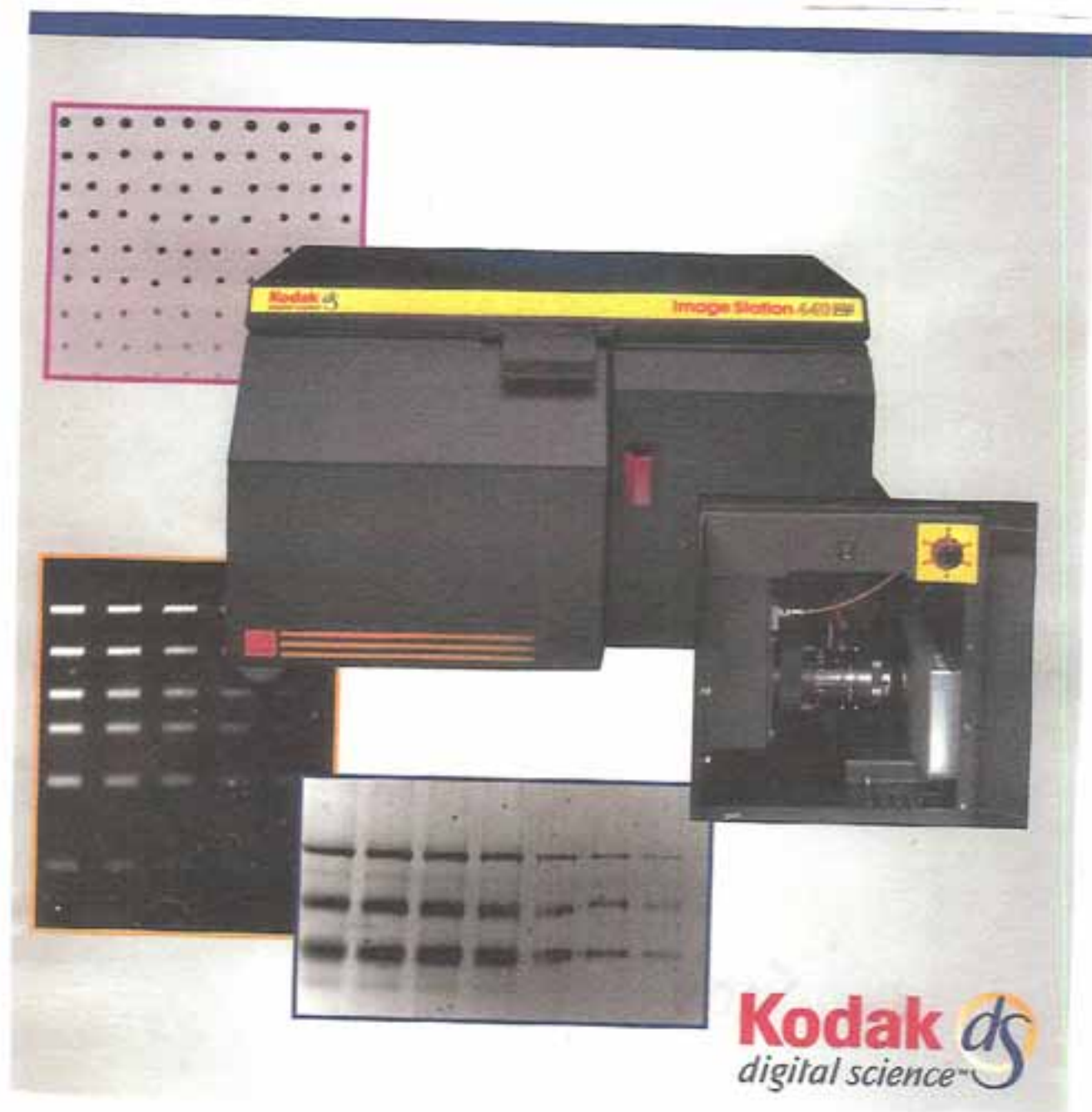


# Kodak Digital Science™ Image Station 440CF



***Kodak Digital Science***  
**Image Station 440CF**

**Windows User's Manual**

Copyright Eastman Kodak Company, 1998. All rights reserved.

Products are for research use only. They are not suitable for human or animal diagnostic or therapeutic use.

This document contains proprietary information of Eastman Kodak Company and its licensors. It is their exclusive property. It may not be reproduced or transmitted, in whole or in part, without a written agreement from Eastman Kodak Company. No patent license or other license is granted in this information.

The software described in this document is furnished under a license agreement. The software may not be used or copied except as provided in the license agreement.

Information in this document is subject to change without notice. Information concerning products not manufactured or distributed by Eastman Kodak Company is provided without warranty or representation of any kind, and Eastman Kodak Company will not be liable for any damages resulting from the use of such information.

Coomassie is a registered trademark of Imperial Chemical Industries, LTD.

Goo Gone is a trademark of Magic American Corporation.

Kodak, Kodak Digital Science, X-Omat, BioMax, COP1 and the *ds* monogram symbols are trademarks of Eastman Kodak Company.

Macintosh is a registered trademark of Apple Computer, Inc.

NEN is a trademark of NEN Life Science Products, Inc.

SYPRO and SYBR are registered trademarks and Oregon Red is a trademark of Molecular Probes, Inc.

Windows and Windows 95 are trademarks of the Microsoft Corporation.

# Image Station 440cf

## Contents

<b>CHAPTER 1:</b>	<b>INTRODUCTION .....</b>	<b>1-1</b>
	About the User's Manual .....	1-2
	Navigating through the User's Manual .....	1-3
	What's in the Kodak Digital Science IS440cf Package .....	1-4
	Registering Your IS440cf System .....	1-5
	Obtaining Technical Support .....	1-6
<b>CHAPTER 2:</b>	<b>SYSTEM OVERVIEW .....</b>	<b>2-1</b>
	Getting Familiar with the IS440cf .....	2-2
	Sample Work Area .....	2-4
	Closed Optical Path Image Chamber .....	2-6
	Capture System Chamber .....	2-8
	IS440cf Acquire Window .....	2-11
	Setting the Acquire Software and Camera Preferences .....	2-15
<b>CHAPTER 3:</b>	<b>CAPTURING AN IMAGE .....</b>	<b>3-1</b>
	Sample Illumination/Detection Guidelines .....	3-2
	Preparing for Image Capture .....	3-6
	Capturing the Image .....	3-13
	Viewing the Image .....	3-20
	Submitting the Image .....	3-22
	Printing an Image .....	3-27



# Image Station 440cf

## Contents

<b>CHAPTER 4:</b>	<b>MAINTAINING THE SYSTEM.....</b>	<b>4-1</b>
	Regular Maintenance .....	4-2
	Changing a Filter .....	4-3
	Changing a Lid Gasket.....	4-5
<b>CHAPTER 5:</b>	<b>TROUBLESHOOTING THE SYSTEM .....</b>	<b>5-1</b>
	Contacting Technical Services .....	5-2
	Common Instrument Questions .....	5-3
	Common Image Questions .....	5-4
	Common Software Questions .....	5-7
<b>CHAPTER 6:</b>	<b>INSTALLING THE SYSTEM .....</b>	<b>6-1</b>
	Temperature & Humidity Requirements .....	6-2
	Electrical Requirements .....	6-2
	Space Requirements .....	6-3
	Installing the IS440cf.....	6-4
	Repacking the IS440cf .....	6-11
<b>CHAPTER 7</b>	<b>WARRANTY &amp; REGULATORY INFORMATION .....</b>	<b>7-1</b>
	IS440cf Limited Warranty .....	7-2
	Software License Agreement .....	7-4
	Regulatory Information .....	7-8

# Image Station 440cf

## Contents

<b>APPENDIX A: DIGITAL IMAGING CONCEPTS .....</b>	<b>A-1</b>
Image Resolution .....	A-2
Spatial Resolution .....	A-4
Dynamic Range .....	A-6
Noise .....	A-7
Binning Pixels .....	A-9
The Image Histogram .....	A-10
<b>APPENDIX B: UV ILLUMINATION .....</b>	<b>B-1</b>
IS440cf UV Illumination .....	B-2
<b>APPENDIX C: IMAGE CORRECTION .....</b>	<b>C-1</b>
IS440cf Image Correction .....	C-2
<b>APPENDIX D: RELATED PRODUCTS .....</b>	<b>D-1</b>
<b>GLOSSARY .....</b>	<b>G-1</b>





# CHAPTER 1: INTRODUCTION

Thank you for purchasing the *Kodak Digital Science™* Image Station 440cr (IS440cr) system. We know you will be pleased with this multi-purpose imaging system which provides sensitive and quantitative imaging of chemiluminescence, fluorescence and chromogenic gels and blots. The IS440cr is as sensitive as autoradiography films for most chemiluminescence applications and detects fluorescent signals at picomole to femtomole levels on gels and blots. The affordable system combines a thermoelectrically cooled Charged-Coupled Device (CCD) camera, an innovative Closed Optical Path Image (COPi™) style chamber, and the powerful *Kodak Digital Science 1D Image Analysis Software* to provide a sensitive and quantitative benchtop imaging solution for your laboratory.

The cooled CCD camera, combined with the 6X zoom lens, captures 12-bit images that can be accumulated to 16-bit files at a pixel density of 752 x 582. This generates an image with a resolution of 58 microns/pixel at full zoom. The IS440cr Image Acquire Software, within 1D Image Analysis Software accumulates and displays multiple image captures so you can view and optimize the quality of your image. The chamber provides a light tight environment, so there is no need to use a darkroom.



## About the User's Manual

The *Kodak Digital Science Image Station 440CF (IS440CF) User's Manual* provides you with all of the information you need to capture images. It is designed to be used in conjunction with the *Kodak Digital Science 1D Image Analysis Software User's Manual*.

Additional computer equipment manuals may also be included in your IS440CF system. These manuals provide information from the manufacturer regarding the computer that is supplied with your IS440CF system. Use these manuals for detailed information about your new computer.

### Conventions

This User's Manual uses the following conventions:

- Menu commands, tool names, and window names are capitalized.
- Tips, Examples and Notes appear in the text like this:

---

**NOTE:** Kodak Scientific Imaging Systems provides maximum performance products for scientific imaging.

---

- Safety Warnings



- This symbol is used in the manual to designate a warning or caution statement.



- This symbol is used in the manual to designate where electrical shock is possible.



---

**WARNING:** The ImageStation provides protection from electrical shock and UV light exposure when operated according to this manual. If the instrument is not used according to these instructions, the protection provided by the instrument may be impaired.

---

## Navigating through the User's Manual

The *Kodak Digital Science IS440cf User's Manual* is divided into the chapters listed below. In each chapter, you will find helpful hints and troubleshooting information.

- Chapter 1: *Introduction* gets you started by providing you with information on what's in your package, how to use your manual and how to get help.
- Chapter 2: *System Overview* details the instrument and software related to the IS440cf.
- Chapter 3: *Capturing an Image* describes how to use the IS440cf to capture and optimize images.
- Chapter 4: *Maintaining the System* provides guidelines for the care and maintenance of your system.
- Chapter 5: *Troubleshooting the System* provides an outline for solving problems you may encounter.
- Chapter 6: *Installing the System* walks through the installation process of your IS440cf.
- Chapter 7: *Warranty & Regulatory Information* provides detailed information on the IS440cf warranty, software licenses and regulatory information.
- Appendix A: *Digital Imaging Concepts* discusses the basics of resolution and histograms and how they apply to the IS440cf.
- Appendix B: *UV Illumination* describes the specifications of the illumination used in the IS440cf.
- Appendix C: *Image Correction* reviews the details of CCD, lens and illumination corrections relating to the IS440cf.
- Appendix D: *Related Products* gives you information on Kodak products related to IS440cf.
- *Glossary* defines terms that are used in the manual.

## What's in the Kodak Digital Science IS440cf Package

Prior to using your new system, please take a moment to ensure that all necessary parts have been received.

**NOTE:** Computer components are shipped separately.

### ***Kodak Digital Science Image Station 440cf***

- IS440cf Chamber and Capture System (1)
- Compression Pad , located inside the instrument lid (1)
- 120 Volt Power Cord (1) or 230 Volt Power Cord (3)
- Camera/Computer Cable (1)
- Light Diffuser (1)
- Test Target (1)
- IS440cf Image Acquire Software Disk (1)
- Calibration Disk (1)
- User's Manual (1)
- Registration Form (1)

### ***Kodak Digital Science 1D Image Analysis Software***

- 1D Image Analysis Software Disks (3)
- User Registration Card (1)
- User's Manual (1)
- Copy Protection Device (1)

## Registering Your IS440cf System

To be eligible for upgrade programs and to receive new product information, you must register your system by completing and returning the enclosed registration cards. For best product support, please take the time to complete and return the enclosed cards.

## Obtaining Technical Support

If you have a problem using the IS440cf system, please refer to the appropriate section of this User's Manual, the 1D Image Analysis Software User's Manual, or utilize the program's on-line help.

If you require additional assistance, contact NEN<sup>TM</sup> Life Science Products Technical Support. NEN is the qualified distribution and support group for the Kodak IS440cf system. Before contacting Technical Support, please have the following information ready:

- Serial number of your IS440cf system.
- Serial number and version number of your 1D Image Analysis Software (Select About 1D under the Help menu while the software is running).
- The make and model of the computer system you are using.
- Operating system software version.
- The type of image you are capturing or analyzing.
- The problem you are having and what you were doing when the problem occurred. Please note the exact wording of any error messages, including any error number displayed.

### NEN Life Science Products Technical Support

#### *World Headquarters*

NEN Life Science Products, Inc.  
549 Albany Street  
Boston, MA 02118-2512 USA  
1-800-551-2121, International FAX 1-617-426-2464  
e-mail: techsupport@nenlifesci.com

#### *European Headquarters*

NEN Life Science Products  
Imperiastraat 8  
B-1930 Zaventem  
Belgium  
32-(0)2-717-7911



***NEN Life Science Products Local Offices***

Argentina 1-816-0820	Italy 167 790310
Australia 1-800-252-265	Japan 3-5820-9408
Austria 01 285 77 85	Korea 2-516-7331
Belgium 080094540	Mexico 5-250-0805
Brazil 11-835-9922	Netherlands 0800 0223042
Canada (English Speaking) 800-677-9912	New Zealand 9-579-3024
Canada (French Speaking) 800-677-8856	Norway 0 23 05 19 30
Chile 2-220-4804	Poland 0 22 779 83 50
China 10-62563836	Portugal 011 303 08 20
Colombia 1214-9804	Puerto Rico 787-721-2128
Denmark 0 32 50 66 10	Singapore/Malaysia 4-457927
Finland 0 22 67 8811	Spain 091 579 0469
France 0800 907762	Sweden 08 625 1270
Germany 0130 810032	Switzerland 0800 555027
Greece 6-49-76-21	Taiwan 2695-9990
Hong Kong 2-793-0330	Thailand 2-274-8331
Hungary 01 3959273	United Kingdom 0800 896046
Iceland 568-8533	United States 800-551-2121
Indonesia 21-723-1018	Venezuela 2-235-2316
	Non-listed European Countries 0032-2-717 79 79





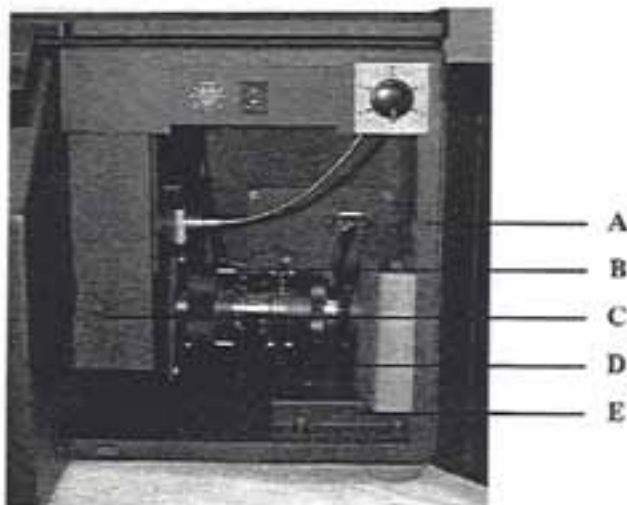
## CHAPTER 2: SYSTEM OVERVIEW

This section provides an overview of the Image Station 440cr system. You will review the principles of operation and get a better understanding of the critical components—the Sample Work Area, the Closed Optical Path Image (COPI) chamber which also contains the UV illumination source, the Capture System and the IS440cr Image Acquire Software.

## Getting Familiar with the IS440cf

### Front View

Front View of the IS440cf



- A** The *Lid* allows access to the work area.
- B** The *Sample Work Area* is 49 x 31 cm and will accommodate imaging of samples up to 25 x 20 cm.
- C** The *Closed Optical Path Image Chamber* isolates the optical path from the outside environment. It contains the 45° mirror which directs the light from the sample to the camera lens. The internal UV light sources are also contained in the COPI chamber.
- D** The *Capture System Chamber* includes the six position Filter Wheel, a 6X zoom lens and a cooled CCD camera.
- E** The *Radiator* dissipates heat from the thermoelectric cooler.

## Rear View

Rear View of the IS440cr



**A** The *Circuit Breaker* protects the unit in the unlikely event that there is an electrical short in the UV light source wiring.

**B** The *Camera Cable Connector* couples the camera to the IS440cr PCI card in the supplied computer with the special Camera/Computer Cable.

**C** The *Power Cord* connector is the receptacle for the power cord which provides electricity needed to power the UV light source.

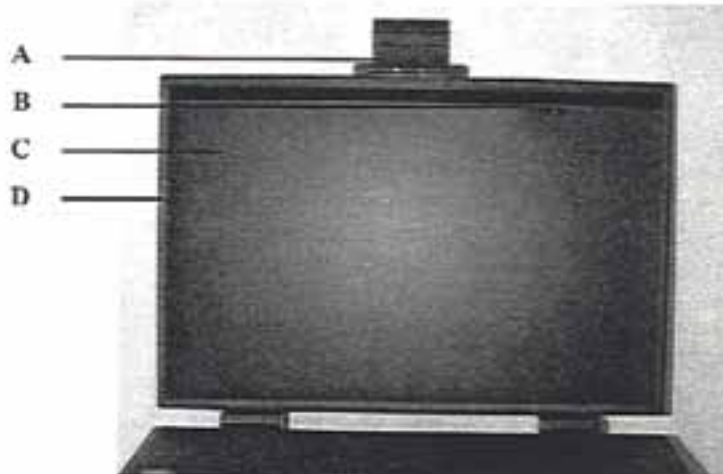
## Sample Work Area

The Sample Work Area is made up of two main components—the Lid and the Platen. Let's review their features.

