selected, its range of colors is displayed to the left of the palette buttons and automatically applied to the image. To apply a different palette to the image, click the NEXT or PREVIOUS buttons.

Note: changes in Black level, White level and Gamma setting can alter the effect of each of the palettes, and can enhance the results produced.

4.6.1 Gray Scale (Palette 0)

This is the default or standard gray scale, consisting of different gray levels, ranging from black to white.

4.6.2 Saturation (Palette 1)

This is a modified gray scale palette in which black is replaced with green, and white is replaced with red. Over- and under-exposed areas of the image are thus shown as green or red, while areas within the linear range of the CCD chip are shown in gray scale. The Saturation Palette is especially useful during quantitation, as areas outside the linear range of the instrument do not give accurate quantitative information. This palette allows the user to avoid those areas during quantitative analysis.

This palette can also be accessed by clicking the Show Saturation checkbox in the Camera Setup and Preview function (accessible by clicking on the Camera icon in the Tool Bar window)

4.6.3 Palettes 2 through 11

These are color substitution palettes in which the gray levels are translated into different color ranges. These palettes can be useful to help distinguish features and highlight details on an image.

Palette 2 maps the gray scale levels to a red/green/blue palette. Values of 0 are mapped to red; saturated to blue, and values in between to green.

Palette 3 maps the gray scale levels to a red/green/blue palette. Values of 0 are mapped to blue; saturated to green, and values in between to red.

Palette 4 maps the gray scale levels to a red/green/blue palette. Values of 0 are mapped to green; saturated to red, and values in between to blue.

Palette 5 maps the gray scale levels to a cyan/magenta/yellow palette. Values of 0 are mapped to cyan; saturated to yellow, and values in between to magenta.

Palette 6 maps the gray scale levels to a cyan/magenta/yellow palette. Values of 0 are mapped to yellow; saturated to magenta, and values in between to cyan.

Palette 7 maps the gray scale levels to a cyan/magenta/yellow palette. Values of 0 are mapped to magenta; saturated to cyan, and values in between to yellow.

Palette 8 maps the gray scale levels to a red palette. Values of 0 are mapped to dark red; saturated to white, and values in between to shades of red.

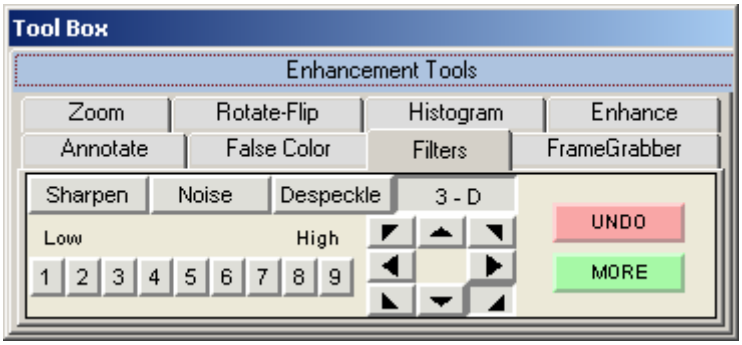
Palette 9 maps the gray scale levels to a blue palette. Values of 0 are mapped to dark blue; saturated to white, and values in between to shades of blue. This palette may be useful when printing an image of a Coomassie-stained protein gel onto a color printer.

Palette 10 maps the gray scale levels to a green palette. Values of 0 are mapped to dark green; saturated to white, and values in between to shades of green. This palette may be useful when printing an image of a SYBR® Green I-stained protein gel onto a color printer.

Palette 11 maps the gray scale levels to an orange palette. Values of 0 are mapped to dark orange; saturated to white, and values in between to shades of orange. This palette may be useful when printing an image of an EtBr-stained gel onto a color printer.

4.7 Image Filters

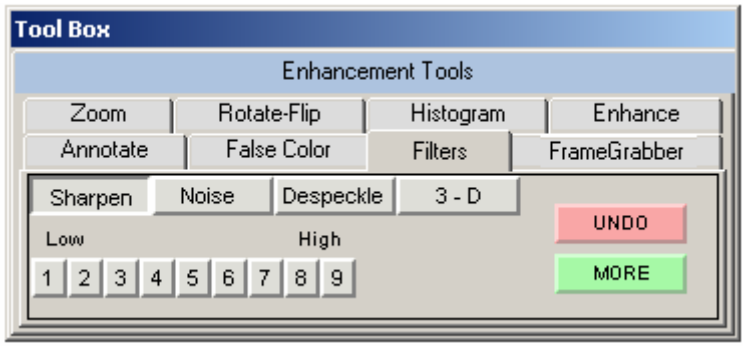
ImageQuant Capture includes a variety of enhancement filters that can improve the appearance of an image. Some filters sharpen detail, others smooth and reduce random noise. Still others help visualize edges and separate closely spaced bands or objects. Depending upon the unique characteristics of an image, the results of each filtering operation vary. Assess the characteristics of the image and then select the filter designed to minimize its imperfections.



