Storm

User's Guide
for Microsoft Windows

um 363-367

Amersham Biosciences

The Storm instrument is covered by one or more of the following U.S. patents: 5,528,050; 5,578,818; and foreign equivalents.

The Storm system is for research purposes only. It is not intended or approved for diagnosis of disease in humans or animals.

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Table of contents

Chapter 3 Getting started

Part two Scanning in the storage phosphor mode

Chapter 4 About storage phosphor screen autoradiography

Chapter 5 Preparing for storage phosphor screen autoradiography

Part three Scanning in the fluorescence mode

Chapter 7 About fluorescence scanning

Chapter 8 Setting up for fluorescence scanning

Chapter 9 Scanning the fluorescent sample

Part five Appendixes

Preface

About this user's guide

The *Storm™ User's Guide* provides information on how to use the Storm system to scan radioactive and fluorescent samples. This guide includes information for the Storm 860, 840, 830, and 820 instruments. The Storm system operates on a computer running the Microsoft™ Windows™ operating system.

- **Part one: Introduction**—Describes the Storm instrument, the safety issues, and how to get started.
- **Part two: Scanning in the storage phosphor mode**—Describes how to prepare a sample and expose it to the storage phosphor screen. Describes how to scan the screen using the Storm instrument and the Scanner Control software.
- **Part three: Scanning in the fluorescence mode**—Describes how to prepare a fluorescent sample. Describes how to scan the sample using the Storm instrument and the Scanner Control software.
- **Part four: Maintaining the Storm system**—Describes how to maintain the Storm instrument, Image Eraser, and exposure cassettes.
- **Part five: Appendixes**—Includes troubleshooting and reference information.

Related publications

In addition to the *Storm User's Guide*, you should be familiar with the ImageQuant™ User Documentation, which consists of the following:

- *ImageQuant User's Guide*—A step-by-step guide for all major functions of the ImageQuant software.
- *ImageQuant Reference*—A reference, by menu item, for each function of the ImageQuant software.
- *ImageQuant Tutorial*—A tutorial that highlights some of the features of the ImageQuant software.
- *ImageQuant Utilities User's Guide*—A user's guide that describes the FluorSep™ and ImageQuant Tools utilities.

Safety

Trained operator

Warning ⚠

The operator of the Storm instrument is assumed to be trained in the correct operation of the instrument and the safety issues. Throughout the *Storm User's Guide,* the word "you" refers to the trained operator.

Chapter 2 in this guide provides important safety information that should be used when operating the Storm instrument. Before using the Storm instrument, read and understand the safety information thoroughly.

Special safety text

Make sure you follow the precautionary statements presented in this guide.

Warning ⚠

Indicates a possibility of physical injury or death to the user or other persons if the precautions or instructions are not observed.

Caution

Indicates that damage to the instrument, loss of data, or invalid data could occur if the user fails to comply with the advice given.

Important

Highlights information that is critical for optimal performance of the system.

Note: Identifies items of general interest.

Safety standards

The Typhoon instrument complies with CE and other applicable standards. For the latest CE conformity information, contact Amersham Biosciences Technical Support. See Assistance for contact information.

Assumptions

The software-related instructions in this user's guide assume you have basic computer skills. You should be familiar with the Windows graphical user interface. You should also know how to use a mouse. If you do not have these skills, refer to the documentation or the Windows Help.

Site requirements

This section lists the site requirements for the Storm system. For more information on site requirements, see section 10.3.

Electrical

Power supply:	15 A 110–120 V~ 10 A 220–240 V~
Line frequency:	50–60 Hz

Environmental

Temperature:	15–45 °C (59–113 °F)
Humidity:	Noncondensing 10%–95%

Assistance

When calling for assistance, be prepared to supply the serial number of your instrument. The serial number is located in the Scanner Information area of the Scanner Control window and on the lower right side of the instrument near the plug (figure 2-2). For contact by phone or fax, use one of the telephone numbers below.

Asia Pacific
Tel: +852 2811 8693
Fax: +852 2811 5251

Australasia
Tel: +61 2 9899 0999
Fax: +61 2 9899 7511

Austria
Tel: 01 576 0616 22
Fax: 01 576 0616 27

Belgium
Tel: 0800 73 888
Fax: 03 272 1637

Canada
Tel: +1 800 463 5800
Fax: +1 800 567 1008

Central, East, and Southeast Europe
Tel: +43 1 982 3826
Fax: +43 1 985 8327

Denmark
Tel: 45 16 2400
Fax: 45 16 2424

Finland & Baltics
Tel: +358 (0)9 512 39 40
Fax: +358 (0)9 512 17 10

France
Tel: 01 69 35 67 00
Fax: 01 69 41 96 77

Germany
Tel: 0761 4903 291
Fax: 0761 4903 405

Italy
Tel: 02 27322 1
Fax: 02 27302 212

Japan
Tel: +81 3 5331 9336
Fax: +81 3 5331 9370

Latin America
Tel: +55 11 3667 5700
Fax: +55 11 3667 87 99

Middle East and Africa
Tel: +30 (1) 96 00 687
Fax: +30 (1) 96 00 693

Netherlands
Tel: 0165 580 410
Fax: 0165 580 401

Norway
Tel: 2318 5800
Fax: 2318 6800

Portugal
Tel: 21 417 70 35
Fax: 21 417 31 84

Russia & other C.I.S. & N.I.S.
Tel: +7 (095) 232 0250, 956 1137
Fax: +7 (095) 230 6377

Southeast Asia
Tel: +60 3 8024 2080
Fax: +60 3 8024 2090

Spain
Tel: 93 594 49 50
Fax: 93 594 49 55

Sweden
Tel: 018 612 1900
Fax: 018 612 1910

Switzerland
Tel: 01 802 81 50
Fax: 01 802 81 51

UK
Tel: 0800 616928
Fax: 0800 616927

USA
Tel: +1 800 526 3593
Fax: +1 877 295 8102

Web site

http://www.amershambiosciences.com

Part one

Introduction

Chapter 1 Introduction to the Storm system

The Storm system is an optical scanner that produces digital images of radioactive or fluorescently labeled samples. This chapter introduces you to the Storm system and contains the following topics:

- The Storm system hardware components (section 1.1)
- How the Storm system works (section 1.2)
- The Storm scan acquisition modes (section 1.3)
- Before you begin (section 1.4)

The Storm system includes from one to three scan acquisition modes, depending on the model (table 1-1).

Table 1-1. Models of the Storm Imaging System

Model	Available scan acquisition modes
Storm 820	Storage Phosphor Screen
Storm 830	Storage Phosphor Screen Red-excited Fluorescence
Storm 840	Storage Phosphor Screen Blue-excited Fluorescence
Storm 860	Storage Phosphor Screen Red-excited Fluorescence Blue-excited Fluorescence

1.1 The Storm system hardware components

The Storm system hardware includes the following components (figure 1-1):

- **Storm instrument—**Scans exposed storage phosphor screens and (in most models) fluorescently labeled samples.
- **Storage phosphor screen—**Collects the image from radioactive samples.
- **Exposure cassette—**Holds the storage phosphor screen in a light-tight environment.
- **Image Eraser—**Erases storage phosphor screens for reuse.

Additional accessories include a SCSI cable, a SCSI terminator, power cords, and tools.

The Storm instrument connects to a computer running the Microsoft Windows operating system.

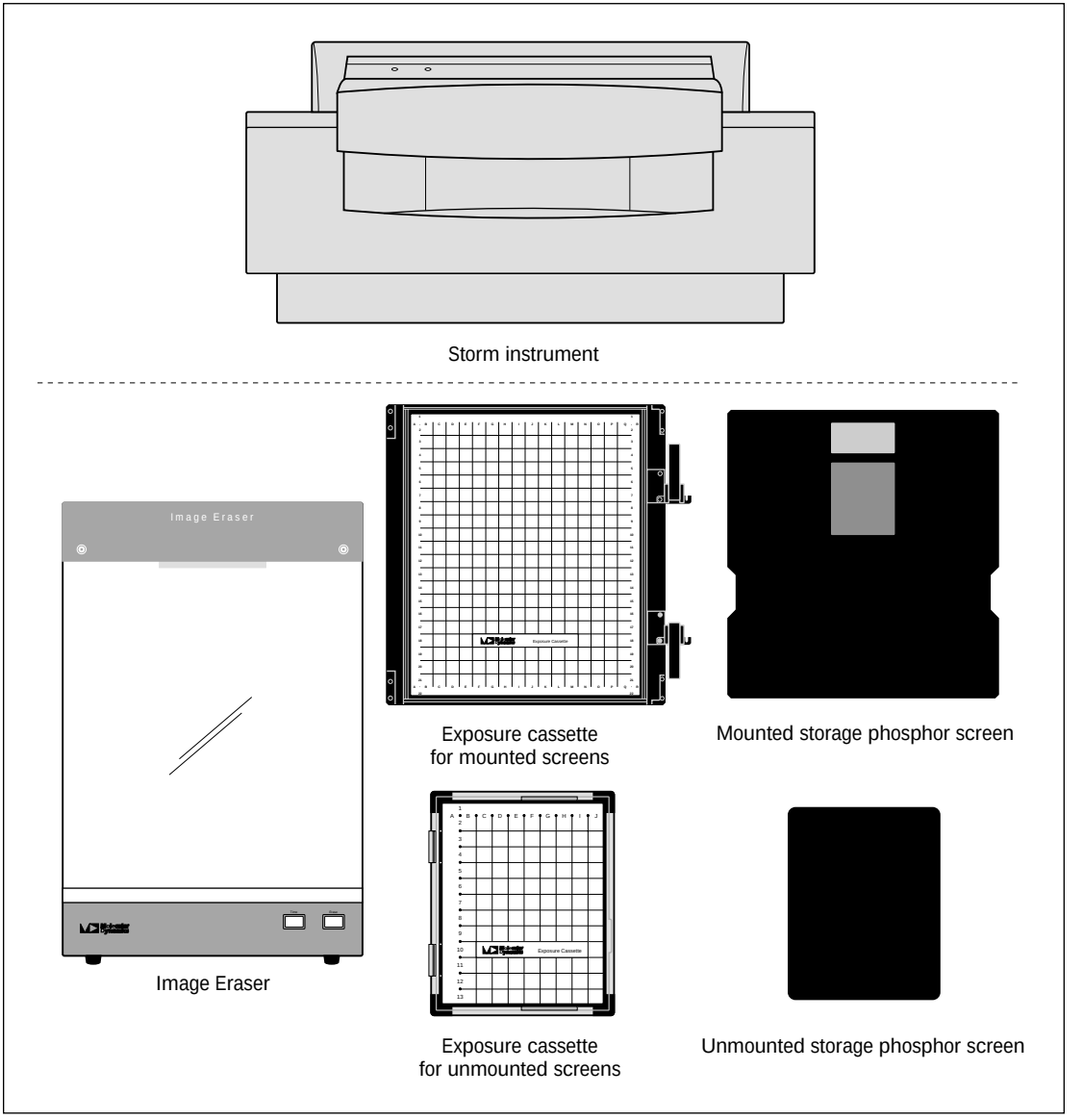

Figure 1-1. The Storm system hardware.

1.2 How the Storm system works

The Storm instrument scans and processes samples in the following sequence:

1. You place an exposed storage phosphor screen or a fluorescent sample onto the glass platen of the instrument.
2. You use the Scanner Control software to select the parameters that are appropriate for the type of sample you are scanning and to start the scan.
3. A beam of light illuminates the scan area one pixel at a time. The beam is red (635 nm) for a storage phosphor screen or red-excited fluorescence scan, or blue (450 nm) for a blue-excited fluorescence scan.
4. The screen or sample emits light in two ways:
   - When red light hits a section of the screen that was exposed to radioactivity, the phosphor emits blue light.
   - When blue or red light hits an area of the sample containing an appropriate fluorochrome, the fluorochrome emits light with a characteristic spectrum.
5. The optical system collects the emitted light and passes it through an optical filter. The filter rejects any excitation light while allowing emitted light to pass through to the photomultiplier tube (PMT).
6. The PMT converts the light to an electric current, which varies with the intensity of the light hitting the PMT. The analog signal from the PMT is then converted into digital information and stored on the hard disk of the computer.

After the scan, you use the ImageQuant software (or equivalent) to map the information to the appropriate pixel location on the monitor and produce an accurate image of the original sample. ImageQuant enables you to quantitate variations in the signal. The level of signal is proportional to the amount of radioactivity or fluorescence present in the sample.

1.3 The Storm scan acquisition modes

Depending on the model (table 1-1), the Storm instrument scans samples using the following scan acquisition modes:

- **Storage phosphor mode—**Creates images from samples labeled with radioisotopes using storage phosphor technology. All Storm systems can scan using the storage phosphor mode. The storage phosphor mode is the only scan acquisition mode on the Storm 820 instrument. Part two describes how to use the Storm system to scan in the storage phosphor mode.

- **Fluorescence mode—**Creates images from samples labeled or stained with fluorescent dyes. Depending on the Storm instrument, one, two, or three excitation modes are available.
  - **Red-excited mode—**Creates images from samples labeled or stained with fluorescent dyes that are excited at 635 nm and have emissions longer than 650 nm. The red-excited mode is available on the Storm 860 and 830 instruments.
  - **Blue-excited mode—**Creates images from samples labeled or stained with fluorescent dyes that are excited at 450 nm and have emissions longer than 520 nm. The reporter enzyme alkaline phosphatase and the substrate ECF™ are examples of such dyes. The blue-excited mode is available on the Storm 860 and 840 instruments.

    Part three describes how to use the Storm system to scan in the three fluorescence modes.
  - **Dual-label mode—**Creates images from samples labeled with two fluorescent dyes. One dye is excited using the red-excited mode. The other dye is excited by the blue-excited mode. The dual-label mode is available on the Storm 860 only.

1.4 Before you begin

Before using the Storm instrument and accessories, familiarize yourself with—

- **Chapter 2—**Important safety information.
- **Chapter 3—**Basic operational instructions for using the Storm instrument and the Scanner Control software.
- **Part four—**Maintenance information for the Storm instrument and accessories.

Chapter 2 Safety

The Storm instrument and accessories have been designed for safe operation. It is imperative that you follow the precautions in this chapter. The topics in this chapter are—

- General safety precautions (section 2.1)
- Electrical safety (section 2.2)
- Laser light safety (section 2.3)
- Hazardous materials precautions (section 2.4)

2.1 General safety precautions

Warnings

The operator of the Storm system is assumed to be trained in the correct operation of the instrument and the safety issues. Throughout the *Storm User's Guide,* the word "you" refers to this trained operator.

Using controls, making adjustments, or performing procedures other than those specified in the *Storm User's Guide* may result in hazardous exposure to laser light, high voltage, or moving parts. Exposure to these hazards can cause severe injury or death.

Please observe the following precautions:

- If the cover or doors of the Storm instrument become damaged, do not continue to use the instrument.
- If the Image Eraser becomes damaged, do not continue to use the eraser.
- To protect your warranty, the Storm instrument and Image Eraser should be serviced only by an authorized Amersham Biosciences Technical Support representative.

If you have problems with the instrument or eraser, contact Amersham Biosciences Technical Support immediately. See Assistance in the preface for contact information.

When you call Amersham Biosciences Technical Support, you will be asked for the serial number of your instrument or eraser. The serial number of the Storm instrument is shown in the Scanner Information area of the Scanner Control window and on the serial number certification label (figure 2-1). The serial number certification label also displays the model number and CDRH (Center for Devices and Radiological Health) compliance information. The label is located on the underside of the sample lid.