### Lid

The lid design provides easy access to the Sample Work Area for positioning a sample in Preview Mode. When closed, the lid creates a light-tight environment for capturing fluorescent or luminescent images. When opened, external room light (with the Light Diffuser) provides even illumination for transmission mode imaging.

IS440cr Lid



- A The *Clasp* creates and maintains the light-tight seal for the instrument.
- B The *Compression Pad Clip* holds the *Compression Pad* in the correct position.
- C The *Compression Pad* is a double-sided pad (black side and white side) with a foam interior. The *Compression Pad* holds thin samples flat and in place when the lid is closed. It can be easily removed to accommodate thicker samples.
- D The *Gasket* surrounding the lid provides the light-tight seal for the instrument.

## Platen

The work surface contains a scratch resistant optical grade material which is the imaging window called the Platen. The Platen is framed in a coated steel panel mounted on the unit base. The work area is water-tight and has been designed for easy cleanup and is well suited for both wet and dry samples.



**WARNING:** Metal utensils or tweezers should not be used on the Platen.

IS440cr Platen

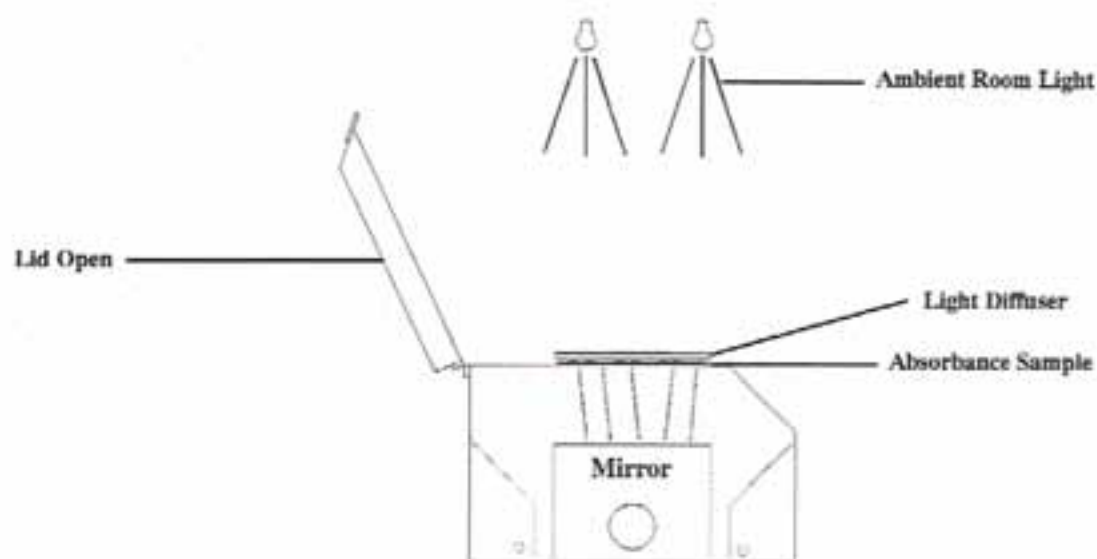


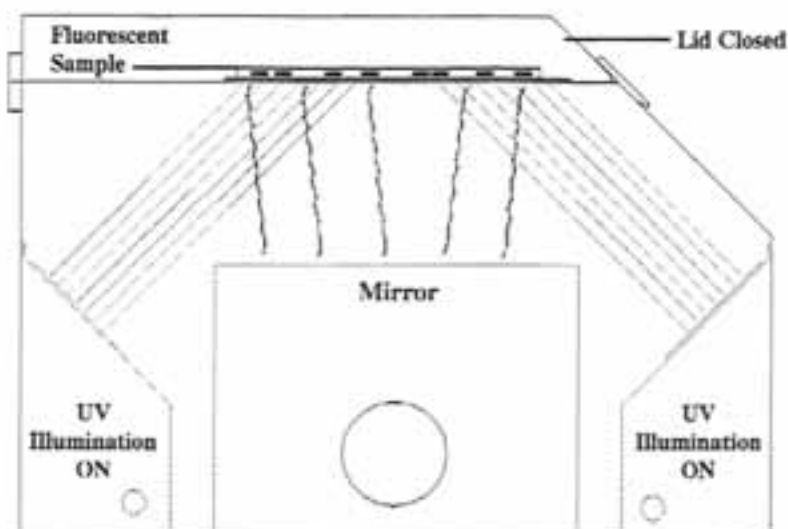
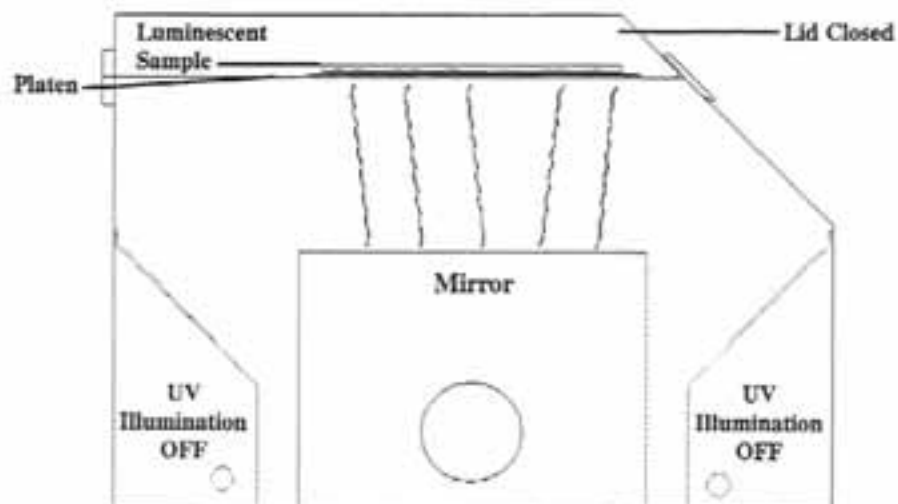
- A The *Platen* is a coated clear plate which accommodates up to a 25 x 20 cm sample for image capture.
- B The *Platen Gasket* lies under the work area and creates the water-tight environment.

## Closed Optical Path Image Chamber

The IS440cr provides three standard forms of illumination including transmission, UV and "no-illumination" modes. Transmission mode utilizes external (room) light (with a Light Diffuser) for absorbance imaging of samples, such as Coomassie gels or autoradiography films. The Light Diffuser, a white plate of translucent acrylic, is placed over the sample to diffuse light uniformly across the sample area. The UV illumination mode utilizes UV bulbs in the illumination chamber to provide 300–400 nm UV excitation of a wide range of fluorochromes. The UV mode can also be used for accurate fluorescence quenching measurements of samples such as TLC or chromogenic blots. The "no-illumination" mode is used for luminescent samples, such as chemiluminescent blots.

### *Schematic of IS440cr in Transmission Mode*



*Schematic of IS440cf in UV illumination Mode**Schematic of IS440cf in "No Illumination" Mode*



## Capture System Chamber

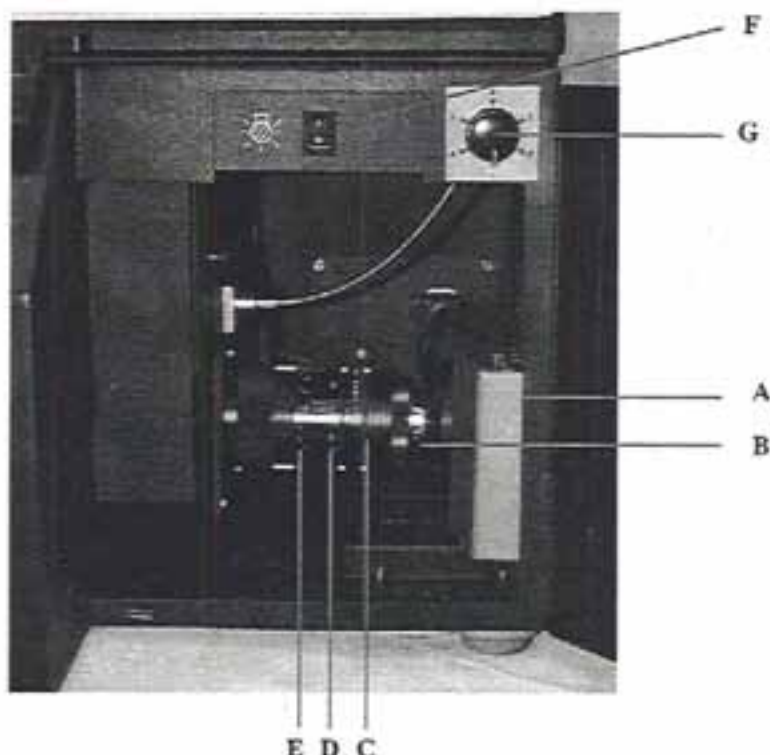
The cooled CCD camera, lens and filter wheel are located in the Capture System Chamber.

### Camera

The IS440cr camera has been custom designed to provide the highest sensitivity for molecular detection. The camera CCD is thermoelectrically cooled to maximize the sensitivity of the camera. The cooled camera collects the image data on a 752 x 582 pixel CCD. Single frame image data is captured at 12-bits with a maximum signal-to-noise ratio of greater than 1,600. The IS440cr Image Acquire Software will also accumulate data from multiple exposures into a 16-bit image. When you capture multiple frames (up to 1 hour per frame), each frame is added to the previous and the result is visualized in the IS440cr Image Acquire Window. In the multiple capture mode, the system provides a maximum linear dynamic range signal-to-noise ratio of greater than 6,000 (3.6 orders of magnitude).

The instrument contains a mirror in the illumination chamber that projects the light from sample into the lens system. The lens system is parfocal at all zoom settings. Therefore once the focus is set, the lens should not need further adjustment. You can manually adjust the focus, zoom or aperture (*f*-stop) settings of the lens. The digital camera has a 6X zoom lens that will capture images from 25 X 20 cm (~350  $\mu\text{m}/\text{pixel}$ ) to 4.2 x 3.3 cm (~58  $\mu\text{m}/\text{pixel}$ ).

## Capture System Chamber

IS440CT Capture  
System Chamber

**A** The *Cooled CCD Camera* is mounted to the base of the instrument. The camera captures 752 x 582 pixel images.

**B** The *Lens* is parfocal ( $f/1.2$ , 12.5 to 75mm). At  $f/1.2$ , the maximum amount of light is passed through the lens.

**C** The *f-stop Ring* regulates the amount of light reaching lens for an exposure.

**D** The *Zoom Ring* adjusts the zoom setting of the lens from 12.5mm to 75mm (6X). The highest zoom level provides a resolution of  $\sim 58 \mu\text{m}/\text{pixel}$ .

**E** The *Focus Ring* adjusts the focus of the lens. Once the lens is in focus (parfocal), no adjustment is required at any of the zoom settings. A mark has been placed on the focus ring to indicate the adjustment at which the Platen surface is in focus.

**F** The *UV On/Off Switch* turns the UV light source On/Off. Always turn off the UV light source when not in use.

**G** The *Filter Selection Dial* changes the filter in front of the lens. The dial should only turn in the clockwise direction.



## Filter Wheel

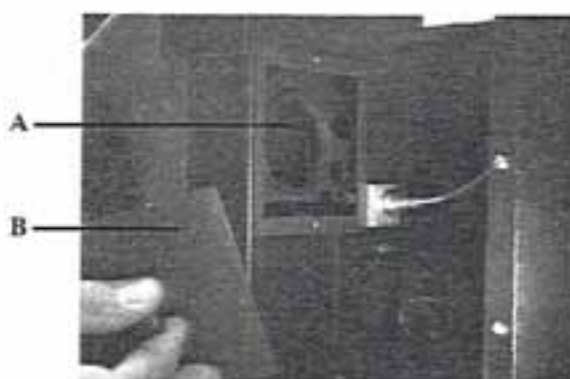
A Filter Wheel is located inside the Capture System Chamber in front of the lens assembly. This wheel has six filter locations, each of which fit a standard 58mm filter. Kodak has provided five filters that are specifically suited for typical imaging needs. However, these filters can be replaced by custom filters if necessary. Position "0" should always be open, without a filter, for use with luminescence and transmission images. To change a filter, remove the Filter Access Panel inside the camera chamber. This light-tight door will give you immediate access to the Filter Wheel.

---

**NOTE:** Only remove the Filter Access Panel when changing a filter.

---

*Filter Wheel and Filter Access Panel*



**A** *Filter Wheel* contains the filters for different detection methods. There are six spaces in the wheel. Position "0" should always be without a filter. The remaining five filters are standard 58mm filters. The filters can be changed if necessary.

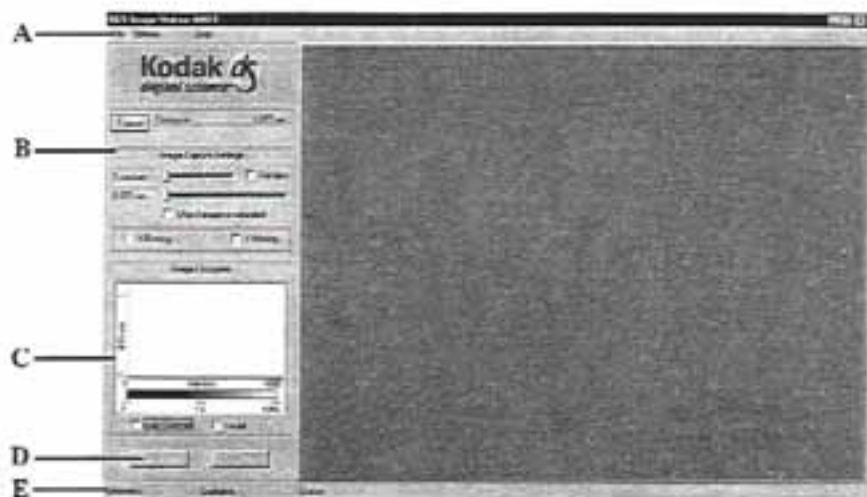
**B** The *Filter Access Panel* provides access to the filter wheel and is light-tight. Remove only the door when changing a filter.

## IS440cf Image Acquire Window

Once you launch the Image Station 440cf Image Acquire Software, the window below appears. Take a few minutes to become acquainted with the window components.

### Overview

IS440cf Image  
Acquire Window



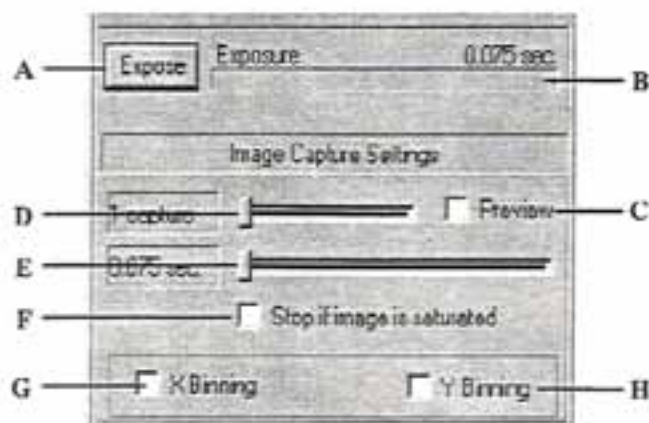
- A** The *Menus* allow you to set preferences, view a full histogram, view the image at a higher magnification or access help.
- B** The *Image Capture Settings* provide selection between Preview and Capture mode and settings for image capture.
- C** The *Image Histogram* displays signal intensity of the image. Use the Histogram to determine exposure time. Use the Contrast Slider or Auto Contrast to adjust the contrast of the image display.
- D** The *Submit and Quick Print Buttons* submit the image to 1D Image Analysis Software and provide a Quick Print dialog box.
- E** The *Status Bar* contains information about the current capture.



## Image Capture Settings

The Image Capture Settings allow you to switch between Preview and Capture modes, set the exposure time, choose single or multiple frame captures and apply pixel binning.

Image Capture Settings  
Available on the IS440cr  
Image Acquire Window



**A** The *Expose/Stop Button* is used to start or stop the camera exposure in Preview or Capture mode.

**B** The *Exposure Progress Indicator* displays the total exposure time and the progress of an exposure.

**C** The *Preview Mode* provides a live-image preview session during which sample formatting and capture adjustments can be accomplished.

**D** The *Capture Slider* sets the number of captures for image accumulation. Maximum is 32 captures. The total number of captures is displayed in the adjacent textbox.

**E** The *Exposure Time Slider* sets the exposure time of a single frame or each frame in a multiple capture. The minimum time is 0.072 seconds and the maximum time is 60 minutes or 3 hours. The total exposure time is displayed in the adjacent textbox.

**F** The *Stop if Image is Saturated* checkbox stops the image scale when the image becomes saturated. This setting is for multiple captures only.

**G** *X Binning* increases the image signal two-fold by adding adjacent pixels in the horizontal direction at the expense of the horizontal resolution.

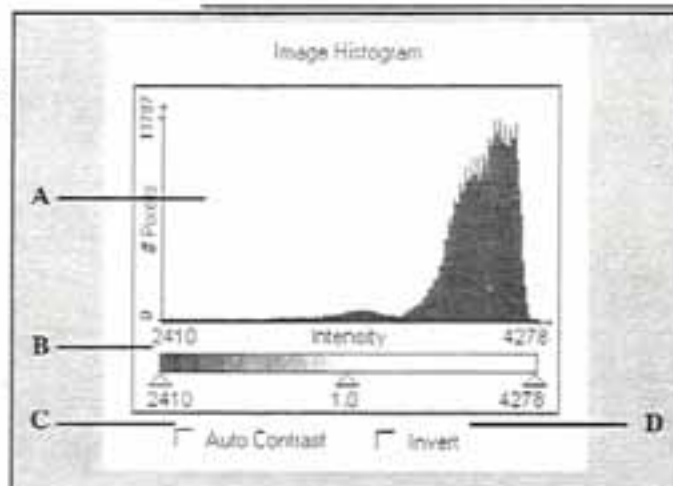
**H** *Y Binning* increases the image signal two-fold by adding adjacent pixels in the vertical direction twofold at the expense of the vertical resolution.

## Image Histogram

The Histogram displays signal intensity of the image. Use the Histogram to determine exposure time and quality of the captured image. Use the Contrast Slider or Auto Contrast to adjust the contrast of the screen image.