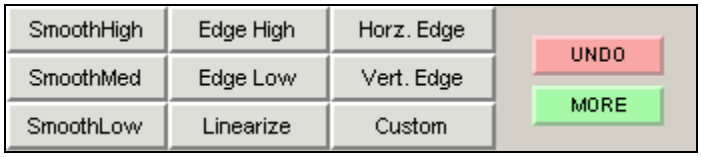
Filter Tools with 3-D (contour) selected

When an image is filtered, the original image information is replaced with the results of the filtering operation. As a result, the original image information is altered. To avoid losing the original image, save it as an original TIFF file *before* applying a filter.

When the FILTERS button is selected in the Tool Box, a pop-up dialog appears. If the desired filter is not shown, choose MORE to display more filters. One or many filters can be applied to a single image.



The Filter Tools with Sharpen highlighted



The Filter Tools with More selected

4.7.1 General Information on How Filters Work

To enhance an image, filters change the value assigned to each pixel. The new value assigned to a pixel is determined based on the values of the other pixels in its local vicinity (or “neighborhood”). The neighborhood is a two-dimensional matrix of pixel values, where each dimension has an odd number of elements. The "pixel of interest" is the one at the center of the neighborhood. This is the pixel whose old value is being replaced with a new one as the result of the filtering algorithm.

The pixels in a neighborhood provide information about the brightness trend. This information is important to the filtering process. The brightness trend is also referred to as the "spatial frequency." Images with high spatial frequency content contain large, closely spaced changes in pixel values. For example, on a black and white checkerboard, the smaller the squares, the higher the frequency content.

Images with low spatial frequency content (for example, images of clouds) contain large areas of slowly changing pixel values.

Most of the filter options available (with the exception of the Noise filters) use a weighted summation process to determine the value assigned to the pixel of interest. Each pixel in a 3x3 neighborhood is multiplied by a "convolution kernel" having the same dimensions. The resulting sum is assigned to the pixel of interest.

						(K1xP1)+
						(K2xP2)+
						(K3xP3)+
						(K4xP4)+
P1	P2	P3		K1	K2	K3	(K5xP5)+
P4	P5	P6	X	K4	K5	K6	(K6xP6)+
P7	P8	P9		K7	K8	K9	(K7xP7)+
							(K8xP8)+
3x3 pixel neighborhood				convolution kernel			(K9xP9)
(P5 is being calculated)							**New Value for P5**

Each element of the convolution kernel is a weighting factor, also called a "convolution coefficient." The size and arrangement of these weighting factors determine the type of transformation the image will undergo. Changing a weighting factor influences the overall sum and, therefore, affects the value given to the pixel of interest.

4.7.2 Sharpening Filters

These filters can increase image sharpness and provide edge enhancement. However image noise may be enhanced as well. These filters accentuate the high-frequency details of an image while leaving the low-frequency content intact. High frequency portions of the image get brighter while low frequency portions become black.

Sharpen level 9 (high) has the largest effect on the image. Sharpen level 5 has an intermediate effect. Sharpen 1 (low) has the most subtle effect on the image. 9 different sharpening levels are available for optimization of the image.

4.7.3 Noise Filters

This filtering process uses the values of the pixels contained in the area surrounding a pixel to determine the new value given to the pixel of interest. The noise filter sorts the pixels in the neighborhood into ascending order and picks the middle or median pixel value as the new value for the pixel of interest. 3 levels of noise reduction are available.

4.7.4 Despeckle Filters

The despeckle filter is a type of smoothing filter based on data rejection. Pixels in the neighborhood (usually the adjacent pixels) are used as a data set upon which the average and standard deviation of the set are calculated. If the pixel of interest (the center of the neighborhood) is different from the neighborhood average (either greater or lesser) by a threshold (a multiple of the standard deviation) it is replaced by the average value.

The effect of the filter is to "smooth" pixels that are much different from their neighbors. Artifacts such as hot pixels, cosmic rays etc. are commonly rejected by this type of filter. The filter strength is controlled by the threshold factor. For large factors very little data is rejected as only very large deviations are required for rejection, where as, low thresholds result in more smoothing. (For example, setting #1 results in outliers of 1 standard deviation greater or lesser than the neighborhood average to be corrected for, setting #2 results in outliers of 2.5 standard deviations to be corrected for and setting #3 results in outliers of 5 standard deviations to be corrected for.)

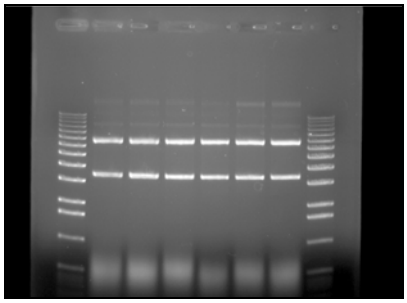
These filters are particularly effective in eliminating random noise contained in an image and produce less blurring than the Noise filter described above.