The serial number of the Image Eraser is located on the right side of the eraser near the plug.

Figure 2-2 shows the exact location of these labels.

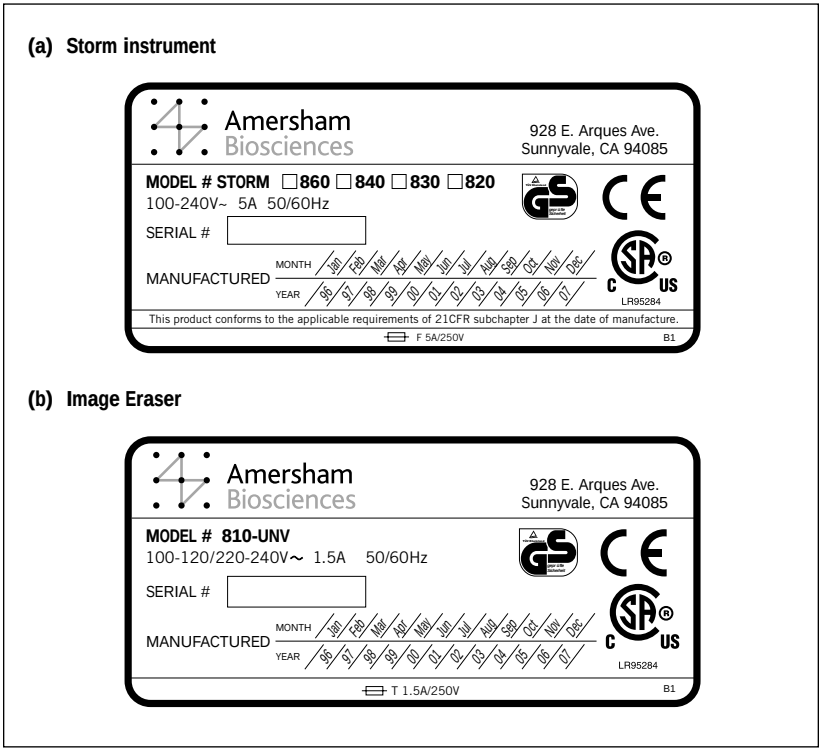

Figure 2-1. The serial number certification labels.

If a label becomes illegible or is missing for any reason, please contact Amersham Biosciences Technical Support for a free replacement label. While waiting for the replacement label, copy the labels shown in figure 2-4 and attach the copy of the label to the instrument.

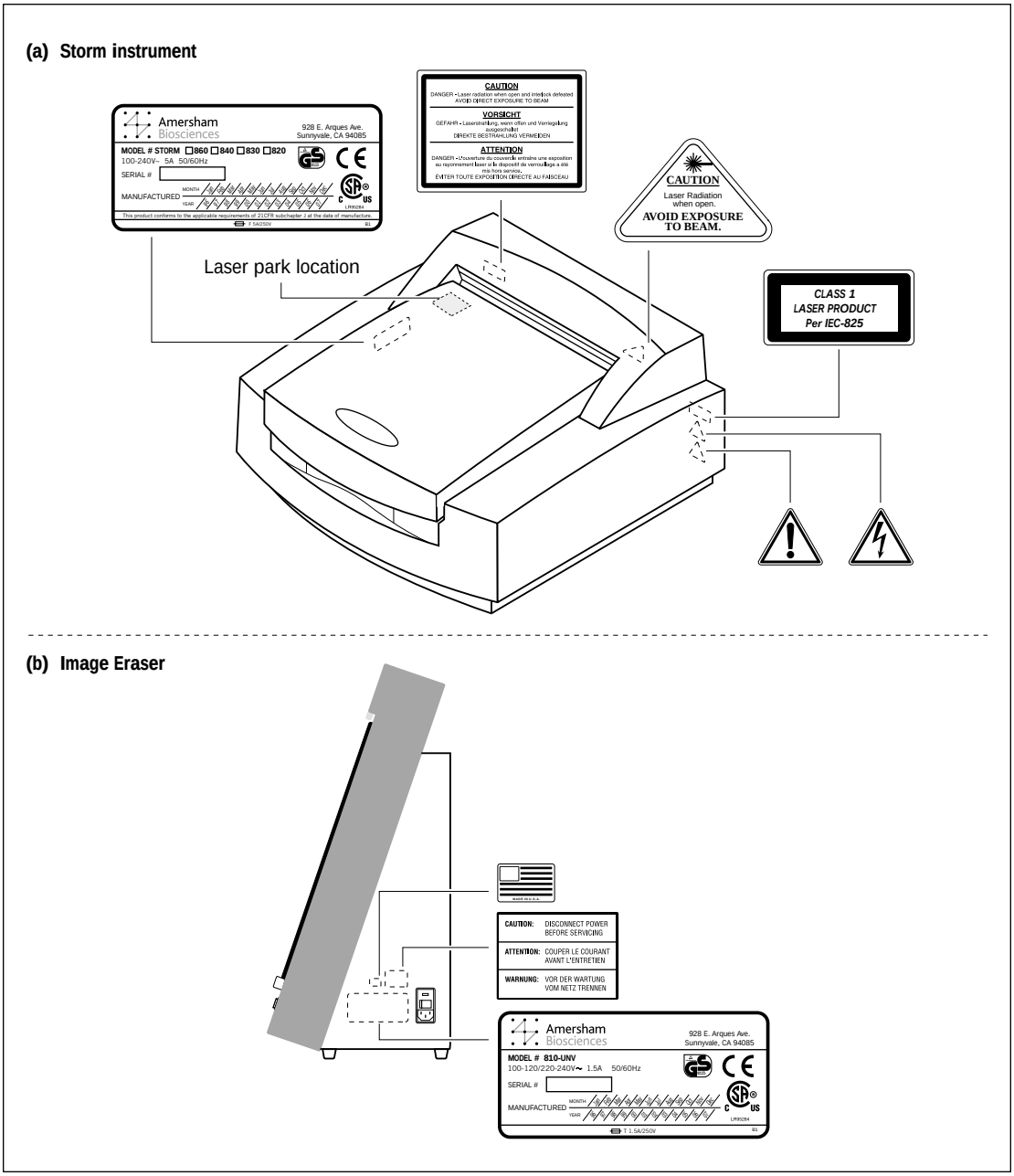

Figure 2-2. Location of important labels on (a) the Storm instrument and (b) the Image Eraser.

2.2 Electrical safety

You should follow the electrical safety information provided below to make sure you are operating the Storm instrument and Image Eraser safely.

2.2.1 Electrical connections

Before you turn on the instrument or computer, check that the instrument, Image Eraser, computer, and monitor are plugged into surge protectors, and the surge protectors are plugged in and turned on.

Plug the Storm instrument into a grounded circuit capable of delivering at least—

- 15 A for a 100–120 V~ power source
- 10 A for a 200–240 V~ power source

Do not use circuits shared by equipment containing compressors, such as refrigerators and centrifuges. Make sure power cords are in good condition and are not frayed.

2.2.2 Fuses

Warning

Before turning on the Image Eraser, make sure that the correct operating voltages are selected. If you select the wrong voltage, you can severely damage the eraser.

Both the Storm instrument and the Image Eraser have fuses in a fuse holder.

- **On the Storm instrument—**The fuse holder is on the lower left side of the Storm instrument and contains two 5A, 250V (, F5A, 250V) fast-blow fuses. The fuse holder is designed to accept both 0.25-in by 1.25-in English fuses (designated 3AG fuses) and 5-mm by 20-mm metric fuses.
- **On the Image Eraser—**The fuse holder is on the right side of the Image Eraser and contains two 1.5A, 250V (, T1.5A, 250V) slow-acting fuses. The fuse holder is designed to accept both 0.25-in by 1.25-in English fuses (designated 3AG fuses) and 5-mm by 20-mm metric fuses.

To change the fuses on the Storm instrument, follow the instructions in section 10.1. To change the fuses on the Image Eraser, follow the instructions in section 11.3.

Warning

If you need to change the fuses, turn off the instrument or eraser and disconnect the power cord. If a fuse requires repeated replacement, the instrument or eraser could have an electrical problem. Do not use the instrument. You could expose yourself to electrical shock. Contact Amersham Biosciences Technical Support. See Assistance in the preface for contact information.

2.2.3 High-voltage hazard and precautions

Inside the Storm instrument, Image Eraser, computer, and monitor are high-voltage electronics. See the computer and monitor precautions before opening the computer or monitor.

Storm instrument

Do not remove the main cover of the instrument. There are no user-serviceable components inside, and you can be exposed to high voltage. The only time you need to access the interior of the Storm instrument is to remove the SCSI cover to add or remove SCSI cables (section 10.2). The SCSI cover must be in place before you operate the Storm instrument.

Image Eraser

Warning

Because the Image Eraser uses high voltage, you should always disconnect the power cord from the eraser before performing any maintenance task.

Figure 2-3 displays the high-voltage hazard label on the Image Eraser. See figure 2-2 for the exact location of the label.

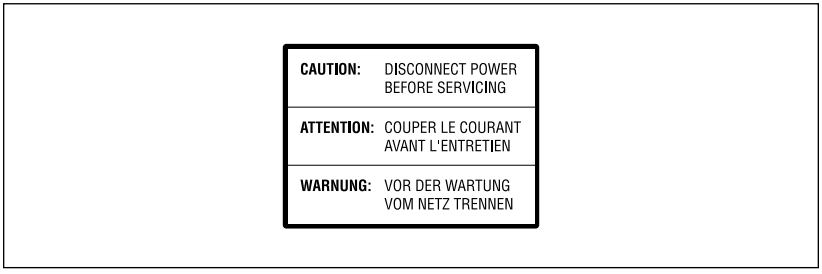

Figure 2-3. High-voltage hazard label on the Image Eraser.

Caution **Disconnect power before servicing.**

Attention **Couper le courant avant l'entretien.**

Warnung **Vor der wartung vom netz trennen.**

When using the Image Eraser, follow the precautions below:

- Plug the power cord into a grounded outlet.
- Before cleaning the Image Eraser, always turn off the power and unplug the eraser.
- Before changing a bulb, always turn off the power and unplug the eraser.

- When changing a bulb, do not remove covers other than the front diffuser panel. There are no user-serviceable parts inside.
- If the covers become damaged, do not use the eraser. Contact Amersham Biosciences Technical Support for repair.
- If fuses must be replaced repeatedly, do not use the eraser. Contact Amersham Biosciences Technical Support for repair.

2.3 Laser light safety

Warning

Using controls, making adjustments, or performing procedures other than those specified herein might result in hazardous laser light exposure.

The Storm instrument is a Class I laser instrument that houses a Class IIIB laser and, under the specified operating procedures, does not allow operator exposure to laser light. The laser, with power up to 5 mW, is accessible in the interior of the instrument.

The safety interlocks in the Storm instrument are designed to prevent you from being exposed to the laser beam. If you open the sample lid while the scanner is in operation, the laser turns off.

Warning

Do not attempt to defeat the safety interlocks on the sample lid or try to gain access to the interior of the instrument through any other opening. Exposure to laser light can cause injury. For example, viewing the laser light directly can cause blindness.

2.3.1 Laser-light warning labels

The labels shown in figure 2-4 warn of exposure to laser light. They are located on the main cover of the Storm instrument under the SCSI cover. The exact locations of the labels are shown in figure 2-2.

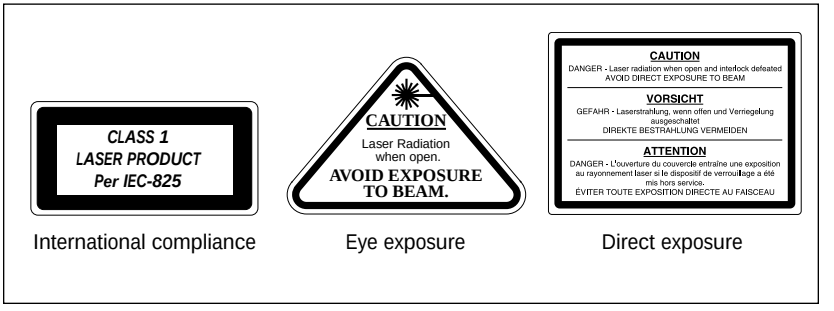

Figure 2-4. Laser-light warning labels.

Caution **Danger—Laser radiation when open and interlock defeated. Avoid direct exposure to beam.**

Vorsicht **Gefahr—Laserstrahlung, wenn offen und Verriegelung ausgeschaltet. Direkte bestrahlung vermeiden.**

Attention **Danger—L'ouverture du couvercle entraîne une exposition au rayonnement laser si le dispositif de verrouillage a été mis hors service. Éviter toute exposition directe au faisceau.**

2.3.2 Safety precautions

The Storm instrument has been designed to protect you from the laser beam during normal operation. Nevertheless, laser power up to 5 mW can be accessed from within the interior of the instrument.

Each model of the instrument contains a red laser. The output of the visible laser diode is specified at a maximum 5 mW at 635 nm in a diverging beam. In addition to the red laser, the Storm 840 and 860 contain a blue LED (light emitting diode). The output of the blue LED is specified at a maximum 10 mW at 450 nm in a diverging beam.

Most of the energy is collected into the collimated beam. When the focused beam is scanning, the time in a 7-mm aperture is approximately 7 ms, the pulse energy is 10.5 μJ for that time period (the limit for Class I is 17 μJ). The average power over the entire scan field is 0.36 μW. The scan field is completely covered by the enclosure and sample lid.

Warning ⚠ **Use of controls or adjustments or performance of procedures other than those specified herein might result in hazardous radiation exposure.**

Please observe the following precautions:

- Do not remove the main cover of the instrument. There are no user-serviceable components inside, and you might be exposed to laser light. You can remove the SCSI cover to add or remove SCSI cables (section 10.2).
- Do not defeat the safety interlocks of the sample lid. During a scan, these safety interlocks protect you from exposure to the laser light by interrupting the scan and turning off the light source.
- Do not continue to use the instrument if the main cover or sample lid becomes damaged and the instrument is no longer light tight. Contact Amersham Biosciences Technical Support immediately to arrange for repair. See Assistance in the preface for contact information.

- To protect your warranty, your Storm instrument should be serviced only by an authorized Amersham Biosciences Technical Support representative. If the instrument is not working correctly, please contact Storm Technical Support.
- If fuses must be replaced repeatedly, do not use the instrument. Contact Amersham Biosciences Technical Support.

2.4 Hazardous materials precautions

Some materials used to label samples can be hazardous. Use good laboratory procedures and follow the manufacturer's precautions when working with these materials.

Before using the Storm system, familiarize yourself with your laboratory's hazardous materials procedures.

Cautions

Do not use volatile organic solvents, such as methanol, chloroform, or acetone. Make sure thin-layer chromatography (TLC) plates are completely dry. Always remove corrosive liquids before loading the sample.

Do not use scintillants or enhancers on your sample. These compounds interfere with the proper function of the storage phosphor screens.

Amersham Biosciences is not responsible or liable for any damages caused by or as a consequence of the use of any hazardous material.