**NOTE:** The Contrast Slider and Auto Contrast only adjust the image display. The original image data remains unaltered and is transferred to 1D Image Analysis Software in its original form.

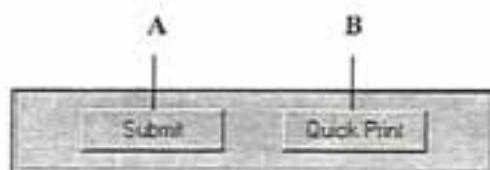
*Image Histogram  
Display and Slider  
Available in the IS440cr  
Image Acquire Window*



- A** The *Image Histogram* displays the range of signal of the image. The histogram can be used to determine the optimal exposure time so the image has the largest possible signal range. The numbers directly below the histogram indicate the range of gray levels for the current image. For multiple capture images, this range can be up to 65,536 signal levels. For single capture images, this range can be up to 4,096 signal levels.
- B** The *Contrast Slider* sets the black and white points and gamma for viewing the image on screen. (i.e. the closer the black and white points the higher the contrast of the image.)
- C** *Auto Contrast* chooses the optimal white and black points and maximizes the on-screen appearance of the image.
- D** The *Invert* checkbox inverts the image from white signals on a black background to black signals on a white background.

## Submit and Quick Print Buttons

*Submit and Preview  
Buttons on the IS440cr  
Image Acquire Window*



- A** The *Submit Button* initiates the transfer of captured data to the 1D Image Analysis Software.
- B** The *Quick Print Button* is used to print the image displayed in the main window. Quick Print allows three print sizes of the image.

## Status Bar

*Status Bar on the  
IS440cr Image  
Acquire Window*



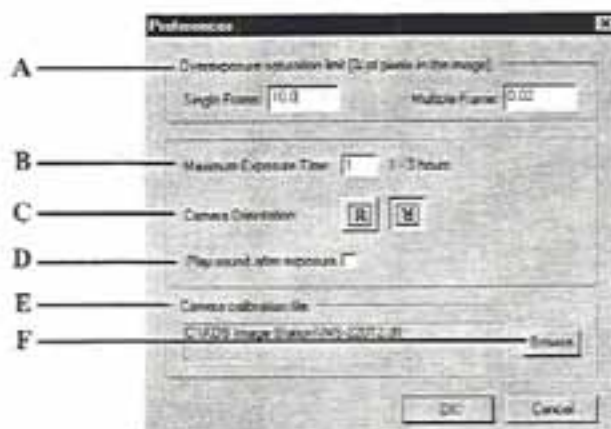
- A** *Exposure* shows the elapsed time for the current image.
- B** *Captures* shows the current number of captures completed.
- C** *Status* shows the current state.



## Setting the Image Acquire Software and Camera Preferences

The Preferences menu in the IS440cr Image Acquire Software allows you to set camera and software parameters including the image saturation level, orientation, maximum exposure time

Preferences Dialog Box



**A** The *Overexposure Saturation Limit (%)* is the percentage of pixels that can become saturated in the image. On the Image Histogram, the image will turn red when the saturation level is reached. The saturation levels for single and multiple frame capture can be set independently using the edit text boxes.

**B** Set the *Maximum Exposure Time* by entering a number up to 3 hour. This value is the maximum exposure time for a single frame capture image. Adjustments made to this text edit box will be reflected in the Exposure Time Slider in the IS440cr Acquire Window.

**C** The *Camera Orientation* selects a normal or mirrored image. This setting controls which orientation the sample placed on the platen will appear in the IS440cr Image Acquire Window.

**D** Check the *Play sound after exposure* box to be audibly notified of the completion of each frame capture.

**E** The *Camera Calibration File* (\*.dft) is specific for each camera and contains information about the CCD's defective pixels. These defective pixels are inherent in every CCD. The \*.dft file compensates and corrects for them. The file is located in the Image Station 440cr folder. The application will ask for the file if it is not in the correct location.

**F** *Browse* allows you to locate the correct \*.dft file. If you can't locate this file, contact technical support.





## CHAPTER 3: CAPTURING AN IMAGE

Now you will step through the procedures for capturing an image including preparing the sample for capture, setting illumination, previewing, capturing, and submitting the image into 1D Image Analysis Software.

## Sample Illumination/Detection Guidelines



The IS440CF captures images for many different applications. When performing experiments, it is important that the appropriate capture conditions are used. The capture parameters relating to three major modes of detection are described below. Later in this chapter, you will find step-by-step procedures for preparing for image capture, setting illumination, previewing, capturing, and submitting the image into 1D Image Analysis Software.

### Luminescence

A luminescent sample needs no additional illumination for capture—it emits light. The amount of light emitted by the sample is directly proportional to the limiting chemical component of the luminescent reaction, usually an enzyme-tagged molecular target. The CCD camera captures the emitted light and represents the signal in the image with very high fidelity (linear response at high precision).

Luminescent samples can vary greatly in signal intensity. Many luminescent signals are dim and may not fill all the signal levels in the Image Histogram in a reasonable period of time. Therefore, luminescent signals may require binning in both the X and Y directions. Generally >100 gray levels is desired, particularly if multiple objects are to be quantitated. In general, the total exposure time for luminescent captures on the IS440CF will be similar to that of autoradiography film. If no significant signal is visible, select an exposure time of up to 1 hour with multiple captures. If no signal is apparent after the first hour of exposure, the IS440CF system is not detecting the signal, verify that your sample is generating signal.

Filters can be used to discriminate and differentially display different kinds of luminescence. However, for maximum sensitivity (usually a priority in luminescence experiments), do not use a filter.

## Fluorescence

A fluorescent sample requires illumination to excite fluorescent molecules (called fluorochromes or fluorophores), which emit light. Internal ultraviolet illumination is provided by the IS440cf. The spectrum of UV illumination is between 300-400nm; for details refer to *Appendix B: UV Illumination* of this manual. The UV illumination is designed to irradiate the sample at a 45° angle (called epi-illumination), and is designed to require that the light interact with the sample in order to eventually enter the lens. Further, the CCD is relatively insensitive to the UV, and it is covered with a filter that blocks infrared radiation that emanates from any lightsource.

Fluorochromes typically emit light in a manner that is proportional to their amount, and the CCD camera captures the fluorescent light and represents the signal in the image with very high fidelity (linear response at high precision).

Fluorescence detection usually generates enough signal within a reasonable period of time to preclude the need for binning. However, binning may be convenient in a particular direction consistent with the spatial resolution of the sample.

For documentation purposes, achieving 500 signal levels using the Image Histogram is desirable. For high quality quantitation, greater than 3,000 signal levels is recommended. In situations where high background fluorescence consumes much of the available gray scale, accumulating images to greater than 60,000 signal levels may be necessary for good quantitation.

Autofluorescence from surrounding materials often presents an interfering background in a fluorescent detection experiment. To diminish the autofluorescent background, a series of filters are provided in the IS440cf Filter Wheel. Selecting one or more filters (multiple images) will optimize the fluorescent signal by discriminating against background and/or will differentiate between multiple fluorochromes within the experimental sample.



## Absorbance

A sample that absorbs light needs illumination to distinguish the absorbing molecules (called chromogens or chromophores). The CCD captures the light that is not absorbed in a highly linear manner. Generally, chromogens that absorb light do so in a manner that is proportional to the negative logarithm of the amount of chromogen. In terms of classical densitometry, this functional dependence is called absorbance or optical density (OD), and is related to the chromogen concentration by an extinction coefficient.

Absorbance can be measured by capturing the light that is transmitted through a sample. Transmission of light through a sample can be accomplished using the IS440cr, simply by opening the lid and permitting external light to be transmitted through a sample. To minimize imaging artifacts, a translucent panel (Light Diffuser) is provided to be placed above the sample on the platen, optimally scattering the incoming light. Because of the high sensitivity of the camera, dim ambient light suffices.

Alternatively, detecting absorbance by reflected light, for samples such as chromogenic blots, may be accomplished using the UV epi-illumination provided by the IS440cr. In the case of a clear sample, white backing of the compression pad may be used to assure light return. This method is an example of fluorescence quenching, which is actually an absorbance methodology.

By using either transmitted or UV illumination, ample signal is provided and short exposure times are expected. Binning is never necessary and exposure times per capture will be approximately 1 second. Increasing the *f*-stop may conveniently extend exposure times. Care should be taken not to saturate the image (histogram turns red). Saturation precludes the appropriate calculation of optical density for absorbance measurements. Utilize the accumulation mode to approach 60,000 signal levels to maximize the range of the optical density.

For improved sample quantitation, it is recommended that filters be used for detection using absorbance methods. For example, a blue dye will present maximum contrast (signal difference) if a red or yellow filter is used.

The following table is a summary of the above, presenting guidelines and helpful suggestions:

**Table 3.1 Sample Illumination/Detection Summary**

Detection Method	Example Applications	Illumination (Lid Position)	Filter	Typical Total Capture Time	Comments
Luminescence	Chemiluminescent Blots	NONE (Closed)	Usually NONE	10 - 1000+ sec., minimal number of captures	Use black side of Compression Pad
Fluorescence	Fluorescent Gels or Blots	UV epi-illumination (Closed)	YES, see Filter Application Table 3-2	10 - 200 sec., multiple reads of 5-20 sec. each	Use black side or no Compression Pad
Absorbance—Transmission	Film Quantitation Stained Gels	External or Ambient Overhead (Open, use Light Diffuser)	Usually YES, see Filter Application Table 3-2	Usually < 1 sec., many captures to fill scale	Use Light Diffuser
Absorbance—Reflectance or Fluorescence Quenching	Chromogenic Blots and Thin-Layer Chromatography	UV epi-illumination (Closed)	YES, usually blue filter	Usually 1-10 sec., many captures to fill scale	Use black side of the Compression Pad with white samples or white side with clear samples



## Preparing for Image Capture

Now you will need to prepare the instrument and sample for image capture.

### Launching the IS440cr Software

A few minutes before image capture, prepare the system by launching the IS440cr Image Acquire Window.



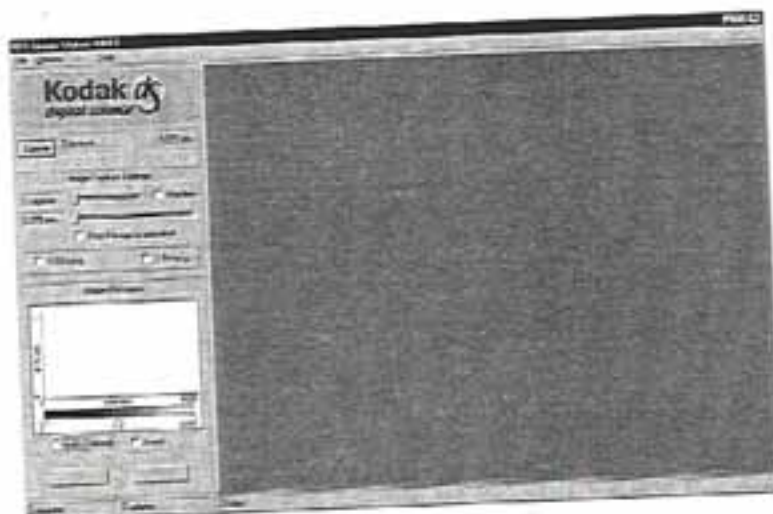
1D Image Analysis Software  
Acquire Hierarchical Menu

1. Launch 1D Image Analysis Software by double-clicking the 1D icon.
2. Select Image Station 440cr from the Acquire hierarchical menu in the File menu.



The IS440cr Image Acquire Window will appear and the CCD camera is powered.

IS440cr Image Acquire Window



### Cleaning the Platen Surface

Be sure that the platen surface is clean prior to any sample placement. Dust, particles or scratches on the platen may introduce artifacts in your data. Extra care should be taken when cleaning the platen to avoid scratches.

1. Open the IS440cf lid.
2. Use an ammonia based spray cleaner. Wipe the platen surface and work area with a lint-free soft cloth or damp paper towel.



**NOTE:** Special care should be taken if your sample is dry. Assure a dry surface by a quick ethanol wipe.

### Orienting the Sample for Capture

Now that the Platen is clean, you are ready to orient your sample on the center of the platen surface. Placement of your sample is similar to placing a letter on a photocopier. The Platen accommodates up to a 25 x 20 cm blot, membrane or gel. If your object is opaque (blot), orient the sample side downward—if translucent (gel), orient the sample either way. If the sample is wet (gel), puddle water or buffer on the platen surface prior to placement of the sample. This will minimize bubble artifacts. The platen area is a watertight and inert surface, ideal for biochemical, luminescence and other photochemical reactions.

1. Open the IS440cf lid.
2. Place the sample on the platen— if opaque, place the sample side down.

**NOTE:** Leave the lid open to use ambient light for visualizing the sample.



3. To view the sample on the screen, select Preview. The exposure time automatically gets set to 0.075 second.
4. Click Auto Contrast.
5. Uncheck Binning.
6. Click Expose. The live image will appear in the IS440cr Acquire Window.
7. Center the image on the platen.

**NOTE:** The Preference menu permits you to flip the image orientation. This may assist you with the natural left-right orientation ambiguity between the sample object and the image.

*Focus Ring Mark*

Focus Ring Mark



**NOTE:** Focusing the camera should not be necessary for most samples placed on the platen— simply adjust the Focus Ring on the camera lens to the special mark.

8. Adjust the Zoom Ring on the camera lens to fill the entire frame with the sample image or features of interest. This will maximize the number of pixels within your sample image.

---

**NOTE:** The Zoom setting relates directly to the image resolution. The IS440cr CCD has a resolution of 752 x 582 pixels. At the lowest zoom setting (12.5mm), the number of pixels are spread over a 25 x 20 cm area producing a resolution of ~350  $\mu\text{m}/\text{pixel}$ . At the highest zoom setting (75mm), the pixels are spread over a 4.2 x 3.3 cm area (~58  $\mu\text{m}/\text{pixel}$ ). An important consideration is the number of pixels that are applied to the smallest feature of interest.

---

9. Record the Zoom setting, you will need it for submitting the image.

### Choosing the Filter and Illumination Conditions

This section contains the basic guidelines on how to set up the illumination and filters prior to image capture.

1. Select the filter using the Filter Selection Dial located at the top of the Capture System Chamber.

Filter Selection Dial



**NOTE:** Only turn the filter dial in the clockwise direction.

The factory-equipped filters are listed with position number inside the door of the Capture System Chamber of the IS440cr.

IS440cr is equipped with five applicable filters. Should a specialized filter be required, the procedure for changing a filter is in *Chapter 4: Maintaining the System*.

2. Choose the appropriate sample background:
  - a. Luminescence
    - Blot (flat)—use the black side of the Compression Pad to apply modest sample pressure.
  - b. Fluorescence
    - Thick samples (gel or other)—remove the Compression Pad from the Lid.
    - Thin samples (blot or other)—use the black side of the Compression Pad to apply modest sample pressure.
  - c. Absorbance
    - Thin samples (transmission)—place the Light Diffuser directly onto the sample to provide even distribution of external light and modest sample pressure.



- Thick samples (transmission)—use the Light Diffuser Risers or added shim to space the diffuser from the sample.
  - UV fluorescence quenching with an autofluorescing sample (blot, TLC)—use the black side of the Compression Pad to apply modest sample pressure.
  - UV fluorescence quenching with a clear sample (thin gel, microscope slide)—use the white side of the Compression Pad to assure light return and to apply modest sample pressure. Assemble gel sandwich wet.
3. If your sample requires illumination, turn on the UV light source using the UV On/Off switch at the top of the Capture System Chamber. For other illumination modes, make sure the UV light source is Off.
  4. Depending on the illumination mode, you may need to close and clasp the lid. Review the *Table 3.1 Sample Illumination/Detection Summary*.



Table 3-2 Image Station 440cf Filters

Position	Filter #	Color	Filter Type	Wavelength*	Applications	Comments
1	# 98	Blue	band-pass	>386,396,403; <470,490,500	DAPI, Hoechst and many blue fluors, red/yellow chromogens or fluorescence quenching. Blue film analysis.	Excellent green/yellow/red attenuation, specific blue fluorescence filter. Fluorescence quenching.
2	# 61	Green	band-pass	>478,482,498; <568,598,604	Green or green-yellow fluors: Fluorescein, Oregon Green. Includes the plurality of all developed fluorochromes. Magenta chromogens.	Excellent blue and red attenuation. Good for differential display, example: Fluorescein without ethidium. Compliments #25/#92/#98.
3	# 16	Yellow	high-pass	516,519,522	Fluorescein and all fluors at higher emission wavelength: green, yellow and red. Differential luminescence. Blue chromogens.	Excellent blue attenuation, good general fluorescence filter, high speed (short exposure times).
4	# 25	Red	high-pass	580, 583, 592	Ethidium and all orange and red fluors. Cyan chromogens.	Excellent blue and good green attenuation. Best for ethidium.
5	# 92	Dk Red	high-pass	618, 621, 627	Far-red fluors, Cy5. Black chromogens/clearest transmission.	Excellent blue and green attenuation. Minimizes scattering artifacts.

\*Wavelengths (nm) at which high-pass filter optical density is 3, 2 and 1 respectively, or low-pass filter optical density is 1, 2 or 3. Both apply to band-pass filter.

For fluorochromes of known emission maximum, the following guidelines may be of some benefit in optimizing signal over background fluorescence. Use a high-pass filter whose optical density is less than 1 OD at a lower wavelength than the fluorochrome emission maximum, but not too much lower (perhaps 5-20 nm). Ideally, choose a band-pass filter whose mid-band matches the fluorochrome emission maximum. For example, ethidium (emission 595 nm) is compatible with filter #25; fluorescein (emission 524 nm) is compatible with filter #16, but expect better results with filter #61 (mid-band is approximately 534 nm).

## Capturing the Image

Now that you have oriented your sample and set the illumination conditions, you are ready to adjust the image capture settings to maximize the image data quality.

Use the *f*-stop, exposure time and binning settings to optimize the image capture. The *f*-stop or lens aperture determines the amount of light that passes through the lens, while the exposure time controls how long the CCD is exposed. You will improve resolution using a higher *f*-stop (smaller apertures), but signal is reduced requiring a longer exposure time. Binning affects how the CCD processes the signal and can enhance the image sensitivity at the expense of spatial resolution. Selecting X and/or Y Binning will combine the values from two adjacent pixel signals in the direction(s) selected. You can expect a four-fold increase in the rate of signal accumulation when both X and Y Binning are selected.