4.7.5 3-D (Contour) Filters

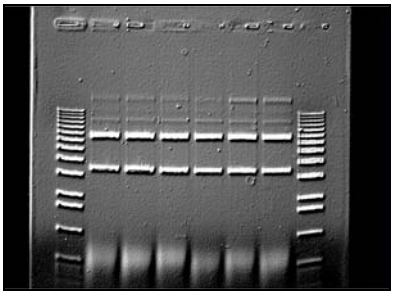
These filters are particularly useful for visualizing faint bands. They produce a "3-D" effect, defining edges and making details easier to see.

3-D level 9 (high) has the largest effect on the image. 3-D level 5 has an intermediate effect 3-D level 1 (low) has the least effect on the image. 9 different 3-D levels are available for optimization of the image.

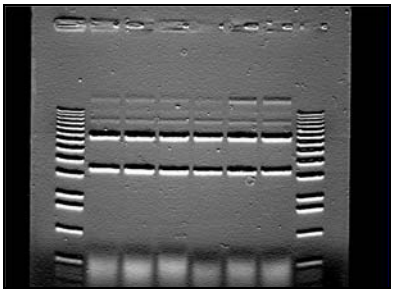
In addition, 3-D allows the user to control the 'direction' of the 3-D shadowing effect. Once the level is refined, just click on a direction arrow to visualize the shadowing adjustment. The default shadow direction is in the lower right hand corner direction and 9 different directions are available.



Original Image



3-D Image Level 5 & lower right shadow



3-D Image Level 5 & upper middle shadow



Original Filter settings



Lower right shadow



Upper middle shadow

4.7.6 Smoothing Filters

These filters are very useful for reducing the visual noise present in an image. When a smoothing filter is applied to an image, rapid changes in intensity are averaged out with the remaining pixels in the neighborhood, thereby decreasing the high frequency content. The visual result is a slight smoothing of the image because sharp pixel transitions are averaged with their surroundings.

Smooth High has the largest effect on the image. Smooth Med has an intermediate effect. Smooth Low has the most subtle effect on the image.

4.7.7 Edge Filters

These filters use Laplacian enhancement to highlight edges, regardless of direction. All edge-enhancement operations attenuate the low frequencies of the image. Regions of constant intensity or linearly increasing intensity become black as a result of these transformations, and regions of rapidly changing intensity values are highlighted.

Note: The White level may need to be adjusted after using the Edge filters in order to see the result of this filtering process.

4.7.8 Horizontal Edge Filter

This filter brightens horizontal edges. This can be useful in pinpointing bands on a gel. The horizontal edge filter (Horz. Edge) enhances image edges by shifting an image vertically by one pixel and then subtracting the shifted image from the original. In an area of constant pixel intensity, the subtraction yields black pixel values. At an edge,

which is an area with large changes in intensity, the subtraction yields light-colored pixel values. The larger the difference in intensities, the lighter the resultant pixels.

Note: After applying the horizontal edge filter the entire image may appear black, and might require reducing the White level in order to better visualize the results.

4.7.9 Vertical Edge Filter

This filter (Vert. Edge) brightens vertical edges using the approach described for the horizontal edge filter (see above), except that the image is shifted horizontally before the shifted image is subtracted from the original. In this case, the vertical edges produce light-colored pixel values. As in the horizontal edge filter, it may be necessary to adjust the White level in order to better visualize the results of this filtering process.

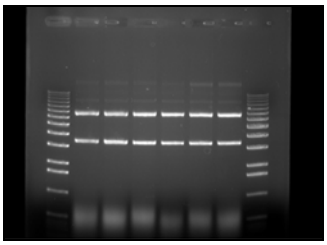
4.7.10 Custom Filter

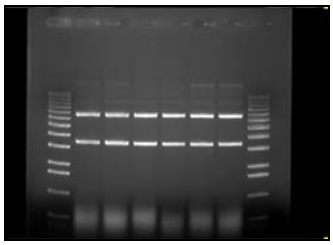
This function also allows the user to customize filters to his/her own specifications. Using the weighting factors of the other filters as a frame of reference, experiment with new weighting factor values.

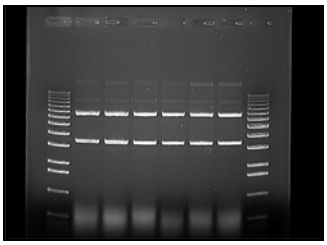
4.7.11 The UNDO Button

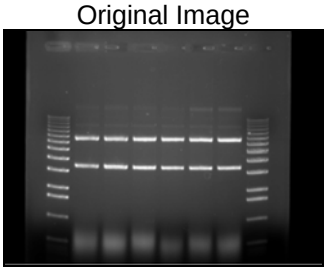
The upper right button is labeled UNDO. This reverses the last filtering process applied to an image. To revert the image to its original state after multiple filters have been applied, press the RESET button on the main interface.