Chapter 3 Getting started

This chapter describes the basic procedures for turning on and turning off the Storm instrument, optional peripherals, and the Scanner Control software. The topics in this chapter are—

- Turning on and warming up the Storm instrument (section 3.1)
- Turning on the computer and optional peripherals (section 3.2)
- Starting the Scanner Control software (section 3.3)
- Quitting the Scanner Control software (section 3.4)
- Turning off the Storm instrument (section 3.5)
- Turning off the computer and optional peripherals (section 3.6)

3.1 Turning on and warming up the Storm instrument

To turn on the Storm instrument, press the on/off switch on the lower left side of the instrument (figure 3-1). The power indicator light on the top of the instrument turns on and remains red during the self-test sequence, which takes about 45 seconds. The light then turns green. (If you have more than one instrument connected to the host computer, turn on all the instruments you plan to use. For more information on multiple devices, see section 10.2.) After you turn on the Storm instrument, wait approximately 15 minutes for the instrument to warm up before you start the first scan.

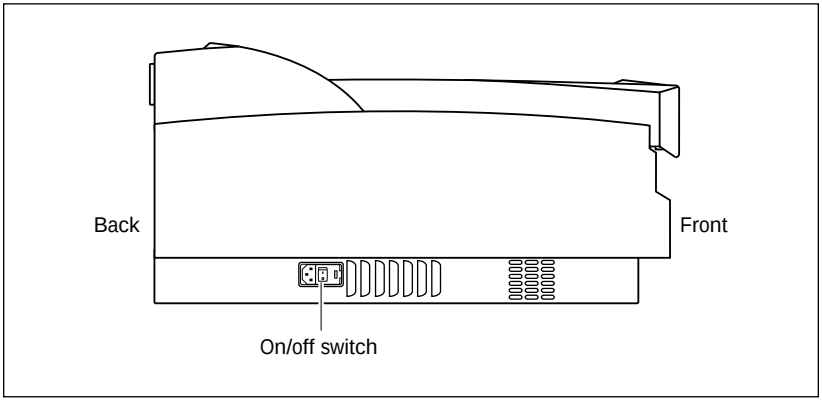

Figure 3-1. Left side view of the Storm instrument.

3.2 Turning on the computer and optional peripherals

The Storm instrument and all optional peripherals must be on and the power light must be green before the computer can communicate with them. After turning on the instrument, turn on the optional peripherals using the manufacturers' instructions.

When the power light on the Storm instrument turns green, turn on the computer and monitor according to the manufacturer's instructions.

Important **If the computer was already on when you turned on the Storm instrument, you must restart the computer.**

3.3 Starting the Scanner Control software

After the computer is turned on, log on to Windows. Locate and double-click the Scanner Control shortcut icon on the desktop. (Alternatively, you can select Scanner Control using the Start menu.) The Scanner Control window appears. To begin scanning a sample, see Part two for the storage phosphor procedures or Part three for the fluorescence procedures.

3.4 Quitting the Scanner Control software

Although it is not necessary, you can quit the Scanner Control software after you finish scanning. To quit the Scanner Control software, choose **Exit** from the File menu, or click the **Close** button ( ). If you want to scan again, start the Scanner Control software.

Important **If you are moving the instrument, use the Park Head and Exit command from the File menu. See section 10.3 for more information on moving the instrument.**

3.5 Turning off the Storm instrument

To turn off the Storm instrument, press the on/off switch on the lower left side of the Storm instrument (figure 3-1) to the off position. You can continue to use the computer after you turn off the Storm instrument. However, if you want to use the instrument again, you must turn on the instrument using the procedure in section 3.1 and then restart the computer.

3.6 Turning off the computer and optional peripherals

Before you turn off the computer, you should save and close any open files, and then close all the running applications. Use the computer manufacturer's instructions for turning off the computer.

After you turn off the computer, use the peripheral manufacturers' instructions to turn off any optional peripherals.

Part two

Scanning in the storage phosphor mode

Chapter 4 About storage phosphor screen autoradiography

This chapter describes storage phosphor screen autoradiography. All models of the Storm instrument use the storage phosphor screen technology. The topics in this chapter are—

- How Storm generates an image from a storage phosphor screen (section 4.1)
- Advantages of storage phosphor screen autoradiography (section 4.2)
- Types of storage phosphor screens (section 4.3)

4.1 How Storm generates an image from a storage phosphor screen

Generating an image from a storage phosphor screen is a two-step process. First, you expose an erased storage phosphor screen to the sample using an exposure cassette. An image is created on the screen from the radiation energy that is trapped in the crystal lattice of the screen.

Next, you use the Storm instrument to scan the storage phosphor screen. The instrument captures the stored information on the screen in the form of a digital image.

The following sections describe the process in more detail.

4.1.1 Storing the image

The storage phosphor screens are composed of fine crystals of $BaFBr:Eu^{+2}$ in an organic binder. When the screen is exposed to a radioactive sample, the radiation excites Eu^{+2} electrons to a state in which they move easily within the phosphor. During the exposure process, Eu^{+2} is oxidized to Eu^{+3}, and BaFBr is reduced to $BaFBr^{-}$. These ions remain oxidized and reduced after the screen is removed from the sample. In this way, the phosphor stores the energy from ionizing radiation. Most of the energy (from ionizing radiation) stored on the screen remains for as long as several weeks.

4.1.2 Releasing the stored information

Storage phosphors release stored energy when stimulated by light of appropriate wavelengths. The Storm instrument scans the screen using a red light at 635 nm. The charged $BaFBr^-$ complexes in the screen absorb light in this range (figure 4-1), freeing electrons and reducing the Eu^{+3} to Eu^{+2*} (an Eu^{+2} ion with an electron in an excited state). As the excited electron falls to the ground state, it releases energy in the form of blue light. The Storm instrument uses a band-pass filter, which allows the light near the peak emissions of the screen to pass through to the detector. The detector collects and measures the light. The emitted light intensity is proportional to the radioactivity in the sample.

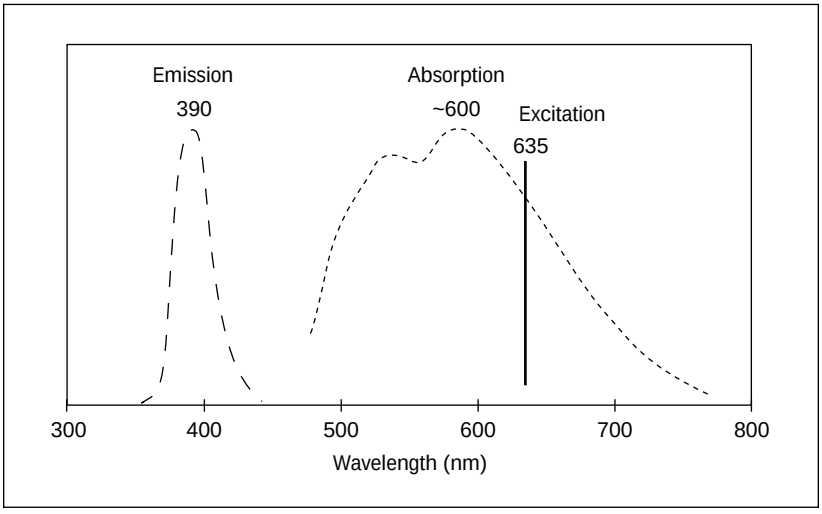

Figure 4-1. Emission (— —) and absorption (---) spectra of the activated storage phosphor screen. The wavelength with maximum stimulation or emission is shown above the curves. (These curves are approximations based on data presented in an article by Sonoda et al., 1983.)

4.2 Advantages of storage phosphor screen autoradiography

Storage phosphor screen autoradiography offers many advantages over traditional film autoradiography—

- Exposure time is approximately one-tenth that of traditional autoradiography using x-ray film.
- Sensitivity is 10 to 100 times that of film, depending on the isotope used and the sample type.
- The linear dynamic range is 1 to 100 000 (5 orders of magnitude). Film has a linear dynamic range of only 1 to 500 (2.5 orders of magnitude). Unlike film, both weak and strong signals can be visualized and quantitated in a single exposure to the storage phosphor screen, which eliminates the need for multiple exposures.
- The storage phosphor screens are reusable.
- The storage phosphor screens are placed on the samples in ambient light, and exposure takes place at room temperature.
- No chemicals, darkroom, or special treatment is required.
- Results are in digital form and can be analyzed qualitatively and quantitatively using ImageQuant.

4.3 Types of storage phosphor screens

The storage phosphor screen detects the beta and gamma ionizing radiation from most isotopes. You can use any of the three types of storage phosphor screens:

- General-purpose (GP) storage phosphor screen (section 4.3.1)
- Low-energy (LE) storage phosphor screen (section 4.3.2)
- Tritium (TR) storage phosphor screen (section 4.3.3)

The TR screen is available in one size: 19 cm × 24 cm (7.4 in × 9.4 in). Unless noted below, the GP and LE screen types are available mounted on an aluminum backing or unmounted in the following sizes:

- The small screen, which is 20 cm × 25 cm (7.8 in × 9.8 in).
- The half-size screen, which is 17.5 cm × 43 cm (6.8 in × 16.8 in).
- The large screen, which is 35 cm × 43 cm (13.7 in × 16.8 in).
- The macroarray screen, which is 24 cm × 30 cm (9.4 in × 11.7 in). The macroarray screen is available in the unmounted style only.

In general, you expose the screen to the sample in an exposure cassette. (See chapter 5 for guidelines on exposing different sample types.) The exposure cassettes come in four styles:

- Small cassette for small mounted screens
- Small cassette for small unmounted screens
- Large cassette for large and half-size mounted screens
- Large cassette for large unmounted or macroarray screens

4.3.1 General-purpose storage phosphor screen

Caution **You should handle the general-purpose (GP) screen carefully. Scratching the surface of the screen will remove the protective overlay. Always follow the guidelines and instructions provided in chapter 5 when handling the GP screen.**

If you use the Storm instrument for a wide variety of applications, you should use the GP storage phosphor screen. The GP screen is protected from radioactive contamination by a durable cellulose acetate overlay that covers the phosphor layer.

The phosphor layer of the GP screen can detect energy from most isotopes. The isotopes include, but are not limited to, ^{32}P, ^{33}P, ^{125}I, ^{35}S, and ^{14}C. Typical samples that can be exposed to a GP screen include ^{32}P Northern blots, ^{32}P Southern blots, ^{125}I Western blots, and a variety of gels.

4.3.2 Low-energy storage phosphor screen

Caution **You should handle the low-energy (LE) screen carefully. Scratching the surface of the screen will remove the thin protective overlay. Exposing the LE screen to wet or moist samples damages the screen. Because wrapping wet samples in plastic film might not prevent damage, make sure the sample is dry before exposing the sample to the LE screen. Always follow the guidelines and instructions provided in chapter 5 when handling the LE screen.**

If you use the Storm instrument for applications that require more sensitivity, you should use the LE storage phosphor screen. The phosphor layer of the LE screen is coated with a thin layer of the durable cellulose acetate overlay and a small amount of iodide, which increases the efficiency of the energy stored and released by the screen.

You can use the LE screen to record ^{33}P, ^{35}S, and ^{14}C emissions. Typical samples that can be exposed to an LE screen include ^{35}S sequencing gels, ^{35}S protein gels, and ^{33}P macroarrays.

4.3.3 Tritium storage phosphor screen

Caution

You should handle the tritium (TR) screen carefully. Because you cannot clean a contaminated TR screen, make sure the sample is as dry as possible before exposing it to the TR screen. You can erase an exposed TR screen that is not contaminated and reuse it. Always follow the guidelines and instructions provided in chapter 5 when handling the TR screen.

You use the TR storage phosphor screen to record ^{3}H emissions. Because the screen is not coated with the protective cellulose acetate overlay, the TR screen can detect the weak energy emitted by ^{3}H. Typical samples that can be exposed to a TR screen include tissue sections on glass and whole body autoradiography.

Chapter 5 Preparing for storage phosphor screen autoradiography

This chapter provides guidelines for sample preparation and instructions on exposing the sample to both mounted and unmounted storage phosphor screens. The topics in this chapter are—

- Guidelines for preparing the samples (section 5.1)
- Preparing the storage phosphor screen for exposure (section 5.2)
- Guidelines for exposing storage phosphor screens (section 5.3)
- Placing the sample in the exposure cassette (section 5.4)
- Placing the screen in the exposure cassette (section 5.5)
- Exposing the storage phosphor screen (section 5.6)

5.1 Guidelines for preparing the samples

The following sections provide guidelines to use when preparing samples for use with the storage phosphor screen.

5.1.1 General guidelines

Use the following guidelines when preparing the samples:

- Follow all the laboratory procedures for the type of sample you are preparing.
- Make sure that thin-layer chromatography (TLC) plates are completely dry before placing them with the screen. To keep flecks off the screen, cover the dry TLC plate with plastic wrap or strong, thin polyester film, such as 5-mm Mylar™.
- Make sure the sample is free from dust and powder. Wear powder-free gloves when handling the samples. Always rinse the gloves thoroughly with distilled or tap water before handling the samples and before preparing reagents. Dust and powder fluoresce and scatter light. This causes artifacts on the images and can interfere with the quantitation.
- Neutralize alkaline denaturing gels and make sure the sample is free from acetic acid vapors and organic solvents. These materials are harmful to the storage phosphor screens and can penetrate the plastic wrapping or Mylar on wet samples.

- Do not use scintillants or enhancers, such as PPO, EN^{3}HANCE™, and Amplify™. These products interfere with the proper function of the screen.

5.1.2 Guidelines for using wet gels with the storage phosphor screen

In addition to the general guidelines in section 5.1.1, use the following guidelines when exposing the screen to a wet gel:

- Do not directly expose the storage phosphor screen to wet chemicals of any kind, especially organic solvents.

Caution **Because organic solvents penetrate plastic, plastic wrap will not protect the storage phosphor screens from organic solutions.**

- Protect the general-purpose (GP) screen from contamination by separating a wet gel from the screen with a piece of plastic wrap or polyester film. Wrap the plastic wrap completely around the gel so that liquid cannot leak out. This precaution reduces screen contamination with minimum attenuation of the signal.
- You can expose a wrapped wet gel in the exposure cassette, but do not lock the clamps on the exposure cassette to secure the screen. Doing so can cause liquid to leak into the exposure cassette. If possible, dry a thin wet gel on blotting paper, such as Grade 3MM Chr by Whatman™ Incorporated, before placing the gel in the exposure cassette.
- Some wet gels are too thick for use in the exposure cassette. Expose these wet gels in a light-tight drawer or other light-tight enclosure.
- Because a low-energy (LE) screen can be damaged by long exposure to liquid, do not expose a wet or moist gel to an LE screen. Wrapping wet gels in plastic film might not prevent damage. Therefore, make sure the gel is dry before exposing the gel to the LE screen.
- Because you cannot clean a tritium (TR) screen, do not expose a wet gel to a TR screen. The TR screen does not have the cellulose acetate layer that protects the GP screen.

5.1.3 Guidelines for using radioactive standards

By exposing your sample in conjunction with a set of radioactive standards, you can convert Storm system counts, which are arbitrary units, to the units of your choice, such as disintegrations per minute.

Keep the following in mind when using standards:

- The range of activities of the standards should bracket expected levels in your sample.
- Increasing the number of steps in the standard series will increase accuracy in the conversion process.
- For accurate quantitation, load the standards as part of the sample.
- If you cannot load the standards as part of the sample, the standards should be in a matrix similar to the final sample. For example, if the sample is a dried 10% polyacrylamide gel, the standards should be made in a 10% polyacrylamide gel and dried as well.

Standards are commercially available from a number of companies that sell radioactive products. Contact these companies for assistance in choosing an appropriate set of standards for your application. Alternatively, you can make your own standards.

5.2 Preparing the storage phosphor screen for exposure

Before exposing the sample to the storage phosphor screen, you should clean, erase, and if necessary, decontaminate the screen.