### Setting the f-stop

Generally, we recommend that you use either *f*-stop 1.2 for speed or *f*-stop 2 for resolution. For flat formats, *f*-stops higher than 2 will not substantially contribute to image resolution. However, you may need to increase the *f*-stop for unusually thick samples. Please note that the exposure time is related to the inverse square of the *f*-stop.

1. Set the *f*-stop by using the *f*-stop ring on the lens. Use these guidelines:
  - **Luminescence**—use the largest aperture (*f*1.2) to maximize signal.
  - **Fluorescence**—when exposure time is unusually long (dim fluorescence), use *f*1.2; for higher resolution, use *f*2.
  - **Absorbance**—use *f*2 or higher to extend exposure time so you have greater control of your dynamic range.
2. Record the *f*-stop setting, you will need it for submitting the image.

---

**NOTE:** The *f*-stop on the IS440cx lens is continuously adjustable (The ring does not mechanically stop at defined positions). It is important that you note the setting for accurate lens correction when the image is submitted to 1D Image Analysis Software. Refer to *Submitting the Image* later in this chapter.

---

3. If image is exposing in Preview mode, click Stop.

---

**NOTE:** If you are satisfied with your image, proceed to *Submitting the Image*.

---

Stop

### Setting Exposure Time and Applying Binning

The optimal settings for binning and exposure time for your image capture are that which will maximize the dynamic range of the image, but may be limited by your experimental conditions. The IS440cr Acquire Software includes an active histogram to aid in determining the exposure time.

1. Select Binning settings in accordance with the *Sample Illumination/Detection Guidelines* described earlier in this chapter and summarized in the *Image Capture Settings Table* at the end of this section. Additional experimental considerations can also influence the choice of Binning settings such as preferential spatial resolution in either the X or Y direction. When necessary, use the checkboxes to select X and/or Y Binning.

---

**NOTE:** Luminescent signals are often dim and may require both X and Y binning.

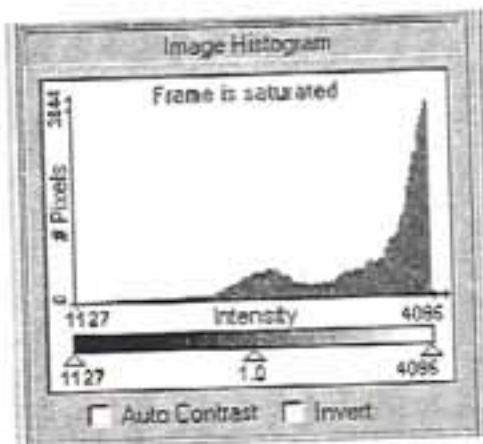
---

2. Select Auto Contrast in the Image Histogram Panel. Auto Contrast enhances the displayed image.
3. Choose an Exposure Time to maximize the use of all available signal levels in the histogram without saturation. Use the Image Histogram to guide you in determining the best exposure time using the following procedure:
  - a. Uncheck the Preview box to enable image accumulation.
  - b. Set the Exposure Time to between 0.075 and 60 seconds. For more details, refer to the *Sample Illumination/Detection Guidelines* section at the beginning of this chapter.
    - **Luminescence**— start with an exposure time of 20 seconds per capture.
    - **Fluorescence**— start with an exposure time of 5 seconds per capture.
    - **Absorbance**— start with an exposure time of 0.6 seconds per capture.



- c. Set the number of captures to 10 or more.
- d. Click Expose. At any time during the session, you can use Stop to terminate the exposures.
- e. Monitor the histogram for the range of signal levels. Be aware that the maximum signal may not be generated from the object of interest.
- If the image reaches saturation immediately (histogram turns red), decrease the exposure time.

*Example 1: The Histogram is saturated.*

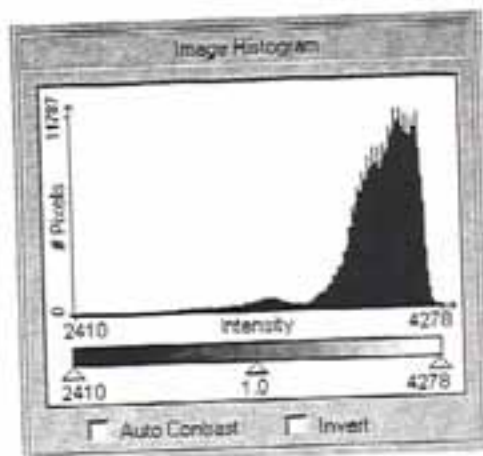


- If the image is not near saturation, estimate the exposure time by extrapolation—using the histogram, calculate the rate of signal accumulation by dividing the maximum signal by the test exposure time, then divide the rate into the desired signal level to determine the total exposure time estimate.

$$\text{Rate of Signal Accumulation [Rate} = \frac{\text{Signal Maximum}}{\text{Test Exposure Time}}]$$

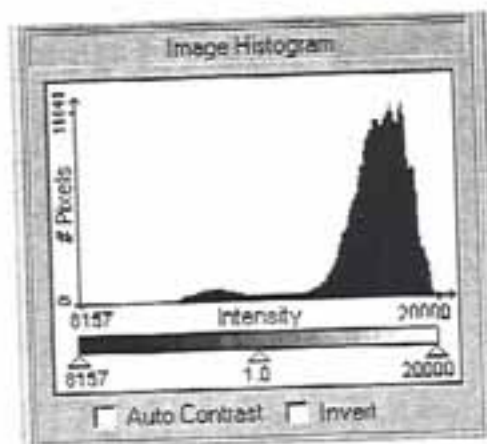
$$\text{Total Exposure Time Estimate} = \frac{\text{Desired Signal}}{\text{Rate}}$$

**Example 2: Single Capture:** If you completed a 10 x 1 second exposure and have a dynamic range of 3000 signal levels, the rate of accumulation is 300 signal levels/second. If you are performing a single frame capture and want 4000 signal levels, the exposure time would be approximately 13 seconds.



- To achieve the maximum dynamic range of the IS440cr, the exposure time estimate determined above (filling the 12-bit scale with a single capture) should be implemented using the accumulation of 16 captures (fills the 16-bit scale with accumulated signal). Intermediate levels of dynamic range may be achieved by accumulating fewer images.

**Example 3: Multiple Capture:** If you completed 6 x 30 second exposures and reached a dynamic range of 20,000 signal levels, the rate of accumulation is 111 signal levels/second. To approach a dynamic range of 65536 signal levels, you will need to expose the image for 590 seconds or approximately 10 minutes. To ensure that you do not saturate single images, the 590 seconds must be accomplished with 16 or more captures, implying 16 x 30 sec captures.





4. Enter the calculated Exposure Time and Number of Captures using the Exposure and Capture Sliders.

*Exposure and Capture Sliders*



---

**NOTE:** For a quick reference guide, see Table 3.3 *Image Capture Settings*.

---

5. You have optimized all settings for a programmed image capture, click Expose.

**Table 3.3 - Image Capture Settings**

Typical examples with relevant capture settings.

Detection Method	f-stop	Binning	Signal level per capture	Exposure time per capture	# of captures	Examples
Luminescence	1.2	Yes	50	1 hour	2	Minimal signal, just enough to measure.
		Usually Yes	2000	3 min.	4	Strong signal, good data to measure minor components.
Fluorescence	1.2-2.0	Usually No	4,000	10 sec.	16	High background, many levels to measure signal over background.
			500	3 min.	5	Time constraints, but good data to resolve minor components.
			500	20 sec.	1	Just need documentation.
			4,096 saturated	2 min.	5	Seeking resolution of minor component, ignoring saturation of bright artifact.
Absorbance	2.0-8.0	No	4,000	0.36 sec.	16	High quality data for broad range of concentrations of chromogens within sample.

## Viewing the Image

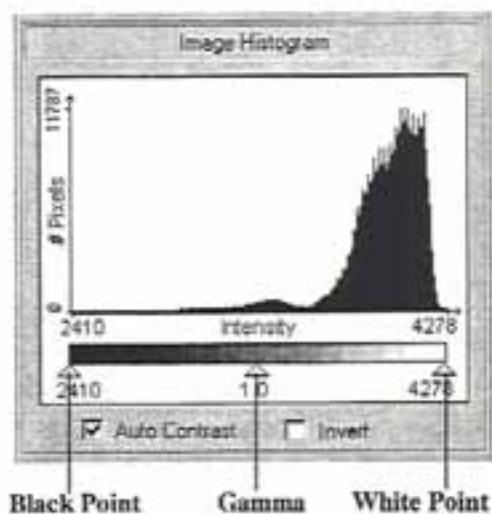
When the image capture has been completed, you can adjust the image as displayed in the window to maximize visualization. These adjustments will not affect the image data file.

### Contrasting the Image

You can automatically or manually contrast your image. The Auto Contrast automatically determines the optimal black and white point from the image data, remapping the signal by linearly interpolating between the two points as a 8-bit image display.

You may also adjust the contrast of the image manually using the Contrast Slider.

*Image Histogram with  
the Auto Contrast  
Turned On*



- The left slider is the black point of the image display. The associated signal is displayed on the lower left side of the Image Histogram.
- The right slider is the white point of the image display. The associated signal is displayed on the lower right side of the Image Histogram.

### Adjusting the Gamma

Unlike contrast, adjusting the gamma of an image disproportionately skews the gray level distribution. Higher gamma values ( $>1$ ), generally lighten the image and improve your ability to visually distinguish between subtle differences in signal in the dark end of the range; a lower gamma ( $<1$ ) aids in distinguishing differences in the light end. Use the center slider on the Image Histogram to adjust the gamma of the image. Adjusting the gamma does not alter the image data file and is only used to enhance the viewed image.

### Inverting the Image

When invert is applied, the brightness value of each pixel is converted to its complementary value (i.e., blacks-to-white, whites-to-black). Invert does not change the histogram or the data file.

### Adjusting the Gamma

Unlike contrast, adjusting the gamma of an image disproportionately skews the gray level distribution. Higher gamma values ( $>1$ ), generally lighten the image and improve your ability to visually distinguish between subtle differences in signal in the dark end of the range; a lower gamma ( $<1$ ) aids in distinguishing differences in the light end. Use the center slider on the Image Histogram to adjust the gamma of the image. Adjusting the gamma does not alter the image data file and is only used to enhance the viewed image.

### Inverting the Image

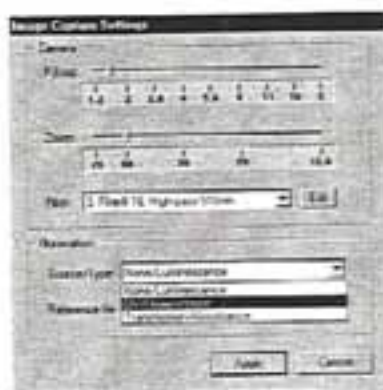
When invert is applied, the brightness value of each pixel is converted to its complementary value (i.e., blacks-to-white, whites-to-black). Invert does not change the histogram or the data file.

- From the Filter pop-up menu, select the filter used when capturing the image.

*Filter Pop-up Menu*

If the filter used is not listed, use Edit to define the type and location of the filters on your filter wheel using the Available Filters dialog box.

- Choose the illumination mode used when capturing the image from the Illumination Source/Type pop-up menu.

*Source/Type Pop-up Menu*

- Select an Illumination Reference File using the Reference File pop-up menu.



---

**NOTE:** You can choose None and then later apply the illumination correction after the image has been submitted to 1D Image Analysis Software.

---

- a. If you have selected None/Luminescence as the illumination mode, the Illumination Reference File defaults to None, since no field illumination correction is required.
- b. If you have selected UV/Fluorescence as the illumination mode we recommend that field illumination correction be applied. Select one of the following options:
  - Choose None. No field illumination correction will be performed at this time. The correction can be later applied in 1D Image Analysis Software (refer to *Generating an Illumination Reference File* in the next section).
  - Choose an Illumination Reference File that is currently open in the 1D Image Analysis Software from the Reference File pop-up menu.
  - Choose Create Illumination Reference File from this Image making the current image capture the Illumination Correction File, click Create. For details, refer to *Generating an Illumination Reference File* in the next section.
- c. If you have selected Transmission/Absorbance as the illumination mode, the Light Diffuser evenly distributes the light across the image. Therefore, field illumination correction is typically not required.
  - Choose None. No field illumination correction will be performed.

---

**NOTE:** If you choose to do a field illumination correction, the same options used in the UV/Fluorescence mode are available, as described above.

---

6. Click Apply. The image will transfer to 1D Image Analysis Software.

### Generating an Illumination Reference File

Improve the quality of your data by applying field illumination correction to images captured in the UV/Fluorescence mode. The illumination non-uniformity is highly reproducible and may be corrected by dividing the sample image by an Illumination reference image. It is important to capture a reference image of the illumination field using the same camera settings (*f*-stop, Zoom) used when capturing the image to be corrected. To generate an Illumination Reference File for UV Illumination:

1. Remove the sample from the Platen and clean the Platen surface and the white backing of the Compression Pad.
2. Turn on the UV lamp using the UV On/Off switch at the top of the Capture System Chamber.
3. Apply the white backing of the Compression Pad to the platen by closing the lid.
4. Select Preview, Choose an Exposure Time that provides greater than 1000 signal levels in the histogram without saturation. Use the Image Histogram to guide you in determining the best exposure time
5. Turn off Binning.
6. Adjust the *f*-stop and Zoom to the settings used when capturing the image to be corrected.
7. Choose a filter position. Either positions #2 or #3 are recommended to assure exposure time of greater than 1 second which will offer better control of the original scale.
8. Increase the exposure time until the histogram is nearly filled, but not saturated (histogram turns red when image is saturated).

Image Capture Settings  
Dialog Box

9. Click Submit. The Image Capture Settings dialog box appears.
10. Adjust the *f*-stop and Zoom Slider to correspond with the



settings on the camera. This will allow the software to document the appropriate settings used when creating this Illumination Reference File

11. Choose the UV/Fluorescence from the Illumination Source/Type pop-up menu.
12. Choose Create Illumination Reference File from this Image from the Illumination Reference File pop-up menu, click Create.

**NOTE:** Multiple Illumination Reference Files, using various *f*-stop and zoom settings, can be generated and saved for future use. See *Appendix C: Image Correction*.

## Printing an Image

The IS440cf Acquire Software will perform a Quick Print and print the image in the main window. When selecting the Quick Print option you will have the choice to print the image in 3 sizes, A6 (SP700), 5" x 7" and US Letter/A4. For each of the page sizes, the image will be scaled so that the entire IS440cf capture is visible on the print. For printing images at other custom magnifications, 1X zoom level or printing image with annotations, print the image using 1D Image Analysis Software.

1. Click Quick Print. The Print Dialog box appears.
2. Choose the paper size.
3. Click OK. The image will print.

**NOTE:** The 5"x7" in option will print the image at this size regardless of paper size. The Fit to Page option will use printer information on paper type, and orientation to scale the printed image.



## CHAPTER 4: MAINTAINING THE SYSTEM

The IS440cr chamber is chemically resistant and water tight and therefore easy to maintain. Periodically, some parts of the IS440cr may need to be replaced. This chapter explains daily maintenance and user serviceable repairs.



## Regular Maintenance

On a regular basis, the IS440cf requires little maintenance and is easy to clean. After each use, the Sample Work Area should be wiped down. Care is needed to avoid scratching the platen. Clean the instrument with a soft lint-free cloth or lens paper.

### Cleaning the Sample Work Area

1. Open the IS440cf lid.
2. Using an ammonia based spray cleaner, wipe the platen, work area and compression pad surfaces with a lint-free soft cloth or lens paper.
3. Be sure the platen area is dry and without dust particles.



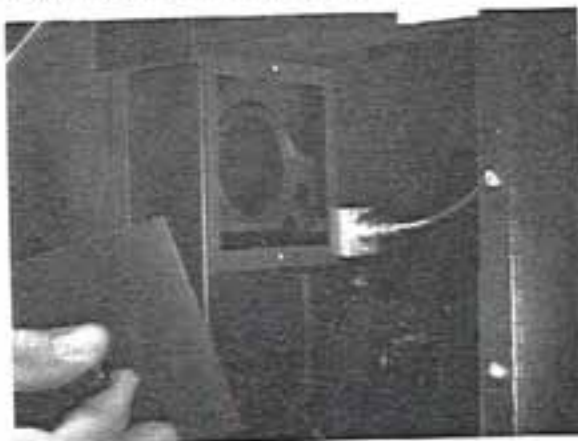
**NOTE:** Always turn off UV light source when not in use.

## Changing a Filter

If you need to replace or use a different filter for your experiments, follow this procedure. Remember that the filter number that is indicated on the dial is not the filter number that you will be changing as described in the chart below.

1. Open the Capture System door.
2. Grasp the knob and remove the Filter Access Panel.

*Removing the Filter Access Panel*



3. Rotate the Filter Selection Dial clockwise so that the filter to be changed is located in the Access Window.

**NOTE:** The filter viewed in the window is two positions forward (clockwise) from the position indicated on the dial.

*Filter Knob Position and Filter at Access Panel Relationship*

<i>Filter Knob Position</i>	<i>Filter at Access Panel</i>
0	2
1	3
2	4
3	5
4	0
5	1

4. Unscrew the filter exposed at the access panel.
5. Carefully screw in the new filter. Be careful not to touch the filter element. Do not overtighten.
6. Replace the access door by aligning the pins in the holes.

## Changing a Lid Gasket

The lid gasket should only be changed if there is a light leak. The following procedure explains how to change the Lid Gasket.

**Replacement Catalog Number :** 196 9641

**Parts supplied:**

gasket  
cyanoacrylate adhesive  
socket drive

### Changing a Lid Gasket

1. Remove the four socket screws holding the lid to the hinge and remove the lid. Perform work on a padded work space to prevent scratching the top of the lid.
2. Note the location of the splice in the gasket which is behind the hinge farthest from the platen. The new gasket will be placed on the lid so that the splice will be in the same location.
3. Gently peel the damaged gasket from the lid attempting to remove the adhesive with the rubber extrusion.
4. Remove all residual adhesive from the lid flange with alcohol, adhesive remover (i.e. Goo Gone™) or other mild solvent.
5. Install the new gasket on a clean, dry flange surface.

**NOTE:** Be careful not to pull or stretch the gasket as it is applied. The lengths of gasket between notches are correctly cut to provide tight mitered corners at each corner.

6. Begin by peeling back the release paper from the first 12" length of gasket and locating the first mitered cut in the gasket in corner A. The gasket should be applied so that the cut sections forming the 90° corner are placed flush to each other. The short section of gasket will fall approximately in the middle of the hinge.