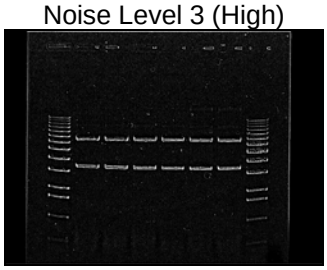
4.7.12 Examples of Filter Results

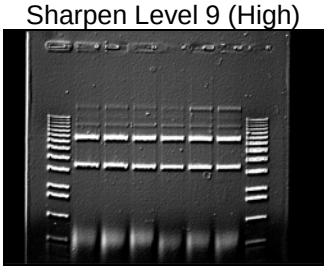

Original Image

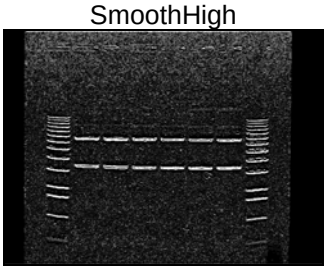

Noise Level 3 (High)

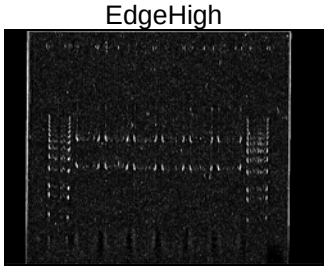

Sharpen Level 9 (High)


SmoothHigh


EdgeHigh

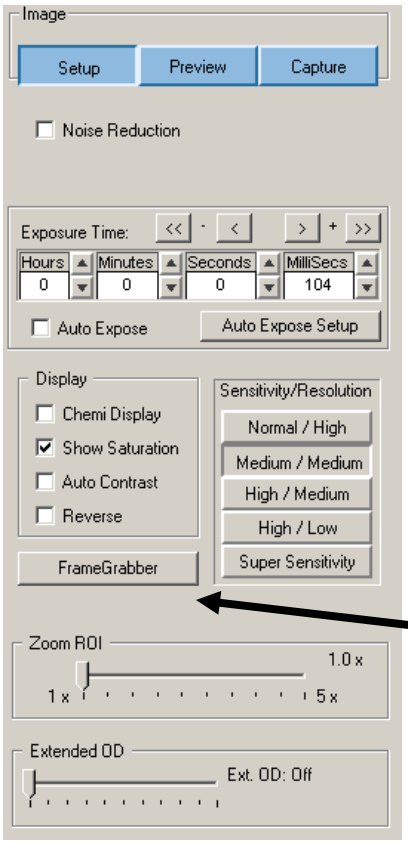

3-D (Contour) Level 5

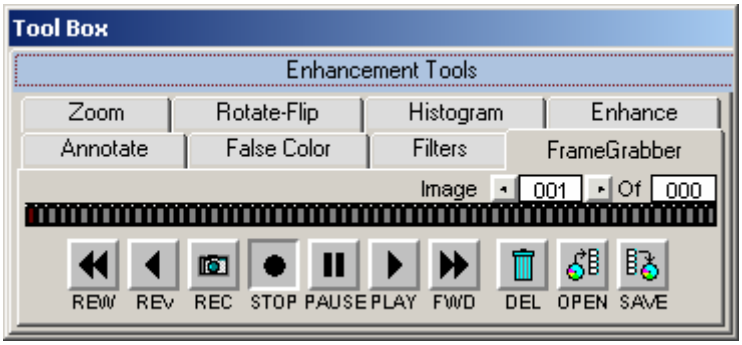

Horizontal Edge


Vertical Edge

4.8 FrameGrabber Mode

If kinetic, multiplex, color, or chemiluminescence experiments are desired where you wish to have the system automatically capture several images at preset exposure times, preset time delay between images, preset lighting sources, and preset filter choices, the FrameGrabber tab can be clicked in Tool Box. (FrameGrabber is also accessible in the camera setup acquisition screen. To access this screen, select the acquire button on the tool bar and then select FrameGrabber on the acquisition screen. ImageQuant Capture stand alone software does not include FrameGrabber acquisition tools)





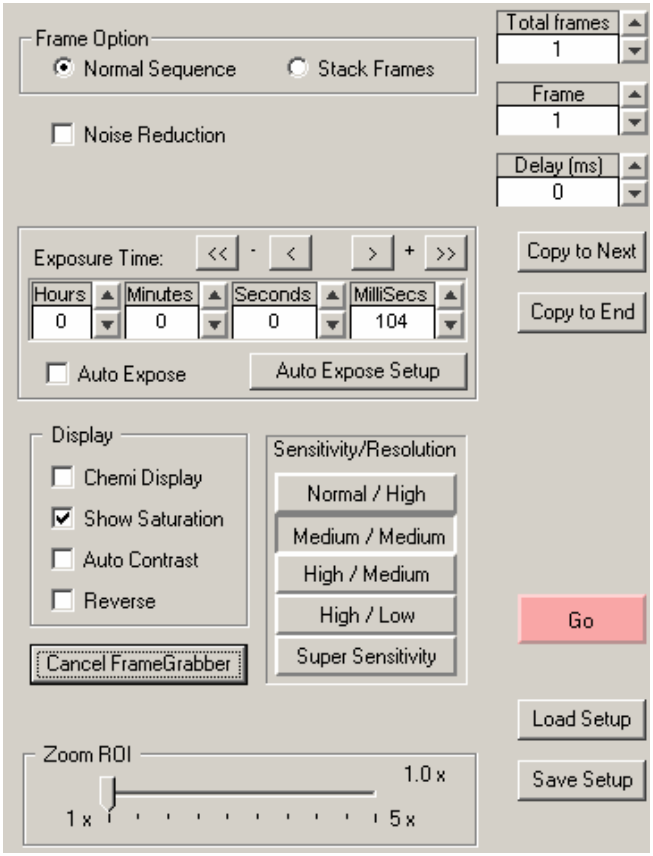
FrameGrabber Setup in the Tool Box Enhancement Tools