5.2.1 Protecting the storage phosphor screen

The storage phosphor screen consists of a relatively soft matrix. To protect the screen—

- Handle the screen on the edges or back only. Do not touch the white phosphor surface.
- Wear powder-free gloves to avoid contaminating the screen with skin oils or powder.
- Do not fold, roll, or gouge an unmounted screen.

5.2.2 Checking for contamination

Between exposures or if the screen has not been used for several days, verify that the storage phosphor screen is clean using the following procedure:

1. Clean and erase the screen using the procedures in sections 5.2.3 and 5.2.4.

Important **Before you erase the screen, check the Image Eraser for contamination from radioactive samples. To clean the eraser, see section 11.1.**

2. Store the screen in a clean, light-tight box.
3. Provide enough time to register the contamination as an image on the screen.
4. After storage, scan the screen and examine the image. If no hot spots appear on the image, erase the screen again and begin the next exposure.

5.2.3 Cleaning the storage phosphor screen

Use the cleaning method appropriate for the type of screen you are cleaning.

- **GP and LE screens—**Use a soft cotton cloth and an intensifying screen cleaner (for example, Kodak™ Intensifying Screen Cleaner). Follow the directions on the bottle. Alternatively, use a small amount of alcohol and distilled water. Do not use a powdered detergent. Any undissolved particles can scratch the surface of the screen. This cleaning procedure removes dust, fingerprints, static electricity, and mild contamination from radioactive samples.
- **TR screen—**Use a gentle gas stream or soft brush to remove any particulate matter from the unprotected surface of the screen.

Caution **To avoid damaging the TR screen, do not expose the screen to liquids of any sort. To avoid damaging the LE screen, make sure you dry the screen completely after you clean the screen using an intensifying-screen cleaner.**

5.2.4 Erasing the storage phosphor screen

After you clean the storage phosphor screen, you use the Image Eraser to erase any residual signal from the screen.

Use the following guidelines when erasing the screens:

- To avoid contaminating the screen or the eraser, make sure that both the screen and eraser are free from radioactive contamination before placing the screen on the eraser.

- Erase the screen immediately before exposure as well as after scanning the screen in the instrument. Cosmic radiation creates background on screens left unused for long periods of time.
- Erase the screen until the recorded signal is fully removed.
- Because you cannot decontaminate a TR screen, you should protect the eraser and the screen from possible contamination by placing plastic wrap or polyester film between the TR screen and the eraser surface.

To erase the screen—

1. Holding the screen by its edges, and with the white side facing the eraser,
2. Place the screen on the eraser as shown in figure 5-1. Rest the screen on the lip of the eraser. To erase two small screens at once, suspend an unmounted screen from the clip at the top of the eraser. Do not use the clip on the tritium screens.

Caution **Do not use the clip on the TR screen. The clip can damage the surface of the screen.**

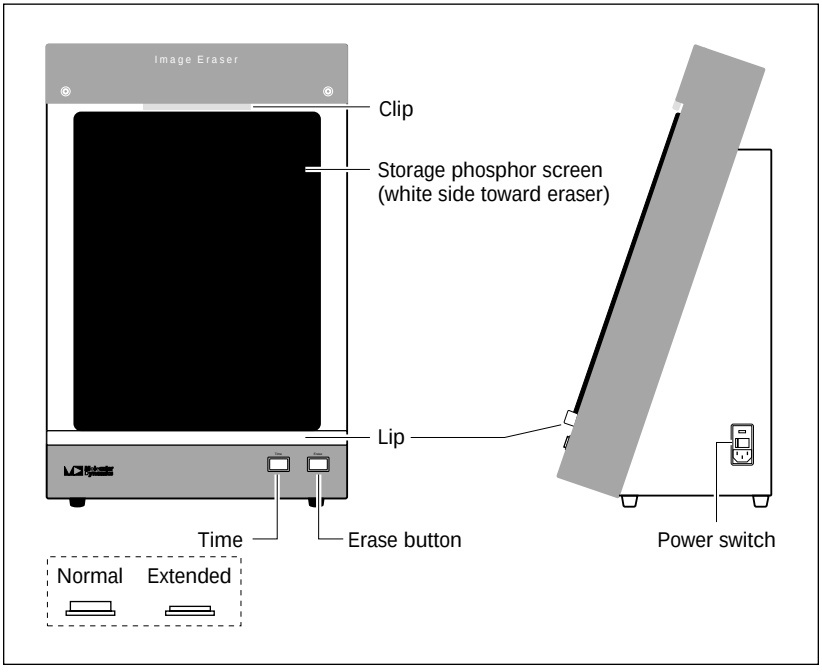

Figure 5-1. Erasing the storage phosphor screen.

3. Set the eraser time by pressing the **Time** button. Figure 5-1 shows the button positions for Normal (out) and Extended (in). Select—
   - **Normal** for typical samples. The Normal setting takes approximately 10 minutes.
   - **Extended,** if the background or residual image is high (for example, the original image contained readings of 10^4 counts and higher). The Extended setting takes approximately 20 minutes.
4. Press the **Erase** button.

If the Image Eraser is unavailable, you can use a fluorescent light box and double the erasure time. If a fluorescent bulb in the Image Eraser needs to be replaced, you should replace all four bulbs before using the eraser. To replace the bulbs, see section 11.2.

5.3 Guidelines for exposing storage phosphor screens

Use the following guidelines when exposing the storage phosphor screen:

- Use an exposure cassette to expose the screen to the sample. The exposure cassette seals out light and also keeps the sample flat against the storage phosphor screen during exposure.
- To expose multiple samples on the same screen, make sure the samples are the same thickness. If the samples are not the same thickness, the thinner sample will not contact the screen uniformly, which can result in a bad exposure, poor image, and poor quantitation results.
- Some samples, such as wet gels or TLC plates, are too thick to fit in an exposure cassette. For thick samples, expose the screen in a light-tight drawer or other light-tight enclosure.
- Place the screen on the sample correctly the first time.

Caution **Adjusting the position of the screen following initial placement can result in a double exposure. If you must adjust the position, remove the screen and erase it. Then place the screen on the sample again.**

5.4 Placing the sample in the exposure cassette

The exposure cassette comes in two styles: one for mounted screens and one for unmounted screens. Each style of exposure cassette comes in two sizes: one for the small screens and one for the large screens. Select the exposure cassette size and style that matches the storage phosphor screen you want to use with the sample. All exposure cassettes come with grid markings inside to facilitate sample positioning.

Caution **Do not put uncovered wet gels in the exposure cassette. For the GP screen, make sure wet gels are properly covered. Do not expose the LE or TR screen to wet gels.**

To place the sample in the exposure cassette—

1. Use a damp cloth to clean the grid surface inside the exposure cassette and remove any radioactive contamination (section 11.4).
2. Place the sample on the grid using the following guidelines:
   - In general, place the sample in the cassette in the orientation you want to view the scanned image (face up on the grid and with the top of the sample toward the top end of the cassette).

     Note: Remember how you place the sample in the cassette. You will use this information later in the Scanner Control window (section 6.4.4).
   - To avoid possible edge effects, you should place the sample at least 1 cm from the edge of the screen. For example, use the area B2 through J13 for the small screens and the area B2 through Q21 for the large screens, as shown in figure 5-2.
   - To minimize the time required for the scan, place the sample near the upper left corner of the grid (for example, start in grid square B2).
   - To minimize the size of the image file, scan only the grid squares that are covered by the sample. If you do not want to scan the entire sample, scan the squares that contain the part of the sample you are interested in analyzing.
   - To use one screen to expose multiple samples of the same thickness, place the samples close together.
   - Align the lanes and rows of the sample with the edges of the grid so that the scanned image will be straight, as shown in figure 5-2.
3. Make a note of the coordinates of the upper left and lower right corners of the area you want to scan (as shown in figure 5-2). The grid coordinates in the exposure cassette match the coordinates in the Storm instrument. You will use this information later in the Scanner Control window (section 6.4.3).

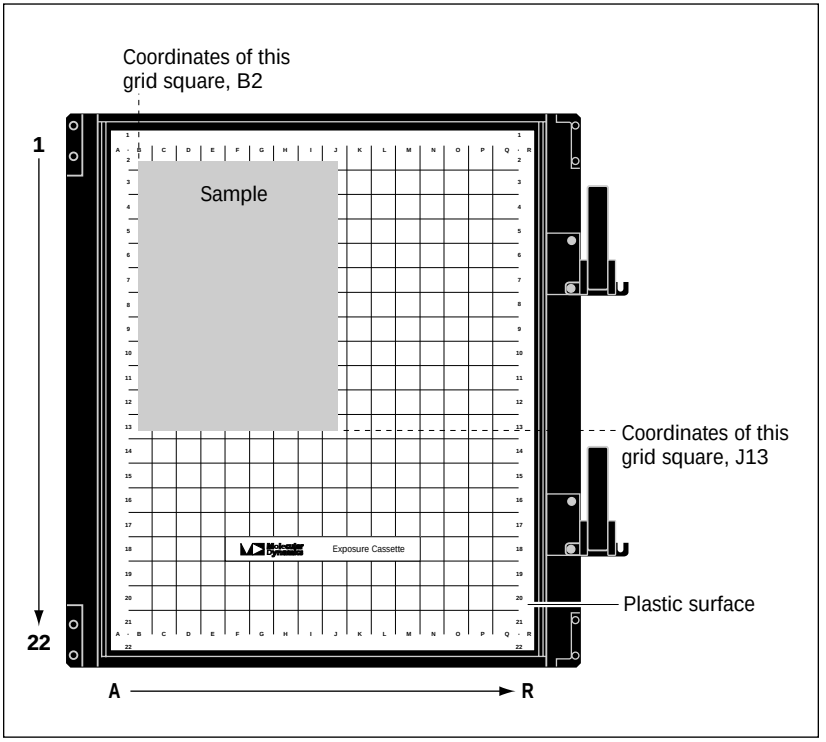

Figure 5-2. Placing the sample in the exposure cassette.

5.5 Placing the screen in the exposure cassette

You should perform storage phosphor screen autoradiography at room temperature. Exposing the screen at subzero temperatures does not provide any advantage.

Cautions

Condensation can destroy the screen. If you are exposing a screen to a frozen sample, place the screen in a sealed, dry environment, such as a sealed bag. After exposure, allow the screen to come to room temperature before removing it from the bag and scanning.

Make sure you place the screen in the correct position when you first set it down. Because the screen is extremely sensitive, adjusting the position can result in a double image.

5.5.1 Placing a mounted screen

To place a mounted screen in the exposure cassette—

1. Make sure the clamp (or clamps) on the cassette are rotated out of the way.
2. Remove the screen from the eraser.
3. Note the groove along the side of the exposure cassette opposite the clamp(s). Place the edge of the screen into the groove. The white side of the screen should face down, toward the sample. Place the top of the label on the screen toward the top of the cassette (figure 5-3).

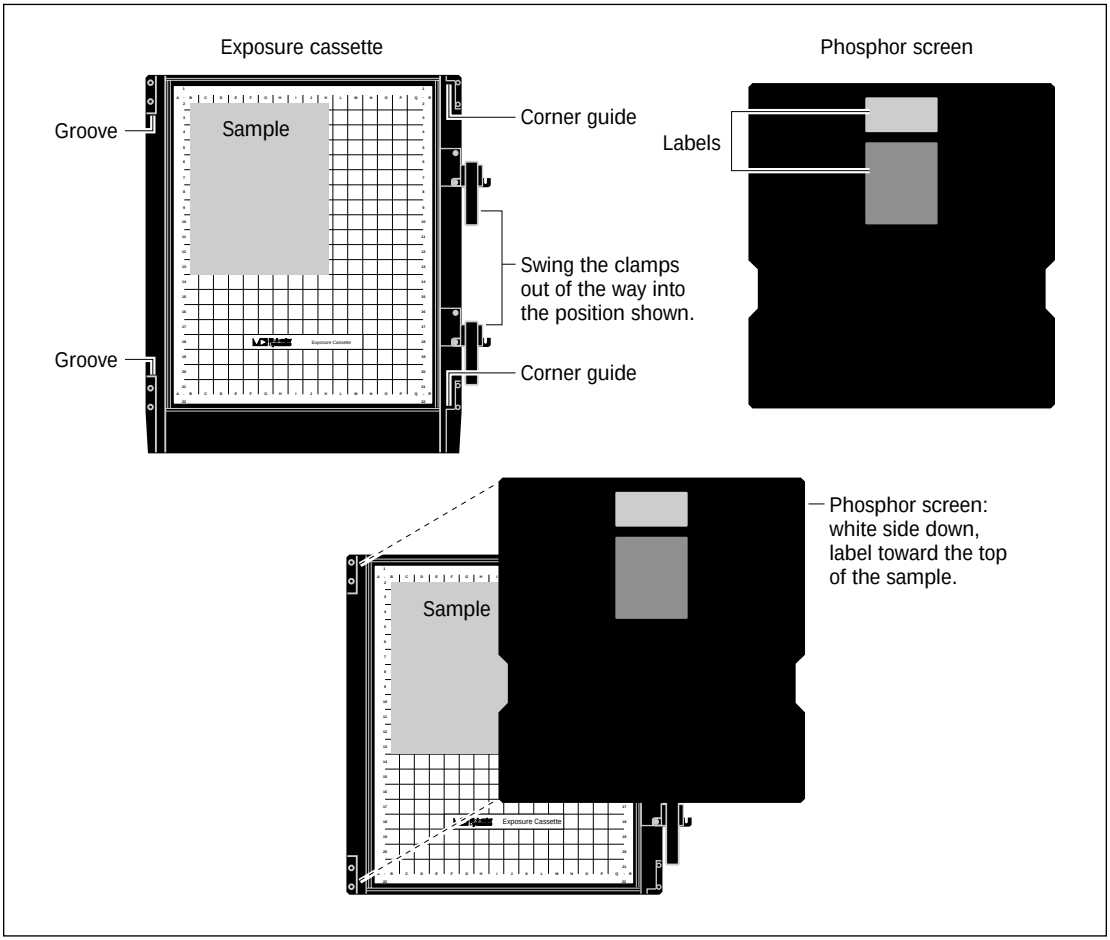

Figure 5-3. Loading the large-format mounted screen into the exposure cassette.

4. Gently lower the screen into place in the corner guides, making sure the screen fits into the guides correctly.

Caution **Do not reposition the screen after it has touched the sample. Because the screen is extremely sensitive, adjusting the position can result in a double image.**

5. Close the exposure cassette by rotating each clamp counterclockwise until it stops at the pin (figure 5-4) and then flipping the lever over to lock the screen into place.

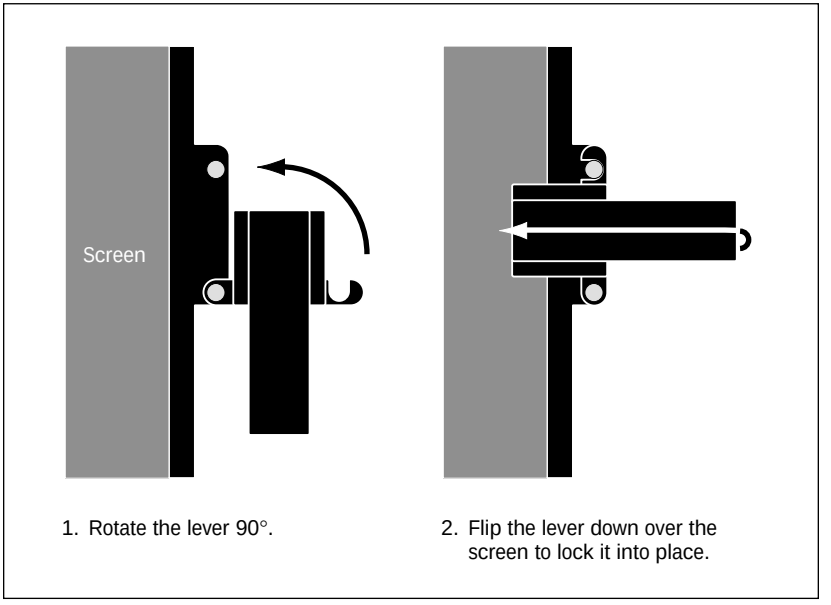

Figure 5-4. Closing the exposure cassette for mounted screens.