*Mounting the Lid Gasket*



7. Gently apply the remaining length of gasket along length 1 of the lid. Peel away more of the backing paper as the gasket is applied to the lid.
8. Locate the second miter cut in corner B and again place the cut surfaces of the gasket together to form a tight 90° fit.
9. Proceed around the remainder of the lid forming tight corners at C and D.
10. As the gasket is applied to length 4 of the lid, there will be an excess length of material that must be trimmed to provide a flush fit with the end of the gasket that was applied initially.
11. Gently apply the gasket along length 4 and lay the extra material over the other end of the gasket.
12. Cut the excess length of gasket so that the cut end will squarely meet the first end of the gasket already in place.



13. Spread the cut ends of the gasket which abut and apply a drop of cyanoacrylate adhesive to the cut ends of the gasket. Be careful to apply adhesive **ONLY** to the cut ends and avoid getting adhesive on the outer surfaces of the gasket.
14. Allow the cut ends to join, holding the cut faces together for 30 seconds until the adhesive cures.



## CHAPTER 5: TROUBLESHOOTING THE SYSTEM

In this chapter, common questions are addressed. The questions are divided into three sections, Instrument, Image and Software. If you still have questions after reading this section and the corresponding information in the manual, contact Technical Support. Please have your serial number and any technical information available.

## Contacting Technical Services

If you have a problem using the IS440cf system, please refer to the appropriate section of this User's Manual, the 1D Image Analysis Software User's Manual, or utilize the program's on-line help.

If you require additional assistance, contact your local NEN™ Life Science Products Technical Support. Before contacting Technical Support, please have the following information ready:

- Serial number of your IS440cf system. The serial number is located on the back panel of the instrument by the power connection.
- Serial number and version number of your 1D Image analysis Software (Select About 1D under the Help menu while 1D is running).
- The make and model of computer system you are using.
- System software version.
- The type of image you are capturing or analyzing.
- The problem you are having and what you were doing when the problem began. Please note of the exact wording of any error messages, including any error number displayed.

### NEN Life Science Products Technical Support

#### *World Headquarters*

NEN Life Science Products Inc.  
549 Albany Street  
Boston, MA 02118-2512 USA  
1-800-551-2121, International FAX 1-617-426-2464  
e-mail: techsupport@nenlifesci.com

#### *European Headquarters*

NEN Life Science Products  
Imperlastredt  
B-1930 Zaventem  
32-(0)-2-717-7911

For a complete listing of NEN local offices, see page 1-7.

## Common Instrument Questions

Problem	Probable Cause	Solution
<i>Cannot access the IS440cf capture system.</i>	Camera-to-Computer Cable is not properly connected.	Check the connections on the back of the IS440cf and at the PCI board interface on the Computer.
<i>UV lamp not working.</i>	No power to the UV light source.	Check that the UV Lamp switch is turned On.  Check that the power cord is properly attached and that the outlet is supplying power.  Check that the circuit breaker switch on the back panel is turned On.
	UV light source is faulty.	Call Technical Support.
<i>Need a different filter.</i>		Follow the procedure to change a filter in <i>Chapter 4: Maintaining the System</i> for the procedure.



## Common Image Questions

<i>Problem</i>	<i>Probable Cause</i>	<i>Solution</i>
<i>Image is black or too dark.</i>	Not enough light for a good contrast exposure.	Open the <i>f</i> -stop to 1.2 or increase the exposure time. See <i>Chapter 3: Capturing an Image</i> .
	Auto Contrast has not been checked.	Check Auto Contrast prior to capturing the image
<i>Image is white or too light.</i>	Too much light during the exposure.	Close the <i>f</i> -stop (larger number for less light). See <i>Chapter 3: Capturing an Image</i> .
<i>Image is blurred.</i>	Image is not in focus.	For thin samples, set focus to the arrow mark on the focus ring. For thicker samples, adjust the focus ring and/or increase the depth of field by increasing the <i>f</i> -stop setting to at least 2.0.
	Lens has been partially unscrewed.	Tighten the Lens.
	Dirty or cracked filter.	Perform an Image with a different filter selected.
<i>Image orientation is incorrect.</i>	Contaminated optics.	Call Technical Support.
	Software preferences are set incorrectly.	In the IS440cf Acquire Software Preferences dialog box, select the normal or inverted buttons depending on your preference.

## Common Image Questions (continued)

<i><b>Problem</b></i>	<i><b>Probable Cause</b></i>	<i><b>Solution</b></i>
<i>Bright imaging artifacts not coming from the sample.</i>	Light leak.	Check that the Filter Access Panel is properly in place.
	Light leak at the Lid Gasket.	Ensure the lid has been closed and latched in place. Ensure the Lid Gasket is clean and free of debris. Verify by performing a 15 minute exposure with the lid closed, X and Y Binning on, no illumination and no sample. Submit and save the image. Expose an additional image with the same settings, but this time place the Light Diffuser over the platen, remove the compression pad and close the lid. If significant increase in artifact signal is seen; the light leak is due to a faulty lid gasket. See Changing a Lid Gasket in <i>Chapter 4: Maintaining the System</i> .
	Light leak elsewhere.	If a signal artifact continues and is not changed in the above procedure, call Technical Support to diagnose the leak and to obtain further instructions.

## Common Image Questions (continued)

<i>Problem</i>	<i>Probable Cause</i>	<i>Solution</i>
<i>Image appears with many speckles in a long "no-illumination" exposure.</i>	Defective Pixel Correction not functioning properly.	Locate and transfer the *.dft file to the correct location. See Camera Setup in <i>Chapter 2: System Overview</i> .
<i>Image appears with many speckles in an UV illuminated image.</i>	Dust or dirt on the Platen.	See Cleaning the Platen surface in <i>Chapter 3: Capturing an Image</i> .
<i>Image partially blocked.</i>	Filter not centered over the lens properly.	Check that the filter dial is lined up with the filter selection properly. View the filter wheel alignment by looking down into the IS440cf platen.
<i>Lines on the image.</i>	Scratched Platen.	Call Technical Support for further instructions.
<i>Upon submitting an image to the ID Image Analysis Software, the background intensity of the image is not even.</i>	Improper Image Reference File was used for correction.	Be sure the proper Image Reference File is used. See Submitting the Image in <i>Chapter 3: Capturing an Image</i> .
	<i>f</i> -stop and Zoom settings used for submitting the image are not the same as those used to capture the image.	Be sure the proper <i>f</i> -stop and Zoom inputs are used. See Submitting the Image in <i>Chapter 3: Capturing an Image</i> .

## Common Software Questions

Problem	Probable Cause	Solution
<i>The IS440cf Image Acquire Software can't locate the calibration file.</i>	The *.dft file was moved, deleted or corrupted.	Locate and transfer the *.dft file to the correct location. See Camera Setup in <i>Chapter 2: System Overview</i> . If the file is unavailable, call Technical Support.
<i>Cannot access the IS440cf Image Acquire Software.</i>	IS440cf driver was moved, deleted or corrupted.	See Installing the Software in <i>Chapter 6: Installing the System</i> .
<i>Copy protection error.</i>	The 1D copy protection device is not responding.	Check the connections and restart the software and computer. Refer to the 1D Image Analysis Software User's Guide.





## CHAPTER 6: INSTALLING THE SYSTEM

This section details the installation of the IS440cr system. This information will be used by your local representative when your equipment is initially installed. Should you need to install the IS440cr system, this section will tell you how to unpack the equipment and what to consider in selecting a new location for the equipment. In addition, it contains instrument performance checks, necessary after installation, and how to repackage and prepare the equipment for a move.

---

## Temperature & Humidity Requirements

The IS440cr system is designed to operate effectively within the temperature and humidity ranges typically found in laboratories and is for indoor use only. For effective operation, the temperature and relative humidity should be:

Temperature: 59 to 86°F (15-30°C)

Relative Humidity: <80%, non-condensing

---

## Electrical Requirements

There are two versions of the IS440cr available:

Catalog Number 167 4373	120VAC*, 60Hz, 1A
Catalog Number 121 5508	100VAC*, 50/60Hz, 1A
Catalog Number 181 1017	230VAC*, 50Hz, 1A

\* Supply voltage fluctuations not to exceed  $\pm 10\%$ .

Refer to your computer operators's manual for its electrical requirements.

## Space Requirements

The IS440cr and computer must be placed on a bench or table that is level and capable of supporting 125 pounds, located not more than 3 feet from an electrical outlet.

The IS440cr system, including computer CPU, monitor and keyboard, requires a minimum space 54 inches wide, 24 inches deep and 26 inches high (137x61x66cm). These dimensions do not allow for a printer or other peripheral device.



**WARNING:** Maintain a minimum of 6 inches clearance around the Radiator to allow for proper cooling.

Additional space adjacent to, above or behind the IS440cr will allow for easier operation.

## Installing the IS440cf

### Unpacking and Setting Up the IS440cf Chamber

1. Remove the instrument from the shipping container. Remove packing materials.
2. Verify components using the list in the *Introduction Section*.
3. Position the IS440cf next to the computer.
4. Connect the serial cable to the connector at the rear of the IS440cf.
5. Attach the AC line cord to the power input module at rear of the IS440cf.

For IS440cf catalog number 181 1017 (230 VAC) select the appropriate modular plug to match your local AC outlet.

### Installing Kodak Digital Science 1D Image Analysis Software

This software program is required when using the *Kodak Digital Science Image Station 440cf*. When you install the software, the KDS 1D folder will be created and placed at the main level of your hard disk. Please refer to the enclosed 1D Image Analysis User's Manual for complete installation instructions.

**WARNING:** If you have a previous version of 1D Image Analysis Software and have made changes to the contents of the KDS 1D folder (added standards, plug-ins or projects), you may want to save a copy on disk prior to installing the new version. Any files that have been edited from the previous version of the software can then be copied into the new KDS 1D folder.

### Installing the PCI Card

The PCI card enables your computer to communicate with your camera.



**WARNING:** The card is stored in an antistatic bag because it is sensitive to static electricity. Be careful when you handle the card.



1. Exit Windows and shut down your computer.
2. Turn off your computer and all the peripherals connected to your computer. Disconnect all power cords from AC source.

**WARNING:** See the documentation that came with the computer for information about opening the computer and PCI installation safety instructions.

3. Remove the computer cover so that you can see the inside of the computer and the accessory card slots.
4. Choose an empty card slot that fits your PCI card.
5. Remove the slot cover from the inside of the computer by removing the screw that holds the slot cover in place.
6. Find the PCI card that came with your IS440cr.



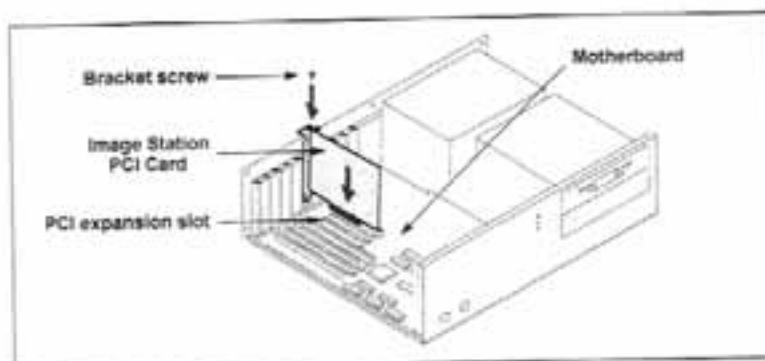
**WARNING:** Static electricity may damage your computer and/or PCI card. Do not take your PCI card out of the bag until you are ready to insert it into the computer. Use a grounded wrist strap when handling either the PCI card or internal computer components. If you do not have a grounded wrist strap, touch the power supply housing immediately prior to handling and inserting the PCI card.

7. Remove your PCI card from the antistatic bag, holding the card by its metal support bracket and card edges

**WARNING:** Do not touch the PCI card's circuitry.

8. Position your PCI card so the bottom edge of the card is lined up over the PCI slot (white) in the computer.
9. Press down firmly on both ends of the PCI card. This card may fit snugly, so you may have to apply pressure.
10. Make sure the card is seated in the slot correctly. When you look at the side of the card it should be evenly aligned with the slot. When you look down at the card, the screw opening in the card edge should sit directly on the PCI slot cover.

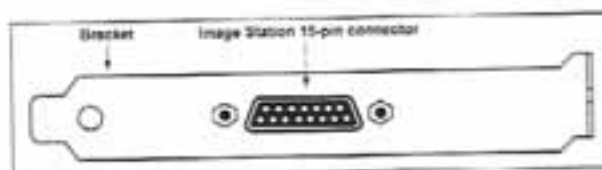
*Installing the PCI card*



11. Replace the screw in the slot cover. The screw holds the PCI card support bracket to the back panel of the computer.
12. Replace your computer cover.
13. Attach one end of the interface cable to the connector on the PCI card you just installed.
14. Attach the other end of the interface cable to the 15-pin connector on the back of the image station.



*Connecting the Interface Cable to the 15-pin Connector*



15. Attach the power cord to the IS440cr and plug the other end of the cord into an appropriate electrical receptacle.



16. Turn on the power to the IS440cf and your computer.
17. When windows starts up it will detect new hardware and ask for a disk. Insert the IS440cf Image Acquire Software Installer Disk. Allow Windows to install the card driver file.
18. Set the monitor for a resolution of 1024 x 768 or better and set the Color Palette to True Color (32-bit).

### Verifying Your Card Installation

Verify the PCI Card was installed in the correct IRQ. The card should be installed by Windows 95 in a unused IRQ. The main conflict to avoid is between the Video Card and the Image Station card sharing an IRQ. To check this do the following:

1. Right click My Computer, select Properties.
2. Click the Device Manager tab.
3. Click Sound, Video, and Game Controllers.
4. Verify that there is a hardware device called Image Station 440cf installed there.
5. Double-click it and select the Resources tab.
6. Make a note of the Interrupt Request (IRQ).
7. Repeat for the other devices, such as modems, ethernet or video cards. Look for conflicts.

### Installing the Kodak Digital Science Image Station 440cf Image Acquire Software

The IS440cf Image Acquire Software is designed to be used from within 1D Image Analysis Software. The software installs like most Windows applications.

To install the IS440cf Image Acquire Software:

1. Insert the Image Station 440cf Image Acquire Software installer disk into your floppy drive.
2. Launch the Setup program on the installer disk. You can do this by double-clicking on My Computer, double-clicking on 3 1/2 Floppy (A:), and double-clicking on the setup program or by Choosing Run from the Start menu and typing A:\Setup at the prompt.
3. Follow the instructions on your screen.
4. Launch 1D Image Analysis Software.
5. Choose Image Station... from the Acquire hierarchical menu in the File menu. The IS440cf Acquire Window appears. If not, you will be presented with the Select Source dialog box. Choose Image Station 440cf from the list and click OK. If KDS Image Station does not appear in the list, restart your computer and reinstall the IS440cf Image Acquire Software.
6. You are ready to begin using the IS440cf Image Acquire Software.

---

**NOTE:** After acquiring the IS440cf Acquire Window for the first time, installation, you may be presented with an alert box informing you that the Camera Calibration File could not be found. If this happens, click Yes to locate the calibration file. This file should be in your Image Station 440cf folder on your hard disk. If this file is not located on your hard disk, exit the installation and proceed to the section, *Installing the Camera Calibration File*.

---

---

**NOTE:** You may need to change the view options in order to see file extensions. You can do this by choosing Options from the Windows View menu. Once the Windows view options dialog box has opened, select the View tab and uncheck the Hide File extensions for known file types option. Also select the Show All Files button. When you have done this click Apply then OK to close the dialog box.

---

### Installing the Camera Calibration File

Each and every CCD camera is unique. All CCD devices contain a certain number of defective pixels. It is necessary to remove these pixels from the image in order to get the best possible contrast on an image acquired with the camera. The Camera Calibration File keeps track of these pixels.

---

**NOTE:** You only need to follow this procedure if the Camera Calibration File is not installed on the your system by the installer you can drag it to your Image Station 440cr folder on your hard drive.

---

1. Open the Image Station 440cr folder on your hard disk.
2. From the Windows Menu go to the View menu and select the Options... item. Click on the View tab and uncheck the Hide File Extensions for Known File Types option. Also select the Show All Files button. When you have done this click Apply then OK to close the dialog box.
3. Insert the IS440cr Image Acquire Software disk in the floppy drive.
4. Open the IS440cr Image Acquire Software disk. There are 4 files on this disk: ReadMe.txt, Setup.exe, ImageStation95.inf, and #####.dft (##### is the serial number of your camera which is printed on the installer disk).
5. Copy #####.dft from the installer disk to your Image Station 440cr folder on the hard drive.

6. Launch 1D Image Analysis Software.
7. Choose Image Station... from the Acquire hierarchical menu in the File menu. Select Image Station 440cr. The IS440cr Acquire Window appears and the CCD camera is turned on.

---

**NOTE:** During installation, you may be presented with an alert box for the Select Source dialog, choose KDS Image Station from the list and click OK. If Image Station 440cr does not appear in the list, restart your computer and reinstall the Image Station 440cr Image Acquire Software.

---

8. When the alert box appears informing you that the Camera Calibration File could not be found, click Yes to browse the disk and locate the Camera Calibration File. This is the file which you copied to your Image Station 440cr folder in step 5. Select it in the browser list and click OK to dismiss the browse dialog box.

You are now ready to capture images using your IS440cr.

## Repacking the IS440cf

If you plan to ship your IS440cf, packing the components correctly are essential to protecting the imaging chamber and computer. Inappropriate packing will void the IS440cf warranty. Follow these directions when packing the IS440cf for shipment.

- Remove any samples from the platen, and dry thoroughly.
- Close and latch the lid.
- Remove the power cord and serial cable from the IS440cf.
- Cradle the camera using foam padding
- Secure the filter access door to the filter housing with masking tape.
- Put the IS440cf in its original plastic bag to keep it clean.
- Pack the IS440cf in its original box. If you have discarded the original packing material, call technical support to arrange for delivery of new packing material.





## **CHAPTER 7: WARRANTY & REGULATORY INFORMATION**

This section contains the IS440CF warranty and software licenses agreements information.

## IS440cf Limited Warranty

### Warranty Time Period

Kodak warrants the *Kodak Digital Science IS440cf* to function properly for one year from date of installation. Specific system components warranted by Kodak are the IS440cf chamber, the hardware components installed within the chamber, the serial data cable, power cord, PCI card and Image Acquire Software and the 1D Image Analysis Software. The computer provided with the IS440cf is covered under separate warranty provided by the computer manufacturer.