FrameGrabber button in the camera setup acquisition screen

Once **FRAMEGRABBER** is selected, a display box will appear for independent control of all lighting, filters, and exposure delay for each image frame.

The **TOTAL FRAMES** setup provides you with the ability to determine how many individual frames (images) you want for the sequence. There are a maximum of 50 frames (images) and a minimum of 1 frame that can be captured with each sequence.

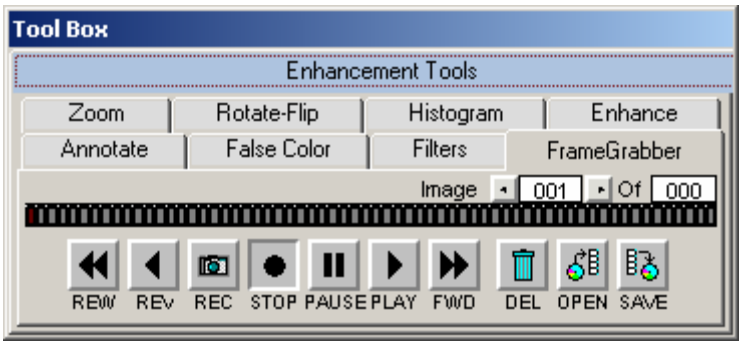
The **FRAME** selection is used for setting up the conditions for each frame (image). For example, if three (3) images are to be captured, you would choose FRAME 1 and setup all of the desired lighting and filter requirements. You can then click on FRAME 2 and repeat the above. Or, you can click on **COPY TO NEXT** to help speed up the setup process. **COPY TO NEXT** copies all settings from the previous frame to the current frame.



Usually, for chemiluminescence imaging, all lighting is off and the filter wheel is positioned for the chemiluminescence position for all frames. Thus, the only variable that is changing from one frame to the next is the exposure time. In this situation, **COPY TO NEXT** is a useful tool to save time in the setup process.

If you are performing kinetic experiments where you want to have a predetermined delay between captured images, then you can use **EXP DELAY** to configure this function. The default **EXP DELAY** is set for the shortest possible delay (19 milliseconds), but can be configured up to 50 minutes between each image. Also, if your exposure delay and/or exposure time and/or lighting options/filter position are consistent for the entire sequence, then once you set up the first frame, you can select the **COPY TO END** selection to automatically choose the first frame settings for the entire sequence of frames (images).

Once the sequence is set up to the desired configuration, click on the **GO** button. The software will then begin the image acquisition process for each frame of the image. When capture is complete, the FrameGrabber box will disappear and the TOOL BOX will automatically display the FRAMEGRABBER tools. This will allow you to play back the sequence, save or load the sequence, or record a new sequence.



Once all images have been captured, the above FrameGrabber display box will become displayed. The remaining buttons will perform the following tasks:

REC	Opens the Camera Setup and Preview screen to record a sequence.
PLAY	Display a continuous loop of all of the captured images.
STOP	Stop the sequence at the current frame display
PAUSE	Pause the playback of the sequence at a user defined image
REW (rewind)	Rewind the sequence to the first image
REV (reverse)	Play the sequence in a continuous loop in reverse
FWD (forward)	Forward the sequence to the last image
DEL (delete)	Delete the sequence on the display. THIS FUNCTION WILL NOT DELETE A SEQUENCE SAVED TO THE HARD DRIVE.
LOAD	Load a previously saved sequence. THIS FUNCTION WILL NOT LOAD INDIVIDUAL IMAGES PREVIOUSLY CAPTURED IN NORMAL CAPTURE MODE.
SAVE	Save a sequence of images.

4.8.1 Saving an Individual Image from a Sequence

After you load, play, and stop a sequence at the desired image, it is possible to save the individual image seen on the screen. Use the SAVE AS button located on the tool bar to save the image in the desired location and file format on the local or network drives.



Note: This will save the current image only. To save the entire sequence, select the 'save' icon on the FrameGrabber tab in the tool box.