5.5.2 Positioning an unmounted screen

To position an unmounted screen in the exposure cassette—

1. Remove the screen from the eraser.
2. Hold the white side of the screen facing down, toward the sample. The top of the label on the screen should be toward the top end of the cassette.
3. Gently lower the screen into place over the grid area in the cassette. The screen should be centered over the grid area and should lie straight and flat in the cassette (figure 5-5).

Caution **Do not reposition the screen after it has touched the sample. Because the screen is extremely sensitive, adjusting the position can result in a double image.**

4. Close the exposure cassette and press the lid shut until the lock clicks.

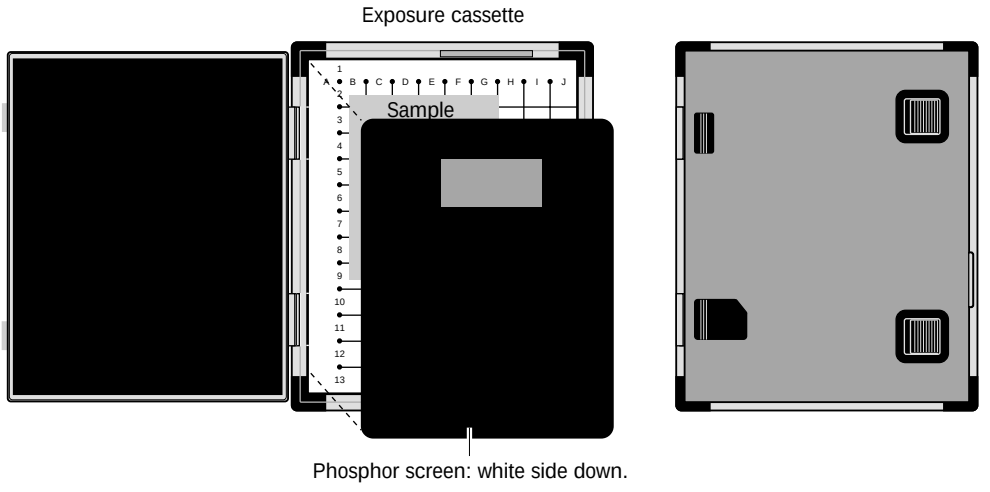

Figure 5-5. Loading an unmounted screen into the exposure cassette.

5.6 Exposing the storage phosphor screen

The storage phosphor screen requires approximately one-tenth the exposure time of normal x-ray film. When determining the exposure time, consider a one-hour exposure to the storage phosphor screen to be equal to an overnight exposure to x-ray film. Because the storage phosphor screen has a wide linear dynamic range, it is unlikely that you will overexpose the sample to the screen. This allows you to capture both the strong and weak sample signals with only one exposure to the sample.

Chapter 6 Scanning the storage phosphor screen

This chapter provides step-by-step instructions for scanning a storage phosphor screen. The topics in this chapter are—

- Verifying the Storm instrument is clean (section 6.1)
- Removing the exposed storage phosphor screen from the exposure cassette (section 6.2)
- Loading the storage phosphor screen into the instrument (section 6.3)
- Selecting the scan parameters in the Scanner Control window (section 6.4)
- Using templates (section 6.5)
- Starting the scan and checking the progress (section 6.6)
- Cleaning up after the scan (section 6.7)
- Storing the storage phosphor screen (section 6.8)
- Analyzing or preprocessing the scanned image (section 6.9)

6.1 Verifying the Storm instrument is clean

You should check the Storm instrument for all types of contamination before placing the storage phosphor screen in the instrument.

6.1.1 Checking for radioactive contamination

Periodically, you should check the instrument for contamination from radioactive samples.

To check the instrument for radioactive contamination, use the following procedure:

1. Select a storage phosphor screen that is free from radioactive contamination.
2. Erase the screen, scan the screen to make sure it is not contaminated, and then erase the screen again. (If, during scanning, an image that looks like a gel or a blot appears in the Scan in Progress window, the screen might be contaminated. Erase the screen. Rotate the screen or place the screen on a different part of the glass platen, and scan the screen again. If the same image appears at the new position in the Scan in Progress window, the screen is contaminated. Select a different screen and repeat this step.)

3. Place the white side of the screen on the glass platen of the Storm instrument.
4. Leave the screen in the instrument overnight.
5. Scan the screen. If an image appears in the Scan in Progress window that looks like a gel or blot, the instrument is contaminated. **Note:** If a white or gray image appears, the image was probably created by background contamination, and the instrument is not contaminated.

To clean the instrument, follow the instructions in sections 6.1.2 and 6.1.3.

6.1.2 Cleaning the glass platen

The glass platen of the Storm instrument should be kept free of contamination from a radioactive sample. In addition, you should clean the glass platen before and after you scan each sample.

Important **To protect your hands from the ethanol and hydrogen peroxide used in this procedure and to avoid transferring oils from your hands onto the glass platen, you should wear gloves. If you scan fluorescent samples using the Storm instrument, make sure the gloves are powder free.**

To clean the glass platen—

1. Grasp the lid release under the center front of the sample lid and pull the release forward until the lid opens (figure 6-1). Raise the lid all the way.
2. (Optional) If you use the instrument for fluorescent scanning and fluorescent material has come in direct contact with the glass platen, moisten a lint-free cloth with 10% hydrogen peroxide and wipe the glass several times.
3. Clean the glass with distilled water and a clean, lint-free cloth or paper. If visible spots remain, clean the glass first with 75% ethanol and then with distilled water.

Caution **Because window cleaners contain ingredients that can fluoresce, do not use window cleaners to clean the glass platen. In addition, the use of acetone or the excessive use of ethanol can shorten the lifetime of the instrument. Protect the glass from scratches. Scratches interfere with accurate imaging and quantitation.**

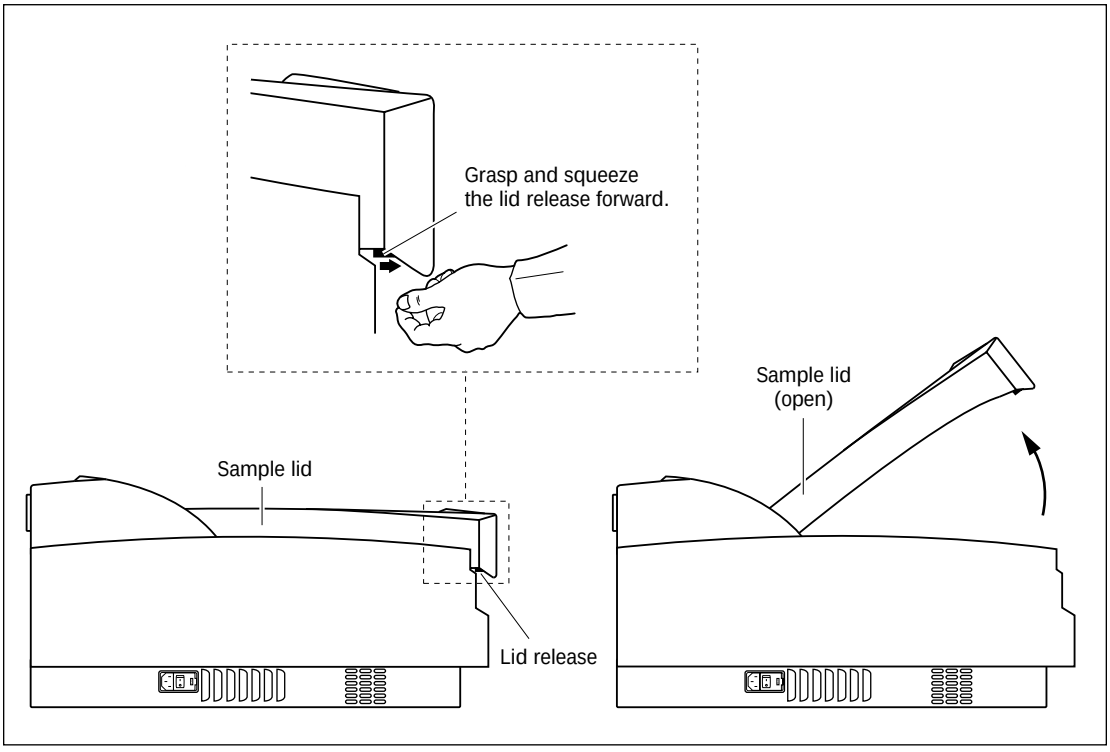

Figure 6-1. Opening the sample lid.

6.1.3 Cleaning the sample lid

Under normal circumstances, the surface of the inner lid should not come in contact with contaminants. However, it is good laboratory practice to check the surface periodically for contamination. For example, you can perform a wipe test, or use the procedure that you used to check the glass platen (section 6.1.1), except place the screen in the instrument with the phosphor side up.

The surface of the inner lid should be clean and free of contamination from radioactive samples. If necessary, clean the surface with a damp (not saturated) cloth moistened with a small amount of distilled water. If visible spots remain, clean the surface first with 75% ethanol and then with distilled water. **Note:** You should wear gloves to protect your hands when cleaning the sample lid using 75% ethanol. To prevent liquid from seeping inside the lid or rolling down onto the glass platen, do not spray liquid on the lid.

6.2 Removing the exposed storage phosphor screen from the exposure cassette

Important **The image on an exposed storage phosphor screen is light sensitive. When transferring the screen from the exposure cassette to the instrument, keep the screen face down. After placing the screen on the glass platen, close the lid immediately. Exposure to direct light will erase some of the signal on the screen.**

To remove the storage phosphor screen from the exposure cassette—

1. Open the latch on the cassette.
   - **For mounted screens—**Flip the lever(s) on the cassette (figure 6-2), and then rotate the clamp(s) clockwise so that they no longer cover the screen.
   - **For unmounted screens—**Open the latch and raise the lid of the cassette.
2. Remove the screen. Handle the screen by the edges only. Do not touch the white surface of the screen. If the sample sticks to the screen, gently peel off the sample.

   Note: To keep unmounted screens from moving and causing a double image, press down on one edge of the screen, and then lift up the opposite edge of the screen.
3. Protect the screen from direct light and proceed immediately to the next section.

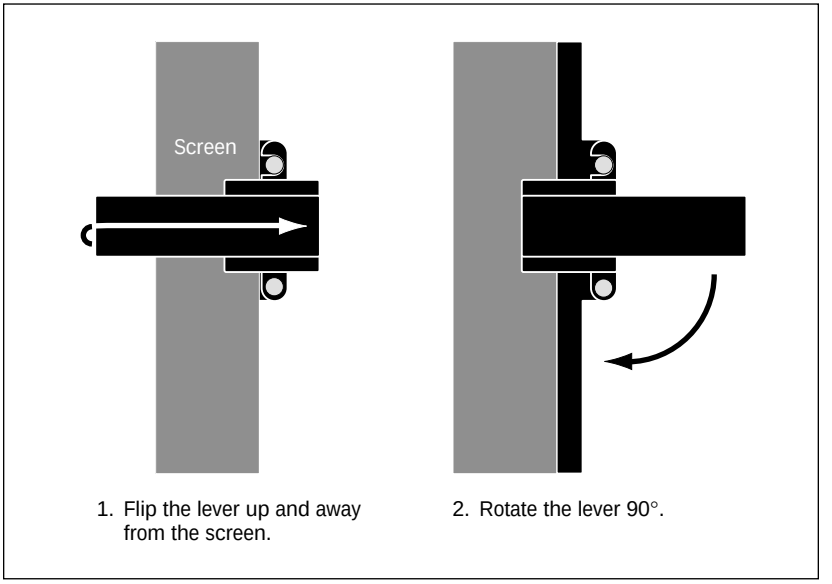

Figure 6-2. Opening the exposure cassette for mounted screens.

6.3 Loading the storage phosphor screen into the instrument

To load the storage phosphor screen into the Storm instrument—

1. If the sample lid is not already open, grasp the lid release under the center front of the sample lid and pull the release forward until the lid opens (figure 6-1). Raise the lid all the way.
2. Align the screen so that the white, phosphor side of the screen is face down and the top of the label is toward the back of the instrument (figure 6-3).
3. To align the upper left corner of the—
   - **Unmounted large or small screen—**Align the top and left edges with the back and left etched lines on the glass platen (figure 6-3).
   - **Mounted large screen—**Align the top and left edges of the metal plate with the inner edge of the black grid labels at the back and left edges of the glass platen.

     Note: To pick up a large mounted screen from the glass platen, use the small tabs attached to the metal back. If your screen does not have tabs or the tabs are damaged, contact Amersham Biosciences Technical Support.

- **Mounted small screen—**Insert the L-shaped adapter by aligning the outer edge of the adapter with the inner edge of the black grid labels at the back and left edges of the glass platen. Next, align the top and left edges of the metal plate with the inner edges of the adapter.

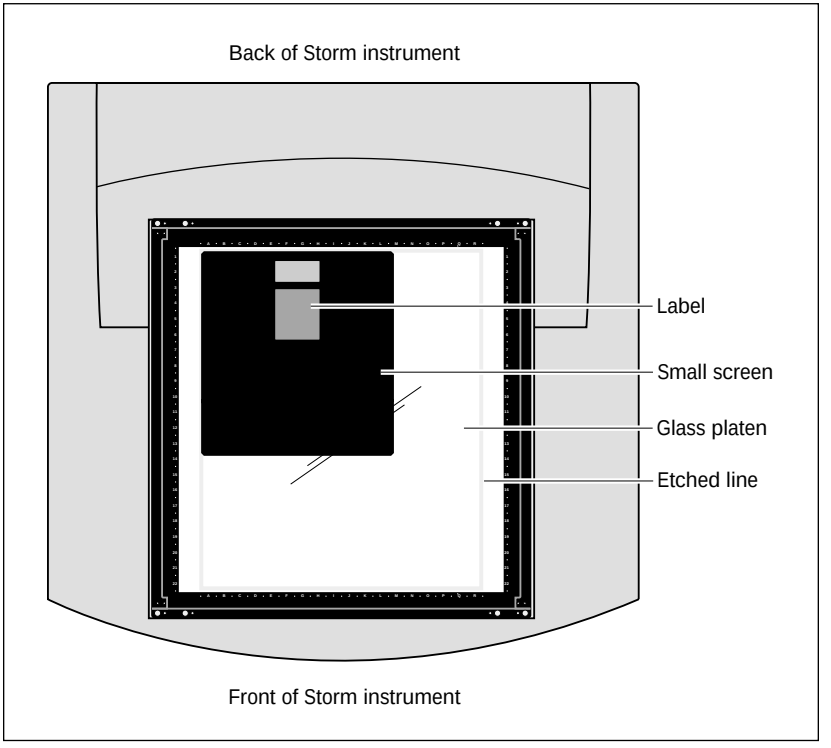

Figure 6-3. Load the screen onto the glass platen of the Storm instrument (small unmounted screen as viewed from above).