### Warranty Repair Coverage

If the equipment does not function properly, as determined by Kodak through its authorized agent, NEN, during the warranty period, shipping instructions and packing materials will be provided to the customer to ship the IS440cf to a regional repair facility, or Kodak will ship replacement part(s) to the customer site. Repair service will include any adjustments and/or replacement of parts as necessary to maintain the equipment in an operating condition consistent with Kodak specifications.

---

**NOTE:** Maintenance parts may be new or reconditioned to perform as new.

---

### Days and Hours of Coverage

Arrangements for service through NEN Life Science Products can be made Monday through Friday 8:00AM to 6:00PM EST in the United States except for locally observed holidays. Hours of coverage outside the United States may vary. Outside the United States, contact your local NEN dealer for hours of coverage.

### How to Obtain Service

If the IS440cf does not function properly during the warranty period call your local NEN dealer to arrange for service.

### Limitations

This warranty does not cover 1) circumstances beyond Kodak's control (such as customer overriding, bypassing or defeating interlock switches on equipment or devices sold by Kodak); 2) problems due to failure of customer to conform to Kodak's site specifications; 3) relocation of equipment or service associated with relocation of equipment; 4) service or parts associated with any unauthorized modifications, attachments or service; 5) rebuilding or reconditioning equipment; 6) misuse; 7) abuse; 8) failure to follow Kodak's operating instructions; 9) supply items.

KODAK MAKES NO OTHER WARRANTIES, EXPRESS, IMPLIED OR OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE FOR THIS EQUIPMENT OR SOFTWARE.

Repair or replacement without charge are Kodak's only obligation under this warranty.

KODAK WILL NOT BE RESPONSIBLE FOR CONSEQUENTIAL OR INCIDENTAL DAMAGES RESULTING FROM THE SALE, USE OR IMPROPER FUNCTIONING OF THIS EQUIPMENT OR SOFTWARE REGARDLESS OF THE CAUSE. Such damages for which Kodak will not be responsible include, but are not limited to, loss of revenue or profit, down-time costs, loss of use of the equipment, cost of substitute equipment, facilities or services or claims of customers for such damages.

## Software License Agreement

You should carefully read the following terms and conditions before using this software package. Using the IS440cf Image Acquire Software and 1D Image Analysis Software indicates your acceptance of these terms and conditions. If you do not agree with them, you should promptly return the diskette package unopened, along with its accompanying materials, and your money will be refunded.

Eastman Kodak Company ("Kodak") provides this program and licenses its use. You assume responsibility for selection of the program to achieve your intended results, and for installation, use, and results obtained from the program.

### License

You may:

- a. use the program on a single machine, or if you have purchased a network license, on the number of machines authorized by your network license (as indicated on the invoice for that license);
- b. copy the program into any machine-readable or printed form for backup or modification purposes in support of your use of the program;
- c. transfer the program and license to another party if the other party agrees to accept the terms and conditions of this Agreement. If you transfer the program, you must at the same time either transfer all copies whether in printed or machine-readable form to the same party or destroy any copies not transferred; this includes all modifications and portions of the program contained or merged into other programs.

You must reproduce and include the copyright notice on any copy, modification, or portion merged into another program.

You may not use, copy, modify, or transfer the program, or any copy, modification, or merged portion, in whole or in part, except as expressly provided for in this license.



If you transfer possession of any copy, modification, or merged portion of the program to another party, your license is automatically terminated.

### **Term**

The license is effective until terminated. You may terminate it at any time by destroying the program together with all copies, modifications, and merged portions in any form. It will also terminate upon conditions set forth elsewhere in this Agreement or if you fail to comply with any term or condition of this Agreement. You agree upon such termination to destroy the program together with all copies, modifications, and merged portions in any form.

### **Limitation of Liability and Remedy**

Kodak warrants that it has full power to enter into this Agreement and to grant you the rights provided herein and that the software will substantially conform to Kodak's published specifications. Kodak further warrants that it will defend you against any claim that the software supplied hereunder infringes a patent, copyright, or other third party intellectual property right, and Kodak will pay any costs and damages that a court finally awards against you as a result of such claim, provided you give Kodak prompt written notice of such claim and tender to Kodak the defense and all related settlement negotiations. Kodak shall have no obligation with respect to any such claim based upon your modification of software or its combination, operation, or use with data or programs not furnished by Kodak or with other than the specified computer system. At any time during the course of any litigation arising out of such a claim, or if, in Kodak's opinion, the software or any part thereof is likely to become the subject of a claim of infringement, Kodak will, at its option and at its expense, either procure for you the right to continue using the software, replace or modify the same so that it becomes non-infringing, or grant you a credit for the software as depreciated, and accept its return. The depreciation will be an equal amount per year over the lifetime of the software as established by Kodak.

EXCEPT FOR REPLACEMENT OF UNMODIFIED COPIES OF SOFTWARE FOUND BY KODAK TO BE DEFECTIVELY REPRODUCED OR DAMAGED PRIOR TO DELIVERY TO YOU, KODAK EXPRESSLY EXCLUDES ALL OTHER REMEDIES OR WARRANTIES OTHER THAN THE FOREGOING, EXPRESS OR IMPLIED, IN RELATION TO THE SOFTWARE (AND ANY SERVICES RENDERED TO SUPPORT THE SOFTWARE), INCLUDING ANY WARRANTY OF MERCHANTABILITY OR OF FITNESS FOR A PARTICULAR PURPOSE.

In particular, Kodak does not warrant that the functions contained in the program will meet your requirements or that the operation of the program will be uninterrupted or error free.

Neither you nor any other third party claiming rights through you will hold Kodak, or any third party from whom it has derived rights in the Software Products, liable for any loss or damage because of any defect or ineffectiveness of the Software Products, including without limitation, any interruption of business, loss of profits, lost data, or indirect, consequential, or incidental damages.

KODAK'S LICENSOR(S) MAKES NO WARRANTIES, EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION THE IMPLIED WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE, REGARDING THE SOFTWARE. EASTMAN KODAK COMPANY'S LICENSOR(S) DOES NOT WARRANT, GUARANTEE, OR MAKE ANY REPRESENTATIONS REGARDING THE USE OR THE RESULTS OF THE USE OF THE SOFTWARE IN TERMS OF ITS CORRECTNESS, ACCURACY, RELIABILITY, CURRENTNESS OR OTHERWISE. THE ENTIRE RISK AS TO THE RESULTS AND PERFORMANCE OF THE SOFTWARE IS ASSUMED BY YOU. THE EXCLUSION OF IMPLIED WARRANTIES IS NOT PERMITTED BY SOME JURISDICTIONS; THE ABOVE EXCLUSION MAY NOT APPLY TO YOU.

IN NO EVENT WILL EASTMAN KODAK COMPANY'S LICENSOR(S), THEIR DIRECTORS, OFFICERS, EMPLOYEES OR AGENTS (COLLECTIVELY EASTMAN KODAK COMPANY'S LICENSOR) BE LIABLE TO YOU FOR ANY



CONSEQUENTIAL, INCIDENTAL, OR INDIRECT DAMAGES (INCLUDING DAMAGES FOR LOSS OF BUSINESS PROFITS, BUSINESS INTERRUPTION, LOSS OF BUSINESS INFORMATION AND THE LIKE) ARISING OUT OF THE USE OR INABILITY TO USE THE SOFTWARE EVEN IF EASTMAN KODAK COMPANY'S LICENSOR HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. BECAUSE SOME JURISDICTIONS DO NOT ALLOW THE EXCLUSION OR LIMITATION OF LIABILITY FOR CONSEQUENTIAL OR INCIDENTAL DAMAGES, THE ABOVE LIMITATIONS MAY NOT APPLY TO YOU.

**General**

You may not sublicense, assign, or transfer the license or the program except as expressly provided for in this Agreement. Any attempt otherwise to sublicense, assign, or transfer any of the rights, duties, or obligations hereunder is void.

This Agreement will be governed by the laws of the State of New York.

Should you have any questions concerning this agreement, you may contact Kodak by writing to Technical Service, Scientific Imaging Systems, Eastman Kodak Company, 4 Science Park West, New Haven, CT 06511, USA.

## Regulatory Information

"This equipment has been tested and found to comply with the limits for a Class A digital device pursuant to Part 15 of the FCC rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense."

"This Class A digital apparatus meets all requirements of the Canadian Interference-Causing Equipment Regulations.

Cet appareil numérique de la classe A respecte toutes les exigences du Règlement sur le matériel brouilleur du Canada."

この装置は、第一種情報処理装置（庶工業地域において使用されるべき情報処理装置）で庶工業地域での電波障害防止を目的とした情報処理装置等電波障害自主規制協議会（VCCI）基準に適合しております。  
従って、住宅地域またはその隣接した地域で使用すると、ラジオ、テレビジョン受信機等に受信障害を与えることがあります。  
取扱説明書に従って正しい取り扱いをして下さい。

### 警告使用者:

這是甲類的資訊產品・在居住的環境中使用時・可能會造成射頻干擾・在這種情況下・使用者會被要求採取某些適當的對策。

## **APPENDIX A: DIGITAL IMAGING CONCEPTS**

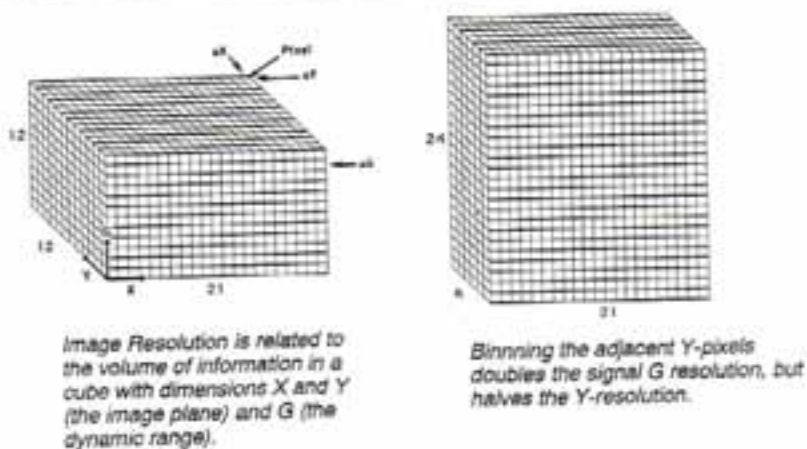
Digital imaging principles as they relate to the IS440c<sup>®</sup> are discussed in this appendix in somewhat greater detail than elsewhere in the manual. Gaining a working knowledge of these concepts will be helpful in making you at home with digital, electronic and quantitative imaging technology. The discussion is presented in an order that starts with the most difficult concept, resolution. The concept of image resolution is central to the understanding of all aspects of digital and quantitative imaging.

## Image Resolution

Image resolution generally refers to the capability to distinguish objects of interest. This discussion focuses on image data in an effort to clarify the point that the information in an image data file has a limited capacity to resolve objects, whether or not those objects may be visibly resolved. In digital imaging, resolution can be discussed with greater clarity since the fundamental concepts and terms are well-defined and amenable to measure. The concepts of bit depth, gray scale, CCD, noise, pixels and many other relevant terms are briefly defined in the *Glossary*. To simplify the following discussion, the term "objects" are physical items co-located on a flat surface that emit light that is captured by an imaging system. The image information resides in an image data file, and the word "image" most often refers to the data file. A subsequent representation of the image may be printed or displayed on screen for viewing; hence, the image data may be visualized as a "perceived image".

As defined above, an object in a plane is emitting a signal. The signal is captured, and an image represents that object within a measurable precision or level of error. The image data may be conceived as having an X,Y plane and a signal (G) associated with each point on the X,Y plane *see Figure 1*. Each of the image variables X, Y and G have an uncertainty or an error introduced by the imaging system. The capability of the imaging data to resolve two objects depends upon the uncertainty of each of these variables. The uncertainty in the X and Y image coordinates is called the spatial component of resolution, and depends upon the number of CCD pixels and the limitation of all elements in the optical path. The uncertainty in the signal G is called the dynamic range component of resolution and depends upon the CCD and supporting electronics. The smaller any of these uncertainties, the higher the precision and the higher the resolution. While the variables X, Y and G are not necessarily separable variables in an image, resolution is generally simplified into spatial and dynamic range considerations.

Figure 1:  
Resolution and Binning



If the coordinate  $X$  (a position  $X$  in the  $X,Y$  plane of the image) has an inherent error or uncertainty  $\Delta X$ , then the  $X$  coordinate may be divided into  $X/\Delta X$  increments, each one of which significantly differs from its neighbor by the margin of error  $\Delta X$ . *see Figure 1* Similarly,  $Y$  is divided into  $Y/\Delta Y$  increments. The fundamental element having a dimension of  $\Delta X$  and  $\Delta Y$  is defined as the smallest spatial element in the image that may be resolved in the  $X,Y$  plane. Similarly, the signal variable  $G$  has an uncertainty  $\Delta G$ , and can be divided into  $G/\Delta G$  increments. The signal error is generally called noise; hence, the number of significantly different signal increments ( $G/\Delta G$ ) is the signal/noise ratio, which is the most relevant measure of dynamic range. Thus, the smallest increment of the signal scale that may be resolved within the spatial element  $\Delta X \Delta Y$  is  $\Delta G$ . Both the spatial and dynamic range components of an image are discussed in later in this section.



## Spatial Resolution

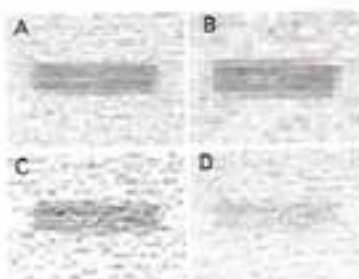
Assuming the spatial resolution is CCD limited, the smallest spatial element in the image is represented by a "pixel", having the dimension  $\Delta X$  by  $\Delta Y$ . Depending upon the image magnification, a certain total number of image pixels of a CCD are applied to an object area, limiting the spatial resolution to a certain number of pixels per object area, often referred to as pixel density. To simplify, the pixel limitation of resolution is usually quoted for only one dimension (as in pixels/mm), assuming that X and Y magnification are identical and the pixels are square. The pixel limited spatial resolution of the IS440cr is variable (zoom lens), ranging from a low resolution of about 2.9 pixels/mm, to a high resolution of about 17 pixels/mm.

Other measures or quotations are often associated with spatial resolution. The measure of dots/inch or dots/mm is often used in the printing industry, and is a true measure of printed resolution; it is clearly analogous to the pixels/mm quoted above. Another common measure used in imaging is line-pairs/mm, a number which corresponds to one half of the above pixels/mm; two pixels are needed to present the same information as one line-pair.

As a general rule of thumb, a minimum of two image pixels or one line-pair must be applied to an object to "resolve" the object in the image. The same applies to the distance between two objects, if the two objects are to be resolved. For example, objects 0.2 mm apart will require at least 10 pixels/mm or 5 line-pairs/mm to discern the space between them (spatially resolve the objects). While this general rule may be a mathematical necessity for spatial resolution, it is not sufficient. Although similar objects within an image captured at a high signal/noise may be well resolved using a spatial resolution of two or more image pixels per object, objects having extreme differences in signal may not be resolved, or objects captured with a low signal/noise may not be resolved.



Figure 2:  
Resolution Depends Upon  
Spatial and Dynamic Range  
Variables



	Pixels/ Distance	Relative Signal	Relative Noise
A	1	1	1
B	1/3	1	1
C	1	1	3
D	1	1/3	1

A relevant example of image resolution is displayed in *Figure 2*, in which two bands are perceived as being nearly “resolved” in the upper left panel (A). If the image is manipulated to reduce the spatial resolution ( $1/3$  pixels/distance (B)), or alternatively manipulated to reduce the signal resolution ( $3\times$  noise) (C) and ( $1/3$  signal (D)), the resolution of the bands is obscured. Hence, image resolution depends on both spatial and dynamic range considerations.

## Dynamic Range

Dynamic range is the name given to the scale of signal or signal/noise ratio of a digital camera system. The maximum signal/noise that is quoted for an imaging system is the ratio of the maximum signal to the minimum noise. The maximum signal of the IS440cf is 12-bits per capture, or 4,096 signal levels. The minimum noise is 2.4 signal levels. Therefore, the maximum signal/noise ratio ( $4,096/2.4$ ) is about 1700, and is related to the number of significantly different signals  $G/\Delta G$  in the above discussion of *Resolution*.

The dynamic range of a camera may be increased by adding different image captures of an object together. The signal scale must be increased to accommodate higher numbers; the IS440cf software provides a 16-bit scale (65,536 levels), providing for the accumulation of multiple images. While signal among accumulated images directly adds, the noise among the images generally does not. If the noise level of a series of independent (but otherwise identical) images is random, then the noise level of the accumulated image will be the product of the noise (of any one image) and the square root of the number of accumulated images. Thus, the signal/noise ratio of an accumulated image is increased by the square root of the number of images participating in the accumulation. The maximum signal/noise ratio of the IS440cf accumulated image is ideally achieved with the addition of 16 images, giving a maximum ratio of  $65,536/(2.4\sqrt{16})$ , which is about 6,800.

## Noise

Image noise is random and/or persistent deviations in signal and is identified with the signal error or uncertainty  $\Delta G$  in the above discussion of *Resolution*. Noise may be measured among pixel signals within an image (for example, background), or among the signals derived from the same pixel from different but otherwise identical images. Noise is most often represented as a standard deviation of a collection of signals about a mean. Noise arising from a digital camera system has two distinct causes:

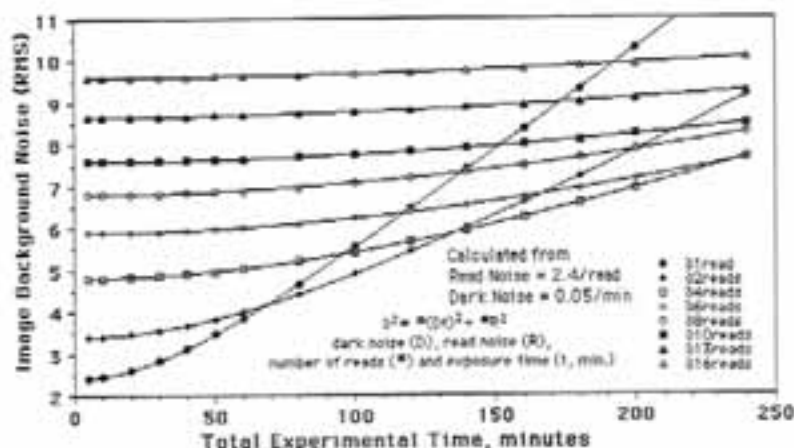
- The supporting electronics contribute "read" noise as the CCD pixel signals are converted to digital signals. The read noise may appear nearly random in a single capture, but usually has subtle non-random components, which become apparent upon adding a large number of images wherein structured features are obvious. For a single exposure (one "read"), the read noise of the IS440cr is about 2.4 signal levels using a 12-bit scale.
- The dark (no light) charge accumulation within a CCD pixel increases with exposure time contributing "dark current noise". Dark current noise is directly related to exposure time, and is significantly reduced with operating temperature (thermoelectric cooling). The "dark" component of noise is generally quoted as a coefficient of time. The IS440cr has a cooled CCD, and the nominal dark noise coefficient is about 0.001 signal levels per second of exposure using a 12-bit scale.