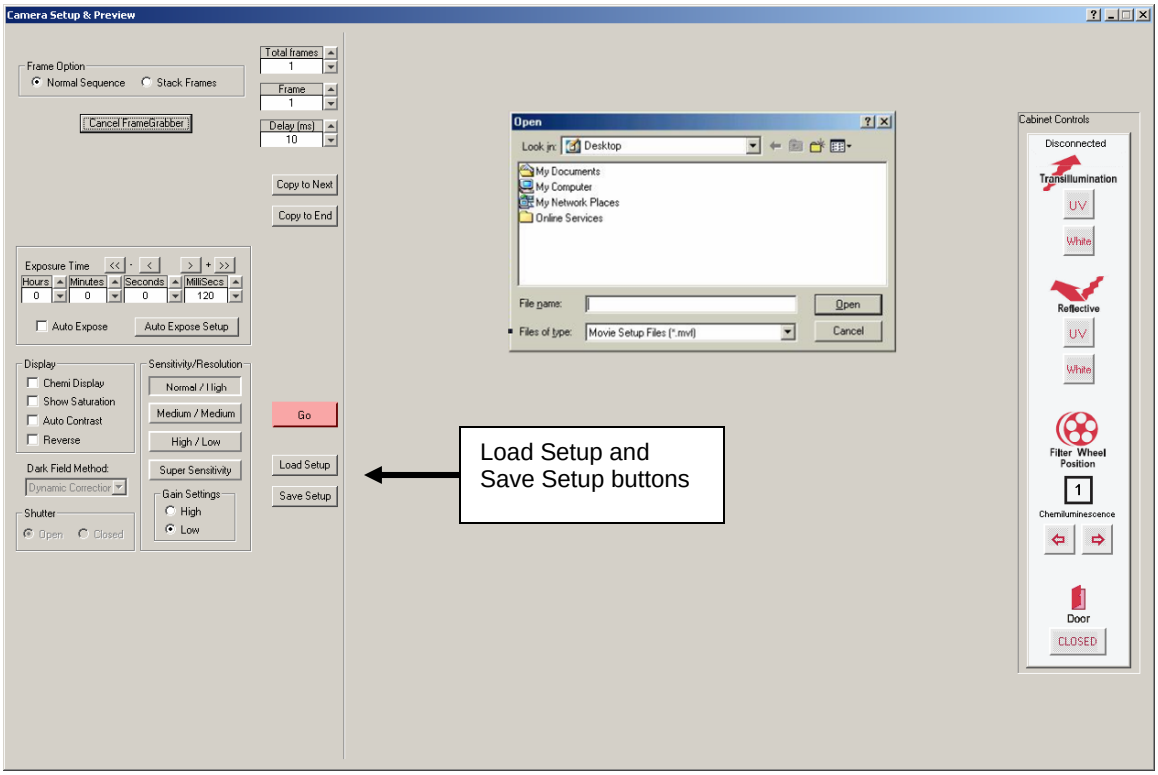
4.8.2 Loading/Saving an Entire Sequence



You can load or save an entire sequence by using the load and save icons in the FrameGrabber tab in the tool box. When a sequence is loaded the loaded frames will be seen as blue in the frame reel. When a sequence is saved a dialog box will appear prompting you to select the location and name for the sequence that you would like to save the images as.

4.9 FrameGrabber: Save/Load FrameGrabber setup routines

Two buttons “Save Setup” and “Load Setup” allow you to save and load all FrameGrabber setup parameters. Files are saved as *.mvf files.



4.9.1 Frame Stacking

At the top of the FrameGrabber setup window is an option for stacking frames. If this selection was chosen during acquisition of the image Stack Frames will use all previous exposure information to sequentially add images to one another. Normal Sequence will not perform this addition. Please note that stacking frames will increase the noise level in acquired images.

Sample case:

Capture 5 frames at 1-5 sec exposure for total time elapsed 15 sec

Display after summation of following frames:

Frame 1 (1 sec)
Frame 2 (1 + 2 sec)
Frame 3 (1 + 2 + 3 sec)
Frame 4 (1 + 2 + 3 + 4 sec)
Frame 5 (1 + 2 + 3 + 4 + 5 sec)

4.9.2 Capturing a Color Image Using the FrameGrabber Function

A color image can be generated by acquiring three images each taken with a red, green and blue emission filter. Once saved, these images are then combined in the Overlay pull-down menu. Open the image captured with each of the three filters as instructed and a RGB (red, green, blue) true color image will be generated.

For color imaging, you will need to use the following *optional* filters:

Red Filter	SYPRO® Red Filter
Blue Filter	Hoechst Blue Filter
Green Filter	SYBR® Green Filter

Chapter 5: Analyzing Images

Once you have acquired an image using ImageQuant Capture, you can transfer it to one of ImageQuant TL's analysis modules using the toolbar options. The ImageQuant Capture toolbar contains 4 buttons: 1D, Array, Colony and Toolbox:



1D
This is a 1D electrophoresis gel or TLC plate analysis module.

Array
The Array module to analyze microplate images, gridded arrays, and dot and slot blot images.

Colony
The Colony Counter module can be used to analyze images by detecting and measuring image features, 2D spots, or colonies. You can display and print measurement data from the detected colonies.

Toolbox
The Toolbox module allows you to perform analysis on a wide variety of images. The flexible nature of this module means that you are not restricted to a single type of analysis when it is used.