4. Gently lower the screen on the glass platen.

   Note: The unmounted screen may not lie completely flat against the glass at this time. When you initiate the scan, the instrument lowers the inner section of the lid to hold the screen flat.

5. Close the sample lid and press it down until the latch clicks. The screen is ready to scan.

6.4 Selecting the scan parameters in the Scanner Control window

You use the Scanner Control window (figure 6-4) to select the scan parameters you want to use when you scan the storage phosphor screen. If the Scanner Control window is not open, see section 3.3.

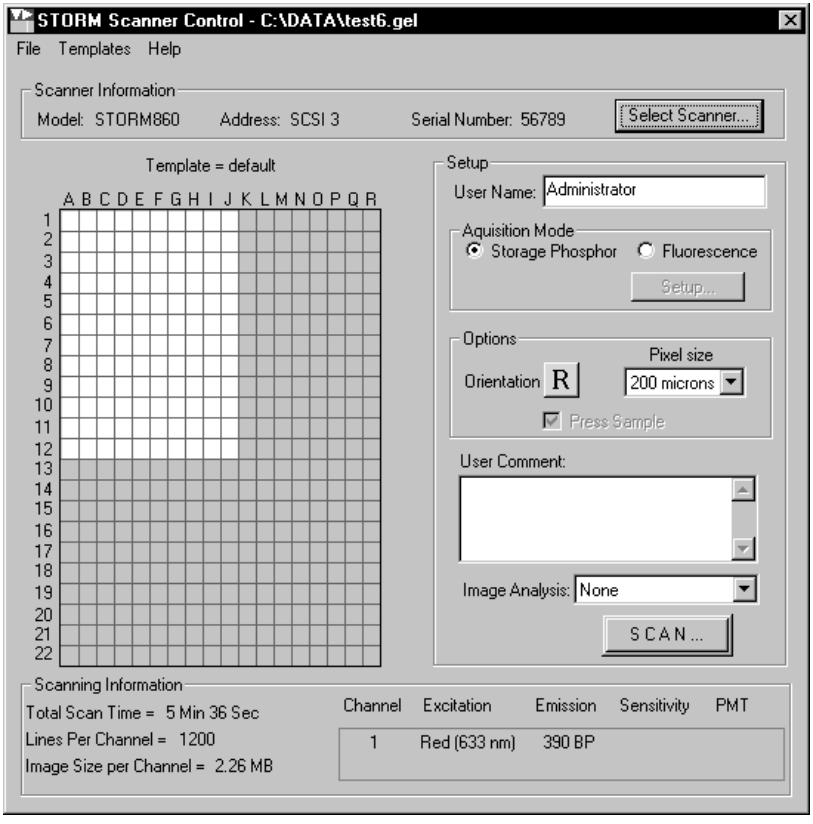

Figure 6-4. The Scanner Control window set for the storage phosphor scan acquisition mode.

6.4.1 Selecting a different Storm instrument (optional)

If you have more than one Storm instrument connected to the same computer, make sure you have the correct Storm instrument selected. The Scanner Information area displays the model number, SCSI address, and serial number of the selected instrument.

To change to a different Storm instrument, click the **Select Scanner** button. In the Select Scanner window (figure 6-5), select the instrument you want to use, and then click **OK.** The Select Scanner window closes, and the Scanner Control window changes to the default parameters for the instrument you selected.

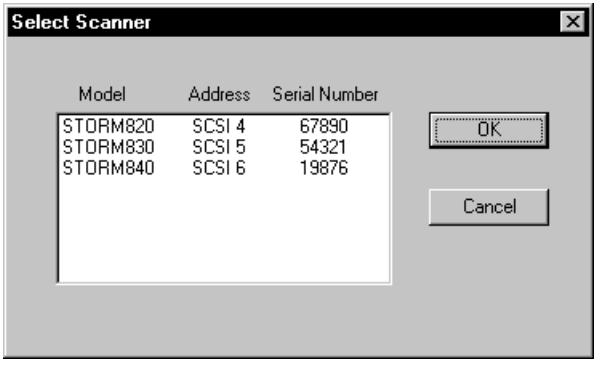

Figure 6-5. The Select Scanner window.

6.4.2 Selecting the storage phosphor scan acquisition mode

The storage phosphor scan acquisition mode is the default mode when you first start the Scanner Control software. If the Storm instrument has been used for fluorescence scanning, you choose the storage phosphor scan acquisition mode by clicking the **Storage Phosphor** button in the Acquisition Mode area of the Scanner Control window (figure 6-4).

6.4.3 Selecting the scan area

The grid in the Scanner Control window allows you to select the area to scan. The letter and number markings correspond to the markings in the exposure cassette and on the glass platen of the Storm instrument.

The white rectangle on the grid designates the area you want to scan. You can either use the current scan area or select a new one.

To select a new scan area—

1. Place the pointer in the grid square corresponding to the upper left corner of the area you want to scan.
2. Drag the pointer to the grid square corresponding to the lower right corner of the area you want to scan (figure 6-6).
3. Release the mouse button. The scan area you selected appears in white. The Scanning Information area displays the approximate image file size, the approximate scan time, and the number of lines per channel.

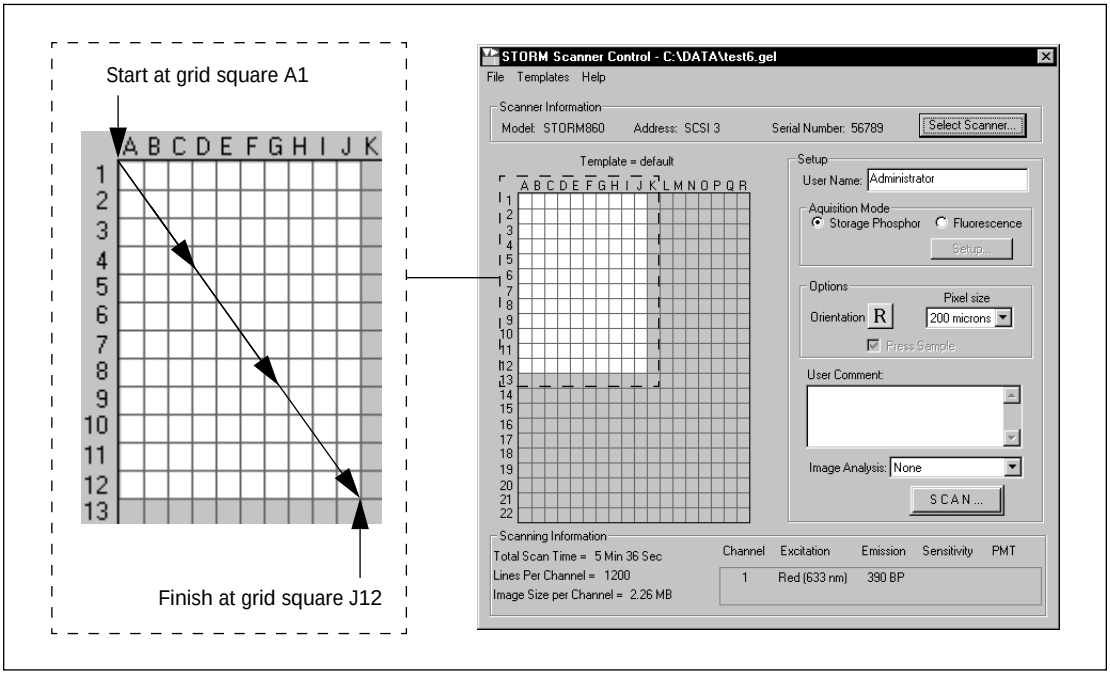

Figure 6-6. Selecting the scan area.

6.4.4 Selecting the sample orientation

You choose the orientation from the Setup Options area in the Scanner Control window (figure 6-4). The orientation buttons allow you to select an icon that represents how you placed the sample in the exposure cassette. Figure 6-7 describes each sample orientation option.

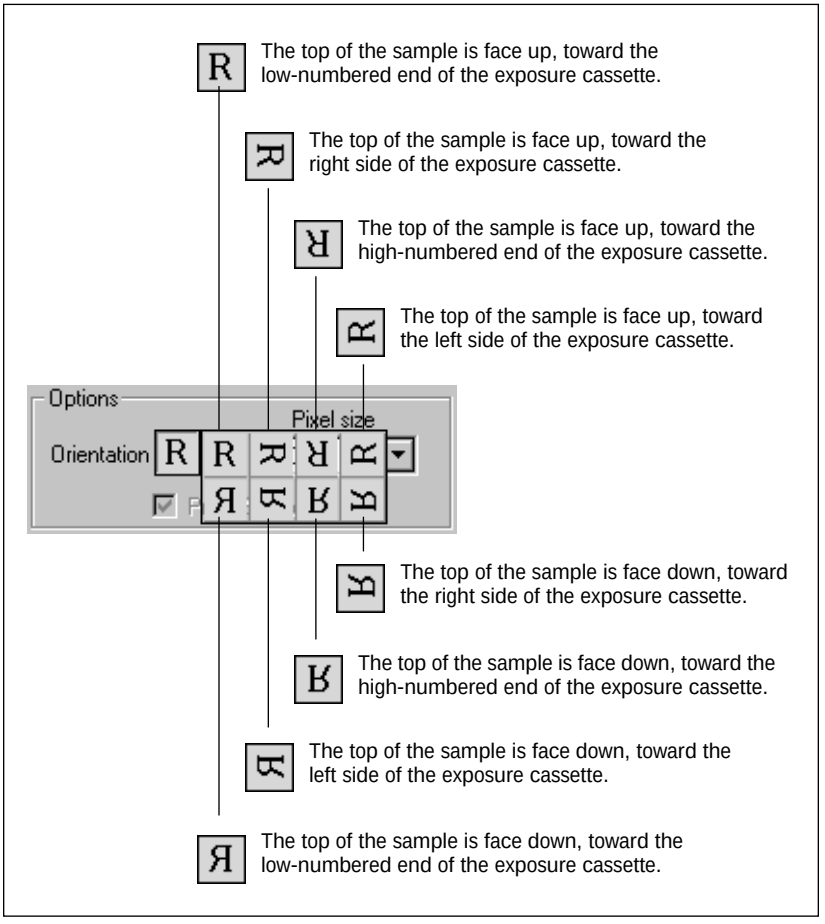

Figure 6-7. The sample orientation buttons.

To display the orientation buttons, place the pointer on the button next to Orientation in the Options area and hold down the mouse button. The orientation buttons appear. Move the pointer to the button that represents how you placed the sample in the exposure cassette and release the mouse button. During the scan, the software maps the pixels to display the image of your sample face up and top-end up.

6.4.5 Selecting the pixel size

Pixel size refers to the size of each individual picture element that is recorded and, together with the thousands of other pixels, forms the image. You choose the pixel size from the Pixel Size list in the Setup Options area of the Scanner Control window (figure 6-4).

- **For most standard electrophoresis samples,** choose **200 microns,** which provides the fastest scan time and the smallest image file size. The 200-µm pixel size produces 50 data points per cm and 100 data lines per grid square.
- **For samples requiring high resolution** (such as DNA sequencing), choose **100 microns.** The 100-µm pixel size produces 100 data points per cm and 200 data lines per grid square.
- **For samples requiring very high resolution** (such as whole body autoradiography), choose **50 microns.** The 50-µm pixel size produces 200 data points per cm and 400 data lines per grid square. After you change the pixel size, Scanner Control updates the image size, the scan time, and number of lines per channel in the Scanning Information area (figure 6-4).

6.4.6 Selecting the image analysis software

You can analyze the scanned image immediately after the scan is finished. To do this, select the analysis software you want to use at the end of your scan from the Image Analysis list in the Setup area of the Scanner Control window (figure 6-4). After the scan is complete, the selected software starts and displays the image.

In the storage phosphor scan acquisition mode, you can choose—

- **ImageQuant Tools—**To view and preprocess the scanned image. See the *ImageQuant Utilities User's Guide* or ImageQuant Tools Help for more information.
- **ImageQuant—**To view and analyze the scanned image. See the *ImageQuant User's Guide* or Help for more information.
- **None—**To leave the Scan in Progress window active so that you can perform another scan using the current scan settings. Scanner Control saves the image you just scanned for later viewing and analysis.

6.4.7 Entering User Comments (optional)

In the User Comment box, type any comment (up to 4 000 characters, including spaces) you want to save with the image. The comments are for reference only and do not affect the scan. After the scan, you can view, but not change, the information in ImageQuant and ImageQuant Tools.

Note: Scanner Control saves the scan parameters with the image. You can view, but not change, these parameters in ImageQuant and ImageQuant Tools. The scan parameters include pixel size, PMT voltage, file size, image type, scan date and time, and the instrument make and model. For more information, see the ImageQuant User Documentation.

6.5 Using templates

A template is a quick way to retrieve the scan parameters you use frequently. The template contains the scan parameters for the selected instrument, which include the grid area, scan acquisition mode, sample orientation, pixel size, and image analysis software. Comments in the User Comment box are not saved with the template.

Important **You cannot use the templates you created with previous versions of the Storm Scanner Control software.**

6.5.1 Creating a new template

To create a template—

1. Select the parameters in the Scanner Control window (sections 6.4.1 through 6.4.6).
2. Choose **Save As Template** from the Templates menu. The Save As Template window appears (figure 6-8).
3. Type a new name for the template in the box. **Note:** Because the Template list displays all the available templates for the selected instrument, you might want to add a label to identify which templates are for storage phosphor and which templates are for fluorescence. For example, a template for storage phosphor can be named TemplateName_Phosphor. (You should type an underscore instead of a space in template names.)
4. (Optional) If you want the template to become the new default, click the **Set as Default Template** check box. **Note:** You can select the system default template from the Templates menu.
5. Click **OK.** The template name appears above the grid area in the Scanner Control window.

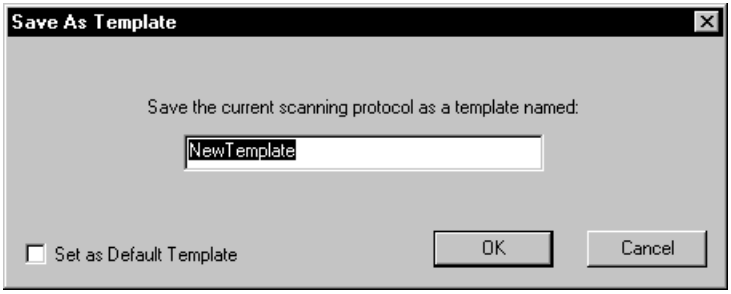

Figure 6-8. The Save As Template window for saving a template.

6.5.2 Selecting a template

If you want to use a different template, choose **Load** from the Templates menu and then select the template name from the list.

Note: The Templates list contains the available templates for the selected Storm instrument. For Storm instruments that scan in both the storage phosphor and the fluorescence mode, selecting a template can change the Scanner Control window to a different scan acquisition mode.

6.5.3 Modifying a template

You can modify a template and use the modified parameters to scan a sample. After you change a parameter, an asterisk appears next to the template name to show that the parameters have been changed. Scanner Control will not allow you to save the modified parameters.

If you want to save a modified template, you must delete the old template. Next, select the new settings and save the template using the name of the template you deleted.

6.5.4 Selecting a template to use as a default

If you want to select a different template to use as the default, choose **Set Default/Load** from the Templates menu and select the template name from the list.