Noise components in imaging generally add according to the square root of the sum of the squares of the components. As a result, the noise within any image may be predicted for the IS440cr, using the relation:

$$\text{Total Noise} = \{N(D^2t + R^2)\}^{1/2},$$

where  $N$  = number of image captures,  $D$  = the dark noise coefficient (signal levels per second),  $t$  = the exposure time per image capture, and  $R$  = the read noise. A graphical representation of the above relation appears as follows.

IS440CF Noise Estimates



Upon studying the graph or manipulating the above relationship, it becomes clear that guidelines relating to the number of captures and exposure time per capture can be established for the IS440CF to minimize noise. There will be some variation among IS440CF cameras, and ambient temperature will influence the dark noise. It is most likely, however, that a single capture will minimize noise for total elapsed times of less than 30 minutes.

Note that the above guidelines are based upon image noise only, and give no consideration to signal or other aspects of the typical image capture. The above guidelines relate to those captures that are signal-limited, typical luminescent captures, wherein noise minimization is crucial. For those imaging experiments with ample signal to fill the available scales in a relatively short amount of time, the above guidelines do not relate. In most fluorescent images, the experimental background noise will be determined by the materials used in the experimental sample rather than the noise contributed by the electronic system; thus, a large number of "reads" will not significantly detract from the image quality, and may lend an element of experimental convenience as the image display and histogram "grows" in significance as the images accumulate.



## Binning Pixels

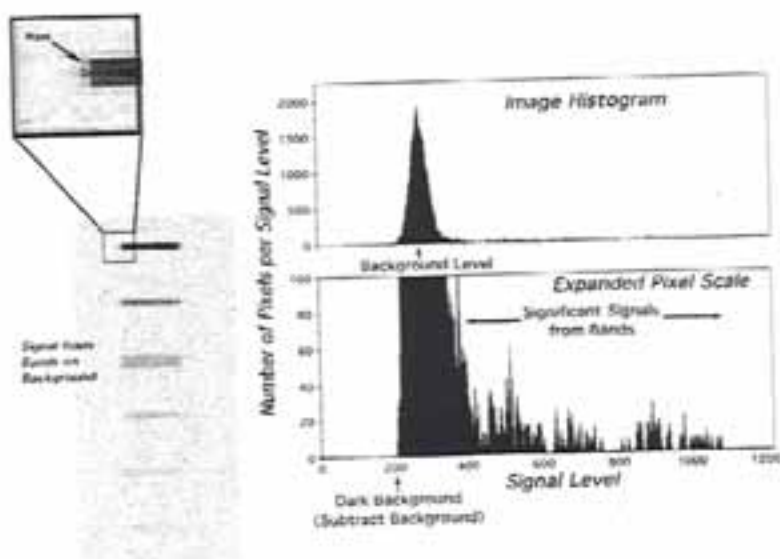
The total amount of information the image file is capable of containing is the summation of all elements in the X,Y,G volume, or the product  $(Y/\Delta Y)(X/\Delta X)(G/\Delta G)$  (*figure 1*). Just as information is the capability to distinguish between alternatives, then the total volume of the cube is related to the image resolution and it is the basis for discerning objects. Note that the information volume may be unchanged if the shape of the cube is changed or distorted (any one dimension may increase with a proportional decrease of another).

The CCD has a special hardware capability that can transform the shape in this information cube. The capability is called binning, and it is the addition of the signal levels of adjacent pixels without the addition of noise. If adjacent pixels are binned in the Y dimension yielding half the Y levels, the number of levels on the signal scale will double. Should both the X and Y dimensions be binned, the signal scale will be quadrupled. The volume of the cube remains the same, hence the image resolution has not changed. If an image has a spatial resolution that far exceeds the need to resolve an object, then the apparent resolution of the object is increased by binning. This increase is usually associated with an apparent increase in detection sensitivity when binning is effectively applied.

## The Image Histogram

The Image Histogram, as used in the IS440cf Image Acquire Software, is a graphical representation of the manner in which pixel signals are distributed on the scale of signal level, *see figure*. The histogram is a frequency diagram, where the number of pixels having a particular signal level are plotted as a function of the signal level, and it is a most convenient graphic with which dynamic range is visualized and manipulated. Following imaging conventions, the scale of signal increases from left-to-right. Perceived image features emitting signal appear bright, thus the visual gray scale has an orientation that is the reverse of the image signal scale. Should the perceived gray scale be aligned with the signal scale, the image will appear with an inverted gray scale and an option to Invert an image is provided in both the IS440cf Image Acquire and the Image Display in 1D Image Acquire Software. It is intended to aid in viewing an image, since the perception of a weak signal over a background is generally aided by viewing the inverted gray scale. Invert has no influence on the image data or histogram.

The Image Histogram





Other viewing aids offered within the histogram are contrast controls and "Gamma". The contrast controls are sliding adjustments beneath the histogram that define the white and black points of an image display, allowing for sampling a range of signal levels. Sampling a range of signals enables the viewing of different data "windows" of the imaging file, and is necessary to fully appreciate the imaging information provided by the extensive signal scale of the IS440cr. The necessity arises from the fact that human perception has a dynamic range of only about 7-bits (128 signal levels of resolution), while the imaging file can have a dynamic range that exceeds 62,000 signal levels. A further aid to viewing an image is an adjustment called Gamma which causes the signal scale to map to a image gray scale in a skewed manner. The benefit of altering Gamma is to enable the viewer to selectively increase the contrast of features of interest within an image.

The appearance of a histogram will depend upon the features within the image. Generally, an image with many features of differing signal levels will have many peaks distributed throughout the signal scale. The most common histogram appearance using the IS440cr will be that of a luminescent image, consisting of several bands on a background of minimal signal as displayed in the *Figure 3*. Note that the image is inverted (dark signal, light background) and the vast majority of pixels (many thousands) have a common, low signal associated with the background peak of the histogram. The mean of the background pixel signals is identified as the background level of the image, and the variation of the signals about the mean (width of the peak) is identified with the image noise caused by the system or the sample. The relatively few pixels (hundreds) associated with the bands are distributed throughout the signal scale, and are noticed only if the scale is expanded.

## Background Subtraction

The background signal level shown in the *histogram figure* is associated with the absence of experimental signal. It is a peak of signal frequency, having a mean background level and a breadth related to image noise. The low signal side of the peak diminishes to a pixel frequency of zero at a signal level of about 200 on a 4,096 scale for a single image capture of the IS440cr. Below the level of zero pixel frequency (200 in this case) there is no image information, and this level is simply the total electronic background. The electronic background is composed of a dark current (electronic charges emanating from the CCD and amplifier), and an intentional voltage offset to assure that the electronic background is always positive.

The electronic background diminishes the total dynamic range of the IS440cr by a small amount, resulting in a net signal level maximum of about  $4096 - 200 = 3896$ . While the background is small, it may be inconvenient or detrimental to a subsequent analysis and it is best subtracted. Electronic background subtraction is managed by the ImageStation Image Acquire Software.

Electronic background subtraction must be executed with sufficient precision to assure that the zero-level of signal equals the actual level of no light. Given an appropriate no-light/zero-signal, lens and illumination correction may be executed with precision (see Appendix C). As many as half the pixel signal levels become zero upon subtracting electronic background from a perfectly dark image. Only the upper half of the background noise peak represented by the histogram (see *histogram figure*) remains, consequently estimates of the background electronic noise are distorted, but are nevertheless consistent throughout the image. Subsequent lens correction will further distort the background noise with a function that increases in a radial manner (from the middle of the image outward). Should an image of uniform background noise be desirable for analysis, the file must be saved prior to lens correction.

Software management of the electronic background is camera specific, and the only the software registered to the specific IS440cr camera properly encodes the appropriate background and defective pixel information.

## APPENDIX B: UV ILLUMINATION

In the following section, you will learn about the principles of UV illumination as they relate to the IS440cr.



**SAFETY NOTE:** According to "Recommended Practice for Photobiological Safety for Lamps & Lamp Systems - General Requirements (ANSI/IESNA)", the UV light emitted from the IS440cr during normal or continuous use is safe for the general population. The safety of the IS440cr relates to both skin and eye exposure. By comparison, the UV exposure is similar to that received from common overhead lighting in laboratories, and is much less than that received by sunlight at sea-level. Hence, no safety warning is issued, nor is any appropriate, for the normal use of the IS440cr. However, as noted in the cited publication, there are certain photosensitive individuals whose tolerance to UV is reduced, but guidelines have not been established.

## IS440CF UV Illumination

The Image Station 440CF supplies UV irradiation to the sample platen from the inside of the chamber. The light is directed at a 45° angle to the underside of the platen, which is generally called "epi-illumination". The illumination system has been designed to optimize the physical features that relate to the fluorescent excitation of samples placed on the platen, among which are:

- A. The optical elements that manage the illumination have been designed to provide a reasonably uniform distribution of excitation energy over the platen area. While it is not possible to design absolutely uniform illumination from light sources at the distance used in this system, the design does achieve a uniformity that is at about the same quality that a camera lens can represent. The 1D Image Analysis Software provides corrections that address both the lens and illumination distortions, thus contributing to a quality analysis of the image data.
- B. The epi-illumination angle requires that the excitation photons interact with the sample and be re-emitted in order to have an angle appropriate to enter the camera lens. Photons simply reflected by a planar surface at the platen will not enter the lens, obviating the required use of special optical filters that reject the excitation energy. However, reflected light from curved surfaces in a sample can enter the lens and will present artifacts in fluorescent images, limiting the useful application of the filters provided in the IS440CF to thin samples. For some applications, specialized filters may be needed.
- C. The energy spectrum of the illumination has been designed to provide a broad range of light (300–400 nm, see figure) within the range of wavelengths called the UVA. The 300–400 nm range of wavelengths effectively excites a very large number of fluorochromes in common usage. Even fluorochromes that are particularly suited to laser-excitation at a much higher wavelength may also be excited by the IS440CF UV illumination.
- D. Compared to commonly used UV transilluminators or hand-held UV light source, the energy spectrum of the IS440CF UV illumination system emits very little energy below 300 nm (see figure). Compared to the 300–400 nm range of light, light below 300 nm contributes very little to the excitation of fluorochromes in common usage, but does substantially contribute to the general





fluorescence of commonly used materials in laboratory samples. Hence, lower autofluorescent background may be anticipated in the fluorescent image applications supported by the IS440cf.

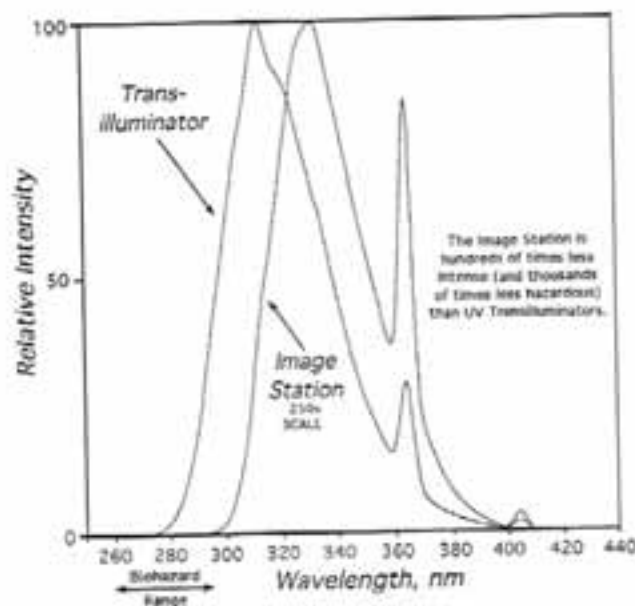
The absence of energy below 300 nm in the IS440cf contributes to its safe usage. Wavelengths below 300 nm are hazardous (see figure and safety statement).

- E. The total energy of the IS440cf UV illumination is very low, nominally  $10 \mu\text{W}/\text{cm}^2$  at the platen surface. However, the digital camera is sufficiently sensitive to assure that fluorescence data can be accumulated over a reasonable period of time with UV illumination, provided. Additionally, the low intensity reduces the possibility that a fluorescent response is the result of multi-photon molecular processes that can contribute to the autofluorescence of solid materials commonly used in laboratory samples (such as nylon).

The reduced energy of the IS440cf UV illumination eliminates the possibility of visualising the fluorescence of a sample, so its usefulness for cutting bands out of gels is precluded, and should never be attempted on an optical surface critical to image quality.

The total energy emanating from the IS440cf platen is more than 250-fold less than commonly used UV transilluminators, contributing to its safe usage (see the following figure and safety statement).

Emission Spectra of UV Illumination





B-4



## APPENDIX C: IMAGE CORRECTION

In the following section, you will learn about the principles of image correction as they relate to the IS440CP.

## IS440cf Image Correction

Image capture with a CCD camera has the distinct advantages of speed and nearly live representation of image data. There are associated disadvantages, the most important of which are the image distortions associated with any lens and defects associated with the CCD sensor. Another important image correction is that associated with the non-uniform illumination of the object.

The removal of defective pixel information is automatic. Defective pixels are characteristic of a particular CCD, have elevated signals (dark current), are always present and amount to about 0.5% of all pixels. Defective pixels in an image are corrected by recalculating their signal value by interpolating the values of their nearest neighbor pixels. For long exposures times (15 minutes or more) occasional "dark" (or high-signal) pixels may be observed.

Generally, you will find those pixels will not be reproduced in other exposures. The "spontaneous" dark pixel cannot be prevented and is likely the result of a cosmic ray.

The lens distorts the image of a flat object in two significant ways: the object intensity is reduced as a function of the distance from the center of the object, and the magnification of the image is slightly increased as a function of the same distance (referred to as spherical aberration or "pin-cushion" distortion). Some distortion is apparent using the widest angle of zoom (12.5 mm). Most important to quantitative imaging is the intensity correction associated with the lens, which is very substantial at the periphery of wide-angle fields. The correction for intensity is provided in the IS440cf system, and is applied at the option of the user upon submitting the captured image to 1D Image Analysis Software. At the time of submission, the *f*-stop and zoom information is requested. Upon accurately providing that information, an appropriate image transform is applied to the image to correct all intensity information throughout the field of view. This correction will apply to any luminescent image and transmission images in which care is taken (proper use of the Light Diffuser) to supply uniform illumination to the platen. Use of a filter should not significantly influence the correction. Except for the very corner of the field at the widest angle and the lowest *f*-stop, the representation of the intensity will conform to a nominal 1% accuracy.

For UV illuminated images, field illumination correction should also be applied. The extent of UV illumination non-uniformity will cause approximately the same amount of quantitation error as the lens distortion of the IS440cr. It is critical to capture a reference image (to be used for image correction) of the illumination field using optical parameters (*f*-stop and zoom) that are identical to an image that is to be corrected. The illumination non-uniformity is highly reproducible and may be corrected by mathematically dividing an image by a reference image captured by the IS440cr, using the protocol outline in the Generating an Illumination Reference File in *Chapter 3: Capturing an Image*.

The reference image used for correction will be filtered to remove noise and artifacts (such as scratches and particulate dust). The filtered reference image is normalized and divided into the experimental image to be corrected. Regions of the experimental image that experienced a greater-than-average illumination are divided by a number greater than one, while those experiencing a less-than-average illumination are divided by a number less than one.

It is recommended that multiple reference images at varying *f*-stops and zooms be captured and clearly designated as a library of images from which reference images may be selected for use in illumination correction. For the sake of accuracy, create reference files at all *f*-stop and zoom settings commonly used, and make certain that the settings are made and documented precisely; it is suggested that only *f*-stop and zoom adjustments that are indexed be chosen for experiments or reference files.

It is likely that such a library could be applied for an extended time period, because the field illumination should be constant for many hundreds of hours. A suggested library of UV illumination reference files is listed in following (15 reference files).

*Suggested Library of UV  
Illumination Reference Files*

<i>f</i> -stop Setting	Zoom Setting
1.2	12.5, 20, 30, 50 and 75 mm
2.0	12.5, 20, 30, 50 and 75 mm
4.0	12.5, 20, 30, 50 and 75 mm

C-4



## **APPENDIX D: KODAK RELATED PRODUCTS**

Kodak products related to Kodak Digital Science Image Station 440CF are included in this section.

## Ordering Information

The following is a partial listing of Kodak products. Please visit our Worldwide Web site at <http://www.kodak.com> or refer to your Scientific Imaging Systems catalog or call (800) 225-5352 or (716) 722-5813 for information on our full line of products.