If the acquired image has been saved, the selected module is immediately accessed when the appropriate button is selected. If the image hasn't been saved, there will be a prompt to save it before the image is transferred from ImageQuant Capture to the appropriate ImageQuant TL module.

Appendix A: Opening ImageQuant Capture Files in Other Software Programs

Files generated by ImageQuant have been tested in the software packages below. For successful imports, the command line is given.

Programs for the Macintosh® operating system were tested on a PowerMac® 8100/100AV. Results may vary for different software versions and/or hardware configurations.

	.TIF	.BMP	.GIF	.MAC	.PCX
Adobe Photoshop 2.51 LE (Mac)	Open	no	Open	Open As/TIFF	no
Adobe Photoshop 3.0 (Mac)	no	Open	Open		
Canvas 3.5 (Mac)	use ResEdit*	no	no	use ResEdit*	no
Microsoft Word 6.0 (Mac)	Insert Picture	Insert Picture	no	Insert Picture	no
Microsoft Word 6.0a (Win)	Insert Picture	Insert Picture	no	Insert Picture	Insert Picture
Microsoft Excel 5.0 (Mac)	no	no	no	no	no
Microsoft Excel 5.0c (Win)	Insert Picture	Insert Picture	no	Insert Picture	Insert Picture
NIH Image 1.59 (Mac)	Open	no	no	Open	no

*For instructions on using ResEdit™, see next page.

Additional packages, such as Claris Works and PowerPoint have also been tested. For these systems, it is necessary to save the file with a ".TIFF" extension in order for them to recognize the file as a TIF format.

Using ResEdit

1. Save image as a TIFF or MACTIFF

2. Obtain a copy of the freeware ResEdit by downloading from Apple Computers through the Internet.

3. Open ResEdit. An animated startup display will show up and continue until you click on the mouse or any key.

4. A dialog box will appear. Open the TIFF image. Another dialog box will appear asking if you want to add a resource fork, click on 'OK.'

5. Next go to the File pull-down menu and click on Get Info for This File.

6. In the File Info window, change the Type to TIFF (instead of TEXT), and the Creator to DAD2 (instead of DOSA). (Must be typed in all CAPS as shown here). Close the window and save changes. Quit ResEdit.

7. After this procedure, the icon will change to a TIFF icon and the file may be opened in Canvas.

Appendix B: Security Features

This feature should only be used by the purchaser or supervisor of the system. Remove this page from the manual and store it in a safe place. When the system is shared by a number of users, security features may be useful for regulating or maintaining a log of instrument use.

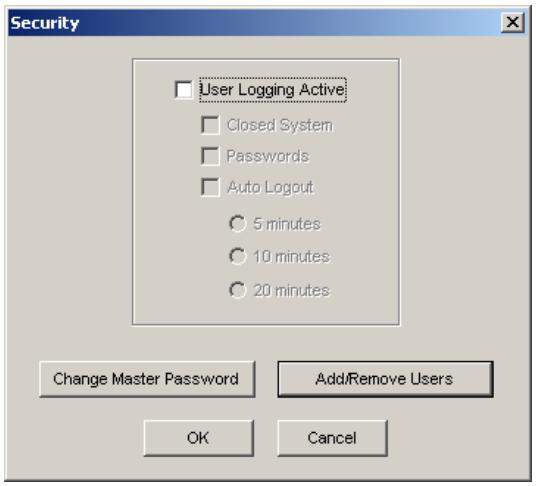
To Change the Security Setup

When you initially receive the system, all security features are inactive and the user name and password to access security settings are both set to "MASTER". To change the security setup, select the Setup pull-down menu and click on Security. A dialog box will appear asking for the system password:



Security Dialog Box

Enter the user name and password, and then click on the Login button. A new dialog box will appear allowing you to configure your security preferences. By clicking on the options in this dialog box, it's possible for a supervisor to change the system password, set the security mode, add and delete names from the authorized list, and activate the Auto Logout feature. When the desired options have been selected, click the OK button. Follow this procedure any time changes to the security mode are necessary.



Security Features Dialog Box

NOTE: You must exit and re-launch ImageQuant Capture in order for any changes to take place.

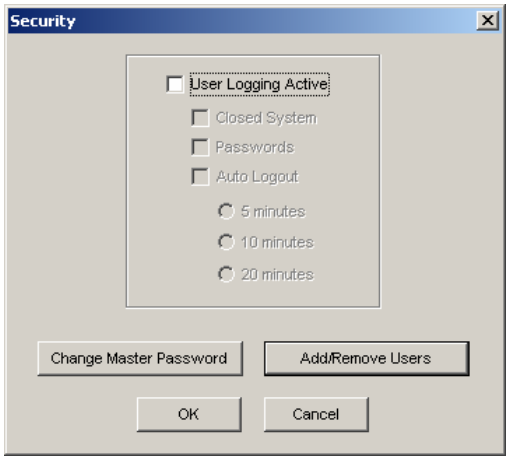
If you decide not to edit the security settings at this time, simply click on the EXIT button to dismiss the dialog box.