6.5.5 Deleting a template

If you want to delete a template, choose **Delete** from the Templates menu. The Delete Template(s) window appears (figure 6-9). Select the template name that you want to delete and click **Delete.**

Note: Because you cannot delete the template displayed in the Scanner Control window, the template name does not appear in the list. To delete the template in use, close the Delete Template(s) window, select a different template from the Load list, and then choose **Delete** again. You should see the template name in the list.

To delete all the templates except the template displayed in the Scanner Control window, click **Select All** and then click **Delete.**

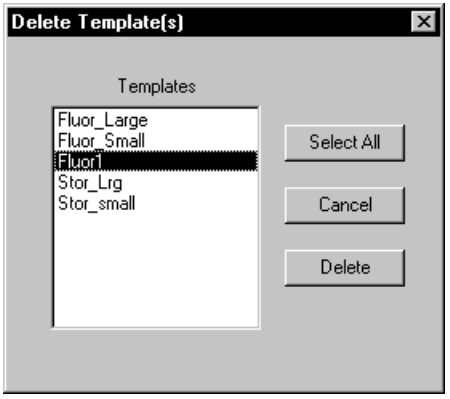

Figure 6-9. The Delete Template(s) window.

6.6 Starting the scan and checking the progress

Warning

Do not attempt to defeat the safety interlocks on the sample lid. These interlocks are designed to protect you from laser light exposure.

Cautions

Never turn off the Storm instrument or disconnect the SCSI cable while scanning. You can severely damage the internal mechanism of the instrument.

Keep the sample lid closed during the scan. Opening the lid shuts off the light source, aborts the scan, and saves the data already collected.

The scanning process, which is rapid and uses low-intensity light, causes little or no photobleaching of most samples. Light hits each position on the sample only while the corresponding pixel is being recorded. The position is only minimally exposed during the rest of the scan.

Scanner Control creates an image file and stores it using the Data File (.gel) type. **Note:** If you type the .ds file extension as part of the file name, Scanner Control changes the file extension to .gel. You use the Dataset File (.ds) type for multichannel images only (section 7.4).

6.6.1 Starting the scan

To start the scan—

1. Make sure that the storage phosphor screen is in place, the sample lid is shut, and all the parameters are correct in the Scanner Control window.
2. Click the **Scan** button. The Save As window appears (figure 6-10).

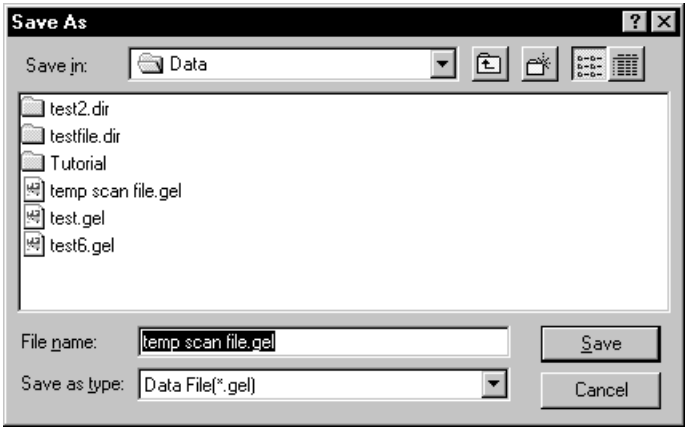

Figure 6-10. The Save As window for entering the data file name for the image.

3. In the Save As window, type a name in the **File name** box.

Caution **If you type a name that has already been used, a message appears asking you if you want to replace the existing image file. Before you click Yes, make sure you do not want the existing image file. Clicking Yes deletes the image and all the associated auxiliary files. If you have analyzed the image using ImageQuant, all the data will be deleted.**

4. (Optional) Change to a different folder. Otherwise, the software saves the image file in the Data folder.

Caution **Saving the scanned data to a folder located on a removable media disk drive can cause a loss of data. For best results, choose a folder on the computer hard drive. After you scan the screen, move the image file to the removable media.**

5. Click **Save** to start the scan. During the scan, the inner lid of the Storm instrument lowers to hold the screen flat against the glass platen of the instrument.

6.6.2 Monitoring the scan progress

After you start the scan, the Scan in Progress window (figure 6-11) appears, and the green Scan indicator light on the top of the instrument blinks. As the Storm instrument scans the storage phosphor screen, Scanner Control displays the image in the Scan in Progress window. In addition, Scanner Control displays the number of data lines scanned and the total scan time remaining (excluding initialization). Scanner messages can also appear as the scan progresses.

Note: To abort the scan, click **Cancel Scan** in the Scan in Progress window. A message appears asking if you want to delete the data file. If you choose to keep the file, the file size will be larger than the actual data. When you view the file size information in ImageQuant, a large file size indicates that the data has been truncated.

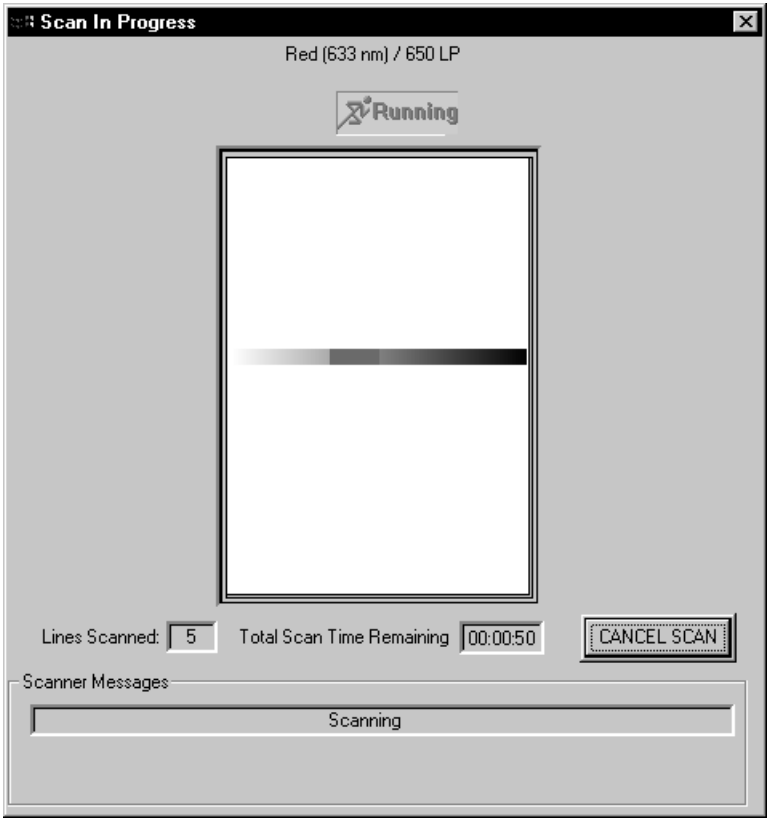

Figure 6-11. The Scan in Progress window for a storage phosphor scan.

6.6.3 Completing the scan

Scanner Control saves the image using the file name you selected in the Save As window. The Scan in Progress window displays a Complete message, and the Scan indicator light on the Storm instrument turns off. At the end of the scan, an image of the sample appears automatically in the software you selected from the Image Analysis list. If you selected None, the Scan in Progress window remains active for more scanning.

6.6.4 Viewing saturated data

Saturated data appear in red in the Scan in Progress window. If the image appears too saturated, you might not be able to analyze the image correctly.

Important **Before repeating the scan, verify in ImageQuant that the image is not too saturated to analyze. See the Gray/Color Adjust feature in the *ImageQuant User's Guide* for instructions on removing saturated data from the image display.**

Because the scanning process destroys the signal on the storage phosphor screen, you must expose a clean storage phosphor screen to the original sample. Alternatively, you can prepare a new sample and expose a clean storage phosphor screen to the new sample. To avoid saturation of the data, expose the screen for a shorter time.

6.7 Cleaning up after the scan

After the Storm instrument completes the scan, open the sample lid and remove the storage phosphor screen. Avoid touching the white side of the screen.

To lift a large mounted screen, pull up on the round tabs attached to the metal plate, and then slide a finger under the edge of the metal backing.

After each scan, you should—

- Remove the sample from the exposure cassette and dispose of the sample using the established procedure in your laboratory.
- Check the storage phosphor screen for contamination (section 5.2.2).
- Clean the storage phosphor screen (section 5.2.3).
- Erase the storage phosphor screen (section 5.2.4).
- Check the Image Eraser for contamination and clean the eraser (section 11.1).
- Check the glass platen and sample lid for contamination and clean the glass platen and sample lid of the Storm instrument (section 6.1).
- Check the exposure cassette for contamination and clean the exposure cassette (section 11.4).

6.8 Storing the storage phosphor screen

Before storing the storage phosphor screen, you should clean, decontaminate (if necessary), and erase the screen. Then store the screen—

- At room temperature.
- Away from sources of radiation, such as strong beta or gamma emitters or x-ray machines.
- In a protective box or in a clean exposure cassette. Damaged screens are unusable and must be replaced.

6.9 Analyzing or preprocessing the scanned image

You should display the image of the sample to determine the quality of the scan. If you selected ImageQuant for analyzing or ImageQuant Tools for preprocessing in the Scanner Control window, the selected software starts and displays the image. Refer to the applicable user's guide or Help for information on how to use the selected software.

Part three

Scanning in the fluorescence mode

Chapter 7 About fluorescence scanning

This chapter describes the theory behind fluorescence. The Storm 860, 840, and 830 can be used to scan for fluorescence emissions. The topics in this chapter are—

- How fluorescence works (section 7.1)
- Advantages of direct fluorescence (section 7.2)
- Optical filters (section 7.3)
- Dual-label (multichannel) experiments (Storm 860 only) (section 7.4)
- Common fluorochromes (section 7.5)

7.1 How fluorescence works

The absorption of light energy by a fluorochrome boosts an electron to a higher energy shell (excited state). The characteristic light absorption pattern of a fluorochrome is shown by its *absorption spectrum* (figure 7-1).

The excited state has a very short half-life, on the order of a few nanoseconds. During the brief lifetime of the excited state, the excited electron generally decays toward a lower energy level within the excited state. When the electron falls to the ground state, the fluorochrome emits light of specific wavelengths. The distribution of fluorescent emissions among different wavelengths is plotted in the emission spectrum (figure 7-1).

The fluorescent emission spectrum is always shifted toward a longer wavelength (lower energy) relative to the excitation spectrum. The difference in wavelength between the maxima of the absorption and the emission spectra is called the Stokes shift. Because of this shift, optical filtration can be used to separate excitation light from the longer-wavelength emitted light.

For more information on how fluorescence is generated, see the Amersham Biosciences publication *Fluorescence Imaging principles and methods.* (See appendix E for a list of references.)

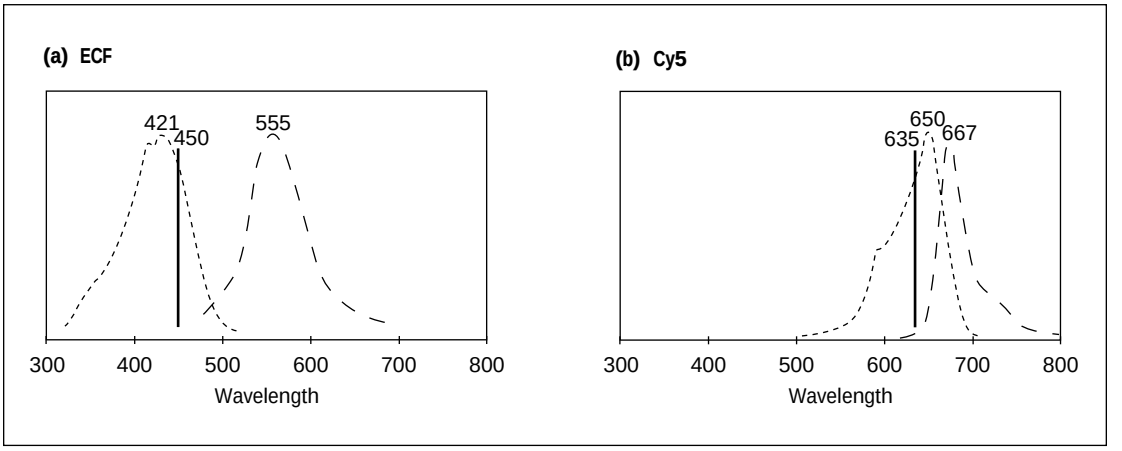

Figure 7-1. Absorption (---) and emission (— —) spectra of the fluorescent product of ECF substrate (a) and of Cy5 (b). The wavelength with maximum absorption or emission is shown above the curves. The vertical line shows the excitation wavelength. (These curves are approximations based on data collected at Amersham Biosciences.)

7.2 Advantages of direct fluorescence

Scanning samples labeled with dyes excitable by 450-nm (blue) or 635-nm (red) light source provides results in digital form. These results can be analyzed qualitatively and quantitatively using ImageQuant.

Scanning direct fluorescence on the Storm system offers several advantages—

- Nucleic acid and protein gels can be analyzed shortly after electrophoresis. You soak gels in dye solution and rinse away the excess dye just as for the well-known ethidium bromide and Coomassie™ protocols. These same gels can be used for follow-on blot experiments.
- You can analyze nucleic acid and protein blots using fluorescently labeled probes, such as Cy™5-labeled DNA probes or antibodies.
- Pixel-by-pixel fluorescent excitation eliminates the fluorescent blooming, which is caused by constant excitation in traditional systems and provides better resolution of closely spaced bands.
- Quantitation is simplified because, unlike instant film, the Storm system provides a linear response to the fluorescent signal intensities.

7.3 Optical filters

The Storm instrument uses optical filters to reject reflected and scattered excitation light while allowing the emitted light from the sample to pass through to the detector. The instrument can use—

- **520-nm long-pass filter—**Passes light with wavelengths longer than 520 nm. The filter is used for blue-excited fluorescence and is installed on the Storm 860 and 840.
- **650-nm long-pass filter—**Passes light with wavelengths longer than 650 nm. The filter is used for red-excited fluorescence and is installed on the Storm 860 and 830.

You use the Setup window in the Scanner Control software to select the appropriate filter (section 9.2).

7.4 Dual-label (multichannel) experiments (Storm 860 only)

You can label the sample with two fluorescent dyes and then use the Storm 860 to create a multichannel image. To provide accurate detection and separation of the signals, you label part of the sample with a fluorochrome that can be collected using the 520-nm long-pass filter and another part of the sample with a fluorochrome that can be collected using the 650-nm long-pass filter. You correct for cross-contamination of the signals by including a standard for each fluorochrome.

You use the FluorSep software to remove the cross-contamination and create two images that can be viewed in ImageQuant. For information on using the FluorSep software, see the *ImageQuant Utilities User's Guide* or the FluorSep Help.

In ImageQuant, you can use the Multichannel commands to view the two channels of data overlaid in different colors, or you can view the two channels side by side in gray scale. For information on using ImageQuant and the Multichannel commands, see the *ImageQuant User's Guide* or Help.

7.5 Common fluorochromes

Table 7-1 lists the absorption and emission maxima of some common fluorochromes. The chemistry of the experiment may alter the absorption and emission spectra slightly.

For more information on selecting fluorochromes, see the Amersham Biosciences publication *Fluorescence Imaging principles and methods Technical Manual*. (See appendix E for a list of references.)