### Kodak Digital Science Image Analysis Software

Kodak Digital Science 1D Image Analysis Software	(Macintosh)	836 7526
Kodak Digital Science 1D Image Analysis Software	(Windows)	894 3771
Kodak Digital Science 1D Image Analysis Software Administrator	(Network-Macintosh)	862 0940
Kodak Digital Science 1D Image Analysis Software Administrator	(Network-Windows)	182 5512
Kodak Digital Science 1D Image Analysis Software User License	(Macintosh)	191 0868
Kodak Digital Science 1D Image Analysis Software User License	(Windows)	140 7584
Kodak Digital Science SQ Image Analysis Software	(Macintosh)	162 9971
Kodak Digital Science SQ Image Analysis Software	(Windows)	872 9535

### Kodak Digital Science Cameras and Accessories

Kodak Digital Science DC120		852 4399
Kodak Digital Science Gel Accessory Kit		177 6871
Kodak Digital Science DC120 Microscopy Accessory Kit	(Macintosh)	800 6066
Kodak Digital Science DC120 Microscopy Accessory Kit	(Windows)	885 5302

### Kodak Digital Science Electrophoresis Documentation and Analysis System 120 (EDAS120)

Kodak Digital Science EDAS 120	(Macintosh)	103 8009
Kodak Digital Science EDAS 120	(Windows)	154 9393

### Kodak Digital Science Microscopy Documentation System 120 (MDS 120)

Kodak Digital Science MDS 120	(Macintosh)	881 5545
Kodak Digital Science MDS 120	(Windows)	885 0539

Kodak Digital Science Color Printers and Accessories

Kodak Digital Science SP700 Color Printer, 120 VAC	815 0294
Kodak Digital Science SP700 Color Printer, 120 VAC, Japan	809 2645
Kodak Digital Science SP700 Color Printer, 220 VAC	169 6293
Kodak Digital Science SP700 Color Printer Print Media Kit, 50 prints <i>-includes printer ribbon and 50 sheets of electronic imaging paper</i>	133 1750
Kodak Digital Science SP700 Color Printer Print Media Kit, 100 prints <i>-includes printer ribbon and 100 sheets of electronic imaging paper</i>	129 9023

Life Science Imaging Films

Kodak BioMax MR-1, 20.3 x 25.4 cm (8 x 10 in) <i>Non-interleaved Pack</i>	50 Sheets	870 1302
Kodak BioMax MR-1, 13 x 18 cm (5 x 7 in) <i>Non-interleaved Pack</i>	50 Sheets	894 1114
Kodak BioMax MS-1, 20.3 x 25.4 cm (8 x 10 in) <i>Non-interleaved Pack</i>	50 Sheets	829 4985
Kodak BioMax MS-1, 13 x 18 cm (5 x 7 in) <i>Non-interleaved Pack</i>	50 Sheets	111 1681
Kodak X-Omat AR-5, 20.3 x 25.4 cm (8 x 10 in) <i>Interleaved Pack (Sheets of paper inserted between sheets of film)</i>	50 Sheets	165 1454
Kodak X-Omat AR-5, 13 x 18 cm (5 x 7 in) <i>Interleaved Pack (Sheets of paper inserted between sheets of film)</i>	50 Sheets	165 1496



# GLOSSARY

**Analog Signal**

A signal that is continuous and uninterrupted. Variations in voltage correspond to variations in brightness. This differs from digital, where a signal is represented in discrete steps of digital value (1's, 0's).

**Analog to Digital Converter (A/D)**

An integrated circuit that transforms an analog (voltage level) to a binary (or digital) value. The IS440cr camera converts the analog signal into a digital signal using an A/D converter.

**Aperture**

The opening within a lens that limits light passage, usually adjustable and referred to as the *f*-stop.

**Autoradiography**

A method of capturing radioactive signals by contact-exposure of film to a flat sample consisting of radioactive objects or labeled molecules.



**Band-Pass Filters**

Clear optical elements that transmit only a limited spectrum of light (limited range of wavelengths) and may be of two general types: colorimetric or interference.

**Binning**

The process by which adjacent CCD signal values are added together and then each pixel value is replaced with the new added value. For example, if two adjacent pixels have an intensity value of 100 and 150, when binning has been applied, both pixels will be assigned a value of 250. Binning can be applied on x and/or y axis of an image. While binning decreases the resolution of the image, it increases the sensitivity of the signal.

**Bit (Binary Digit)**

The smallest amount (unit) of information, measured on a binary scale (integer exponents of 2), where combinations of 0's and 1's are used to code information. Refer to *Bit Depth*.

Eight bits of information is called one byte.

**Bit Depth (Bits-Per-Pixel or Pixel Depth)**

The number of intensity values that can be assigned to each pixel. Images usually fall between 8 and 24 bits. The Image Station 440ce camera produces a 12-bit image in the single capture mode or 16-bit in the multiple capture mode. A 12-bit image has 4,096 gray levels, while a 16-bit image has 65,536 gray levels.

**Black Point**

Corresponds to an intensity value in the image that represents pure black in the screen image. The black point can be adjusted using the histogram sliders to help visualize different features in the image. Features with intensity values below the black point can no longer be seen in the screen image. Adjustments to the black point alter the screen image and do not alter the data that is transferred to 1D Image Analysis Software for analysis.

**Blooming**

If the CCD is overexposed, the CCD pixels do not have the capacity to hold the charge and will "bloom" or spill into the adjacent pixel. Blooming distorts the image. However, excessive signal may often be associated with the property of the object and not the blooming of the CCD.

























**Blot**

A methodology in which molecules/particles of interest are transferred and affixed to a solid support (membrane), usually for the sake of detection and imaging. A liquid sample may be applied to a membrane as a spot, dot or slot (band), defining a formatted sample array as a Dot or Slot Blot. Electrophoresis gels used to length-resolve DNA, RNA or protein molecules may be similarly transferred to membranes by mechanical extrusion or electrophoresis; such blots are respectively called Southern, Northern or Western Blots.

**Brightness**

A relative measure of light associated with a pixel representing its gray level from black and white, through intermediate levels of gray.

Perceived brightness increases from dark to bright, or black to white through intermediate levels of gray. However, the convention of quantitative imaging is quite the opposite, wherein the gray scale is increased from white to black.

**CCD (Charged-Coupled Device)**

An electronic sensor used for imaging light. A silicon crystal, etched to produce micro-electronic circuitry, for the purpose of transforming a real image into an electronic image. A photon impinging upon the silicon is converted to an electron (a negative charge, stable in the crystal matrix), that may be subsequently managed (collected and transported) by the circuitry. The CCD in the IS440cr has  $752 \times 582 = 437,664$  imaging elements (pixels) defined by etchings.

**Chemifluorescence**

Chemically mediated production of a fluorochrome. Fluorescent molecules may be produced by the chemical (enzymatic) conversion of a non-fluorescent molecule (substrate), upon excitation (laser or UV illumination).

**Chemiluminescence**

Chemically mediated production of light. Luminescence or light emission may be produced by a chemical reaction or an enzyme operating on a substrate.

**Contrast**

The contrast of an image represents the perceived differences in intensity between dark and light areas within the image. A low contrast image contains gray levels that are similar in visual intensity, whereas a high contrast image contains extreme differences in visual intensity.

**COPI (Closed Optical Path Image)**

Chamber design used in the IS440cr. Provides a light-tight environment for imaging laboratory samples, reducing the possibility of contamination of the optical path of the instrument.

**Dark Current Noise**

Electronic noise inherent to the CCD sensor and circuitry, which is increased with extended exposure time. Cooling the CCD helps reduce dark current noise. The IS440cr camera is capable of about 1 hour exposure times with only a doubling of noise.

**Digital Camera**

A camera that uses an electronic sensor to record an image in a digital binary format.

**Dynamic Range**

The maximum range of significant digits over which system hardware performs electronic imaging, usually expressed as a signal-to-noise ratio. The IS440cr camera provides a 12-bit (0-4,095 gray levels) signal range for single captures. These single captures can be accumulated by addition in software into a 16-bit file to provide a signal range of 0-65,535 gray levels. With a noise level of approximately 2.4 levels for a single capture, the maximum dynamic range of signal-to-noise is about 1,700 for single captures and about 6,200 for accumulated images.

**Electrophoresis**

Separation of molecules on the basis of charge and size.

**Epi-illumination**

Illumination of an object in a direction other than the optical axis defined by an object and sensor. IS440cr illuminates objects at a 45° acute angle from the optical axis of the lens.

**Floating Point**

A means of representing a signal as real numbers (fractions or decimals). Floating point calculations are important in maintaining the accuracy of analysis data. The 1D Image Analysis Software uses floating point numbers.

***f*-stop**

The ratio of focal length to the aperture diameter in the lens.

**Fluorescence**

The emission of light from a substance having been excited by light absorption.

**Gamma**

A mathematical transformation function that can be used to improve image appearance by decreasing or increasing the contrast of an element of interest in an image. Adjusting the gamma of an image disproportionately skews the gray level distribution, higher gamma values lighten the image and lower gamma values darken the image. Adjusting the gamma does not alter the image data file and is only used to enhance the viewing of the image.

**Gel**

A separation matrix which is typically agarose or acrylamide and is used for electrophoresis.

**Gray Level**

The digital signal assigned to a pixel associated with a level of light (from black to white). For example, an 8-bit and a 16-bit system includes gray level values between 0-255 and 0-65,535, respectively.

**Histogram**

The frequency of the distribution of pixels over the range of signals within an image. The horizontal axis represents the gray level and the vertical axis represents the number of pixels. See Appendix A.

**Illumination/Detection Mode**

Method or style of illuminating a sample for detection by imaging. The IS440cr supports the detection of samples using three illumination modes: (1) No illumination mode for Luminescence detection, (2) Transmission mode (using external illumination) for Absorbance detection, and (3) UV Illumination mode for Fluorescence detection and Fluorescence Quenching/Absorbance detection.

**Image Accumulation**

The addition of multiple exposures (also referred to as frame captures). The data from all the summed images are used to generate the final image for analysis.

**Image Compression**

A computational operation upon image data that results in a reduction of data storage volume. Usually results in some loss of image data.

**Interpolation**

A numerical estimate of a value within a range of empirical data, based on the mathematical trend of data. Contrasts with extrapolation, in which a value outside the range of data is estimated.

**Noise**

Random and/or persistent deviations in signal. In a digital camera the CCD has noise associated with the collection, reading and amplification of the signal that may affect image quality. The IS440cr camera reduces noise by cooling the CCD using thermoelectrical cooling. See also *Dark Current Noise* and *Read Noise*.

**Photon**

The smallest unit of light, or any electromagnetic radiation. Characterized by wavelength.

**Pixel**

The fundamental element in a digital image. In a digital camera, the pixels represents the light sensitive elements on the CCD.

**Read Noise**

Electronic distortions in signal introduced when converting the analog signal to a digital signal.



**Resolution**

The capability to distinguish between objects of interest. It is customary, when describing the characteristics of a digital imaging device or image, to describe the resolution by specifying the number of pixels it captures in the horizontal by vertical direction.

**Saturation**

The limitation of signal imposed by either the CCD or the digital scale in which the signal is being represented. If the signal becomes too large the individual pixels on the CCD will be filled and no additional signal can be detected.

**Thermoelectrical Cooling**

A system that uses electron flow through semiconductors to generate temperature differences. The CCD is mounted on the cool side and the heat is dissipated by cooling fans. IS440cF uses a thermoelectrical cooling system to reduce image noise generated by the heat from within the camera's CCD.

**TIFF**

Tagged Image File Format (TIFF) is an industry standard file format for storing images. Images from 1D Image Analysis Software can be saved in TIFF format for use with other computer programs.

**Transmission**

Mode of image capture, usually absorbance, in which light passes through the illuminated object.

**Ultraviolet Light (UV)**

Generally refers to the spectrum of electromagnetic energy from about 4 to 400 nm in wavelength. Wavelengths of 200-400 nm are useful in the usual laboratory environment.

**Vignetting**

An imaging artifact that is associated with the limitation of the lens. Vignetting generally appears as signal reduction around the edges of the image.

**White Light**

Light that is in the visual part (400-700 nm) of the spectrum (daylight, regular light bulbs).

**White Point**

Corresponds to an intensity value in the image that represents pure white in the screen image. The white point can be adjusted using the histogram sliders to help visualize different features in the image. Features with intensity values above the white point can no longer be seen in the screen image. Adjustments to the white point alter the screen image and do not alter the data that will be transferred to 1D Image Analysis Software for analysis.

**X and/or Y Binning**

*Refer to Binning.*



**A**

- absorbance 3-4
  - exposure times 3-13 to 3-19
- Acquire Software
  - installation 6-8, 6-9
  - launching 3-6
  - license Agreement 7-4 to 7-7
  - Preferences 2-15
- Acquire Window 2-11 to 2-15
  - menus 2-11
  - Overview 2-11
  - Status Bar 2-11, 2-14
- Auto Contrast 2-13, 3-20

**B**

- binning G-2
  - X 2-12
  - Y 2-12
  - applying 3-15
- Black Point 2-13, 3-20, G-2

**C**

- Cable Connector, Camera 2-3
- Camera 2-8, 2-9
  - Cable Connector 2-3
  - Calibration File 2-15
  - CCD 1-1, 2-2, 2-8, 2-9, G-3
  - digital G-4
  - f*-stop Ring 2-9
  - Focus Ring 2-9
  - Lens 2-9
  - Orientation 2-15
  - Preferences 2-15
  - Resolution 1-1, G-7
  - Zoom Ring 2-9
- Camera Cable Connector 2-3
- Capture Slider 2-12

## Capture System Chamber 2-2

- Camera 2-8
- f*-stop Ring 2-9
- Filter Selection Dial 2-9
- Focus Ring 2-9
- Lens 2-9
- UV ON/OFF Switch 2-9
- Zoom Ring 2-9

## Chamber

- Capture System 2-2
- Closed Optical Path Image Chamber 2-2
- Charged-Coupled-Device (CCD) 1-1, 2-2, 2-8,  
2-9 G-3

## Circuit Breaker 2-3

- Closed Optical Path Image Chamber 2-2, 2-6, 2-  
7, G-4

## COP1 2-2

## Compression Pad 2-4

## Clip 2-4

## Contrast Slider 2-13, 3-2, 3-21, G-4

**E**

## Epi-Illumination 3-3, 3-4, G-4

## Exposure

- Button 2-12
- Preferences 2-15
- Progress Indicator 2-12
- Setting the Time 3-15 to 3-18
- Status Bar 2-11, 2-14
- Time Slider 2-12

**F***f*-stop G-5

## Setting 3-14

## File

- Camera Calibration 2-15
- Illumination Reference 3-23 to 3-26

- Filter 2-10, G-2
  - Access Panel 2-10, 4-3
  - changing 4-3
  - choosing 3-10 to 3-11
  - Selection Dial 2-9, 4-3, 4-4
  - Wheel 2-2, 2-10
- Filter Access Panel 2-10
- Filter Selection Dial 2-9
- Filter Wheel 2-2, 2-10
- Fluorescence G-5, 3-3
  - exposure times 3-13 to 3-19
  - quenching 3-4
- Front View 2-2
- G**
  - Gamma 3-13, 3-20, 3-21, G-5
- H**
  - Histogram G-5
    - Auto Contrast 2-13
    - Contrast Slider 2-13
    - Image 2-13
- I**
  - Illumination
    - choosing conditions 3-10 to 3-12
    - guidelines 3-2 to 3-5
    - None 2-7
    - Reference File 3-23 to 3-26
    - summary chart 3-5
    - Transmission 2-6
    - UV, Schematic 2-7
  - Image
    - Capture Settings 2-11, 2-12
    - Capturing 3-1 to 3-28
    - Contrast 3-20, 3-21, G-4
    - Correction C-1 to C-4
    - Histogram 2-11, 2-13
    - Preview Mode 2-12
    - printing 2-11, 2-14, 3-27
    - resolution G-7
    - Submit 2-11, 2-14, 3-22 to 3-26
    - viewing 3-20, 3-21
  - Image Analysis Software, 1D
    - installation 6-4
    - launching 3-6
    - license agreement 7-4 to 7-7
    - package contents 1-4
  - Image Capture 3-1 to 3-28, 3-13 to 19
    - guidelines 3-2 to 3-5
    - preparing 3-6 to 3-12
    - submitting a 3-22 to 3-26
    - viewing 3-20 to 3-21
  - Image Capture Settings 2-11, 2-12
    - Capture Slider 2-12
    - Expose button 2-12
    - Exposure Progress Indicator 2-12
    - Exposure Time Slider 2-12
    - Preview Mode 2-12
    - Stop button 2-12
    - Stop Image if Saturated 2-12
  - Image Histogram 2-1, 2-13
  - Image Resolution A-2 to A-3
  - Installation 6-1 to 6-10
    - 1D Image Analysis Software 6-4
    - Camera Calibration File 6-9, 6-10
    - electrical requirements 6-2
    - IS440 Image Acquire Software 6-8, 6-9
    - PCI Card 6-5 to 6-7
    - Space requirements 6-3
    - temperature and humidity requirements 6-2
  - Invert
    - camera 2-15
    - image 2-13, 3-21

**L**

- Lid 2-2
  - Clasp 2-4
  - Gasket 2-4, 4-5 to 4-7
- Light Diffuser 2-6
- Luminescence 3-2
  - exposure times 3-13 to 3-19

**M**

- Maintenance 4-2
- Manual, User's
  - about the 1-2
  - conventions 1-2
  - navigating 1-3

**P**

- PCI Card 2-3
  - installation 6-5 to 6-7
- Platen
  - Cleaning 3-7
  - Gasket 2-5
- Power Cord 2-3
- Preferences
  - Acquire Software 2-15
  - Camera 2-15
- Printing
  - Quick 2-11, 2-14, 3-27

**Q**

- Quick Print 2-11, 2-14

**R**

- Radiator 2-2
- Rear View 2-3
- regulatory information 7-8

**Related Products D-1 to D-4**

- requirements, installation
  - electrical 6-2
  - space 6-3
  - temperature and humidity 6-2
- resolution
  - camera 1-1, G-7
  - principles A-2 to A-5
- sample
  - illumination 3-2 to 3-5
  - orienting 2-13, 2-15, 3-7
- Sample Work Area 2-2
  - Lid 2-2, 2-4
  - Platen 2-5
- Software
  - 1D Image Analysis 3-6
  - Image Acquire, IS440cr 2-11, 2-15, 3-6
  - license agreements 7-4 to 7-7
- Status Bar 2-11
- Stop button 2-12
- Submit 2-11, 2-14
- System, IS440cr
  - components 1-4
  - front view 2-2
  - installation 6-1 to 6-10
  - Lid 2-2, 2-4
  - maintaining 4-1
  - overview 2-1 to 2-15
  - registering 1-5
  - regulatory information 7-8
  - repacking 6-11
  - requirements 6-2 to 6-3
  - troubleshooting 5-1 to 5-8
  - unpacking 6-4

**T**

- Technical Support 1-6, 1-7, 5-2
- Transmission 3-4, G-7
  - schematic, IS440cr 2-6

Troubleshooting 5-1 to 5-8  
  instrument 5-3  
  image 5-4 to 5-6  
  software 5-7

## U

User's Manual  
  about the 1-2  
  conventions 1-2  
  navigating 1-3  
UV Illumination B-1 to B-4, G-7  
  schematic, IS440cx 2-7

## W

warranty 7-1 to 7-7  
  registration 1-5  
  time period 7-2  
White Point 2-13, 3-20, G-8

## X

X Binning. *See Binning*

## Y

Y Binning. *See Binning*