Setting Various Security Levels

A variety of security levels are available. Each is described below, in increasing order of security. Also shown are the proper settings in the dialog box to choose each security level.

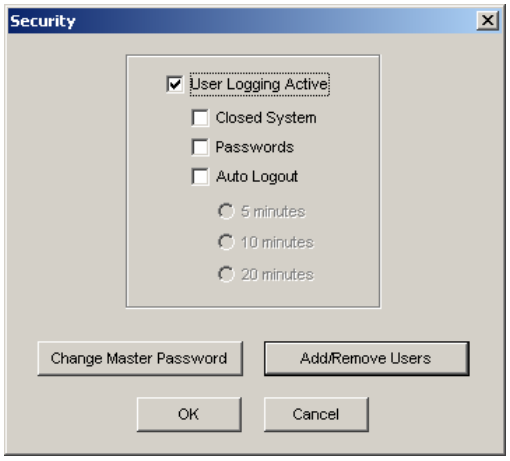
Option 1: No Security Functions

No log in or log out is required and anyone can use the system:



Option 2: Open System with User Name Log In and Out

This security level keeps the system open but requires users to log into the system by typing a name. In this mode, a list of users with their log in and log out times is generated.

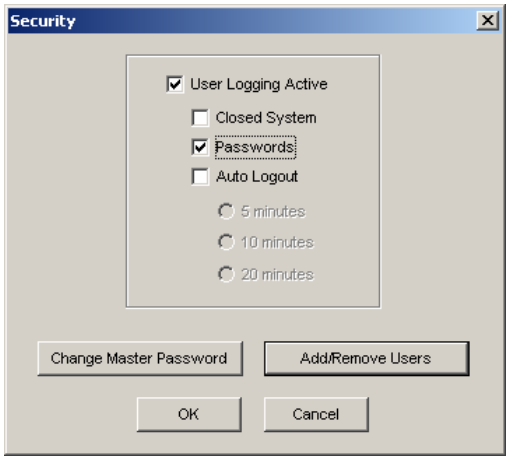


Each time a new name is used, it is added to the master user list. Since this list requires a password (in case the system is later changed to a closed, password-protected system), ImageQuant Capture will automatically assign the user's name (in uppercase) as the password, and the password field is disabled.

When finished using ImageQuant, the user can choose Logoff from the File menu, or can log out by exiting the system. Otherwise, he/she will remain logged in (unless Auto Logout is active).

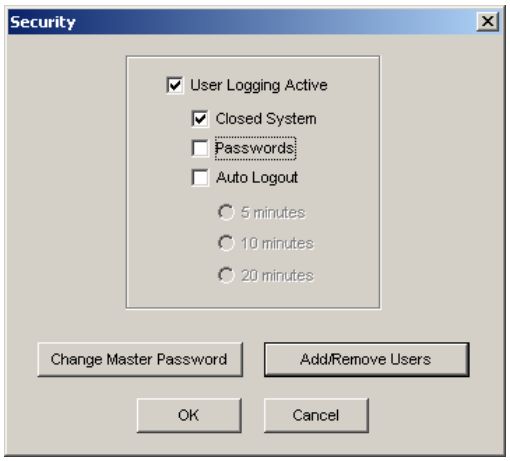
Option 3: Open System with User Name and Password Log In and Out

This security level keeps the system open but requires users to log into the system by typing their names and passwords. When someone enters a name and assigns a password to that name, the same password is needed whenever that name is used to log in. Only one password can be assigned to a name. A list of users with their log in and log out times is generated.



Option 4: Closed System with User Name Log In and Out

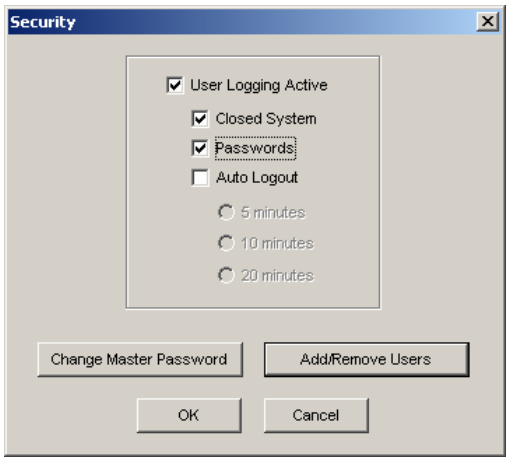
A user can log in only by entering an authorized name. A user log is generated.



In closed systems, the system supervisor may want to add or remove names from a list of authorized users. To do this, click the Add/Remove Users button. A dialog box will appear with a list of all the current users.

Option 5: Closed System with User Name and Password Log In and Out

The system only accepts authorized names and their passwords at log in. The system supervisor can add or remove names from a list of authorized users as described above. Only one password can be assigned to each name. A user log is generated.



Note: passwords are case sensitive (The login dialog defaults to uppercase but the user can override this by pressing the shift key while typing.)