Table 7-1. Fluorochrome Absorption and Emission Maxima*

Fluorochrome	Absorption maximum (nm)	Emission (nm)
Acridine Orange-dsDNA	502	526
ECF™ substrate	440	560
BODIPY™ 493/503	493	503
BODIPY FL	503	512
Cy™5	649	670
ECL Plus™ Western substrate	420	460
FAM™	495	535
Fluorescein	490	520
NanoOrange™	485	590
OliGreen™-ssDNA	480	520
Phycoerythrin (R)	480	578
PicoGreen™-dsDNA	480	520
ROX™	590	605
SYBR™ Green I-dsDNA	497	520
SYPRO™ Orange	472	570
TET™	519	545
TOTO™-1-dsDNA	513	532
Vistra Green™	490	520
YO-PRO™ -1-dsDNA	491	509
YOYO™ -1-dsDNA	491	509

* Values listed are from the manufacturers' catalogs or from data collected at Amersham Biosciences.

Chapter 8 Setting up for fluorescence scanning

This chapter describes how to prepare the sample for fluorescence scanning and how to place the sample in the Storm instrument. The topics in this chapter are—

- Preparing the sample (section 8.1)
- Cleaning the glass platen and sample lid (section 8.2)
- Loading the sample (section 8.3)

8.1 Preparing the sample

The following sections provide guidelines to use when preparing samples for fluorescence scanning.

8.1.1 General guidelines

Dust fluoresces and scatters light, which causes artifacts on images and can interfere with quantitation. To avoid this—

- **Wear powder-free gloves—**Because most powder used in laboratory gloves fluoresces, you should wear powder-free gloves. Always rinse the gloves with distilled or tap water before handling the sample and before preparing the reagent.
- **Filter solutions—**Remove dust particles by filtering all the stock solutions used to prepare the sample, sample matrix, and buffers. Use clean, rinsed containers. Some reagents, such as fluorescent labels, are supplied dust free and require no further filtration. When diluting clean reagents to working concentration, use distilled water collected in a rinsed container so that you do not have to filter the solutions again.
- **Avoid fluorescent indicator dyes—**Many of the commonly used electrophoresis tracking dyes fluoresce. Whenever possible, put the tracking dye in a separate lane. Alternatively, dilute the indicator dyes as much as possible.
- **Avoid excessive exposure to light—**Fluorochromes differ greatly in their sensitivity to light-induced degradation (photobleaching). When working with sensitive fluorochromes and fluorescently labeled samples, use low-light conditions.

8.1.2 Label guidelines

Use the following guidelines when selecting the label for the fluorescence scanning:

- **Blue-excited fluorescence—**Detects samples labeled with fluorochromes that have emissions longer than 520 nm. Use the Blue (450-nm) excitation setting on the Storm 860 and 840.
- **Red-excited fluorescence—**Detects samples labeled with fluorochromes that have emissions longer than 650 nm. Use the Red (635-nm) excitation setting on the Storm 860 and 830.
- **Dual-label fluorescence—**On the Storm 860, you can label the sample with two fluorochromes and create a multichannel image. Use the Blue (450-nm) excitation setting with a fluorochrome that has an emission longer than 520 nm. Use the Red (635-nm) excitation setting with a fluorochrome that has an emission longer than 650 nm.

8.1.3 Low-fluorescence sample support guidelines

For fluorescent samples requiring high sensitivity or highly accurate quantitation, the following material sources have been tested and found to have low background.

- **Gels—**Background fluorescence contributed by the gel matrix increases with gel thickness. Always use the thinnest gel practical for your experiment, especially for agarose gels. Make sure your glass plates are absolutely clean before you pour the gel. Grease and fingerprint oils from the plates can stick to the gel surface and attract dust and fluorescent dyes.
  - **Agarose—**FMC SeaKem™ Gold has very low background. If another type of agarose has properties that are useful for your application, scan a test gel to make sure the background is low enough for your purposes.

    Make sure the agarose is completely dissolved and well-mixed before pouring your gel. Also, avoid generating bubbles when mixing and pouring. Uneven agarose concentration and bubbles affect light scatter and can cause artifacts and interfere with quantitation.
  - **Polyacrylamide**—Polyacrylamide gels are usually clear and thin (less than 1 mm). The background contribution from the gel material is generally very low.
- **Solvents—**Spectroscopic-grade solvents have the lowest autofluorescence.

- **Membranes—**You should scan a test piece of each type of membrane you plan to use and check that the background is low enough for your purposes.
- **Transparency support—**To avoid contaminating the glass platen and sample lid, you can place a sample, such as a membrane, between two pieces of transparency material (section 8.3.3). Although it is not generally a problem, you should check that the background generated by the transparency material is low enough for your purposes.

8.2 Cleaning the glass platen and sample lid

You should clean the glass platen and sample lid before placing your sample on the plate. In addition, you should protect the glass from scratches because these interfere with accurate imaging and quantitation.

Important **If the Storm instrument is used for both fluorescence and storage phosphor scanning, you should check for radioactive contamination (section 6.1.1).**

8.2.1 Cleaning the glass platen

The glass platen of the Storm instrument should be kept free of sources of fluorescent background (such as dust, dried buffer, and skin oils). It is good practice to clean the glass platen before and after you scan each sample.

To clean the glass platen—

1. Grasp the lid release under the center front of the sample lid and pull the release forward until the lid opens (figure 8-1). Raise the lid all the way.

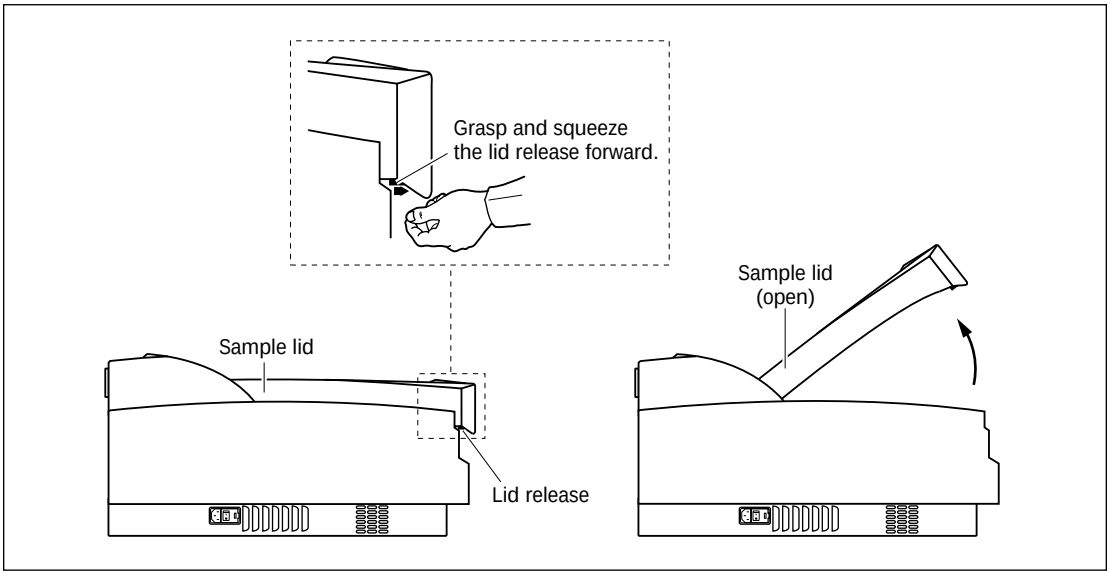

Figure 8-1. Releasing and opening the sample lid.

2. (Optional) If fluorescent material has come in direct contact with the glass platen, moisten a lint-free cloth with 10% hydrogen peroxide and wipe the glass several times.

3. Clean the glass with distilled water and a clean, lint-free cloth or paper. If visible spots remain, clean the glass first with 100% ethanol and then with distilled water.

Caution **Because window cleaners contain ingredients that can fluoresce, do not use window cleaners to clean the glass platen. In addition, the use of acetone or the excessive use of ethanol can shorten the lifetime of the instrument. Protect the glass from scratches. Scratches interfere with accurate imaging and quantitation.**

8.2.2 Cleaning the sample lid

The surface of the inner lid should be clean and free of fluorescent contamination. If necessary, clean the surface with a damp (not saturated) cloth moistened with a small amount of distilled water. **Note:** You should wear powder-free gloves to protect your hands when cleaning the sample lid using 75% ethanol. If visible spots remain, clean the surface first with 75% ethanol and then with distilled water. To prevent liquid from seeping inside the lid or rolling down onto the glass, do not spray liquid on the lid.

8.3 Loading the sample

Before you load the sample, determine how to position it on the glass platen.

Caution **Make sure you place the sample in the correct position when you first set it down. Any fluorescent material left on the glass platen when you move the sample can result in a double image.**

8.3.1 Determining the sample orientation

Determine whether to place the sample face up or face down on the glass platen. The Storm system illuminates the sample and collects data from underneath the sample—

- For a one-sided, opaque sample (such as a membrane or TLC plate), place the sample face down.
- For a **transparent** sample (such as a polyacrylamide gel), place the sample either face up or face down.
- If the sample is **physically uneven on one side** (such as an agarose gel), place the smooth side down. This allows the sample to lie flat.

Note: Remember the orientation of the sample on the glass platen. You will use this information in the Scanner Control window (section 9.1.4).

8.3.2 Determining the sample placement

Use the following guidelines and figure 8-2 to determine where on the glass platen to place the sample:

- To minimize the time required for the scan, place the sample near the upper left corner of the maximum scan area. Each lettered or numbered grid segment is marked by two dots. Use the following to determine the maximum available scan area:
  - **Blue-excited fluorescence—**Within the etched outline on the glass platen and, on the right side, to the extra dot in section Q of the image area grid (figure 8-2). The narrow segment (approximately 3 cm) to the right of the extra dot in section Q is not scanned by the Storm 860 and 840 in the chemifluorescence/blue-excited fluorescence mode.
  - **Red-excited fluorescence—**Within the etched outline on the glass platen when scanning with the Storm 860 or 830.
- To minimize the size of the image file, scan only the number of grid squares covered by the sample. If you do not want to scan the entire sample, scan the squares that contain the part of the sample you are interested in analyzing.

- To record multiple samples (gels, membranes, and so on) during the same scan, place the samples close together. Make sure the samples are the same thickness. If the samples are not the same thickness, the selected focal plane might not be optimal for each sample, which can result in a bad scan, poor image, and poor quantitation results.
- Align the lanes and rows of the sample with the edges of the grid on the glass platen so that the scanned image will be straight.

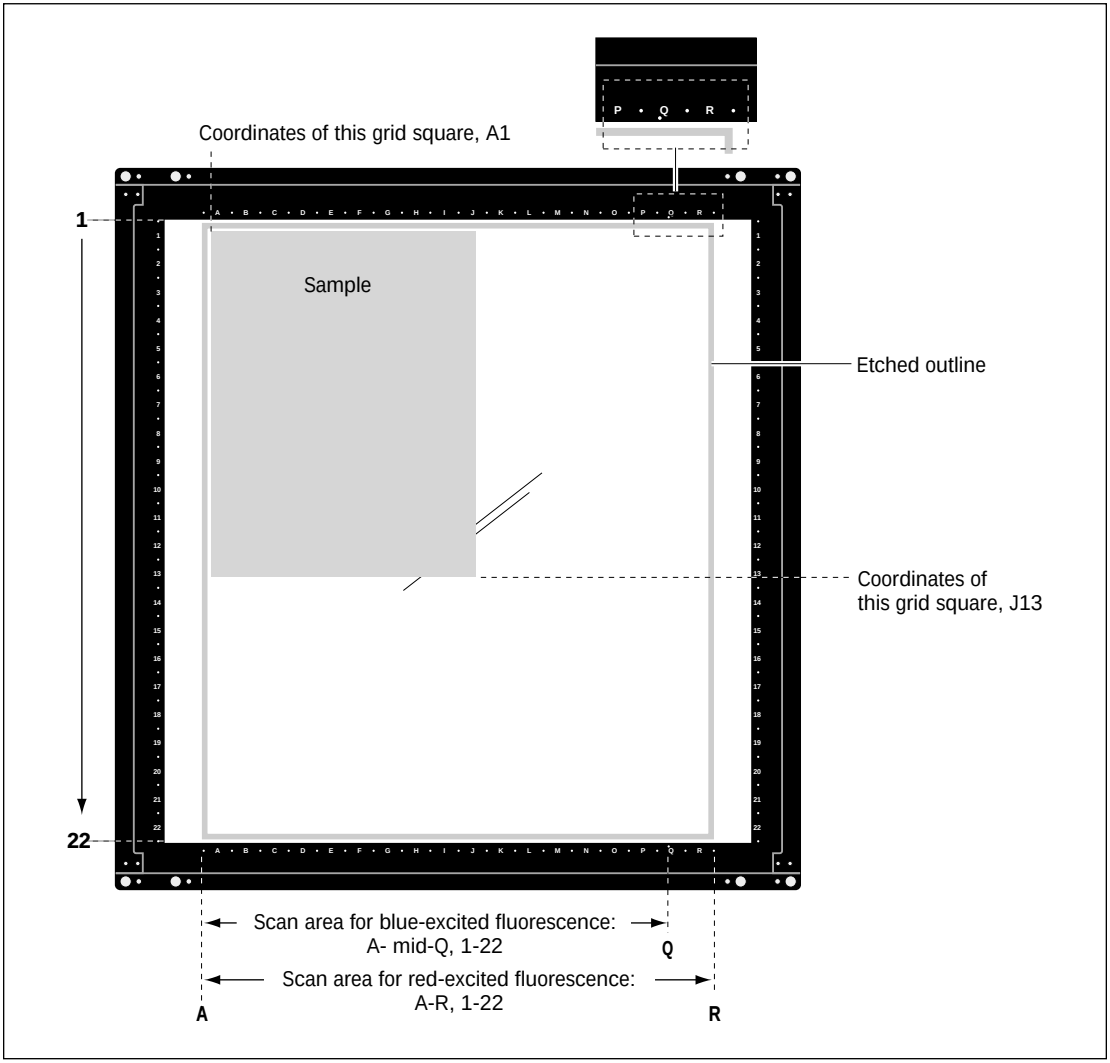

Figure 8-2. Sample placement on the glass platen of the Storm instrument.

8.3.3 Placing the sample on the glass plate

You can place the sample directly on the glass platen. However, for gels and membranes, you can avoid contaminating the glass platen and possible double images if you place the gel on a low-fluorescence electrophoresis glass plate or place a membrane in a low-fluorescence plastic bag.

You place the sample on the glass platen so that the sample or sandwich gel creates a smooth surface on the glass platen. Avoid trapping air bubbles between the sample and the glass platen. Air bubbles can appear on the scanned image. Before placing a wet sample on the glass platen of the instrument, squirt a little buffer or distilled water on the glass. The buffer or distilled water lowers the chance of trapping air bubbles between the sample and the glass platen.

Important **Make sure you wipe off the excess buffer or distilled water. Using too much liquid can cause the sample to move on the glass platen during the scan, which could affect the quality of the collected data.**

Placing a gel or membrane

To place a gel or membrane on the glass platen—

1. If the sample lid is not already open, grasp the lid release under the center front of the lid and pull the release forward until the lid opens (figure 8-1). Raise the lid all the way.
2. (Optional) For wet samples, squirt a little buffer or distilled water on the glass platen.
3. Hold the sample by the edges or use a clean plastic spatula (for gels) or forceps (for membranes) to handle the sample. Gently lower the sample onto the glass starting at one edge. Do not trap air bubbles under the sample or scratch the glass.

Important **Do not touch the glass platen or the part of the sample that will be read by the Storm instrument. Oil from fingerprints and powder from gloves, even thoroughly washed gloves can leave a print that can be detected.**

4. Make a note of the coordinates of the **upper left** and **lower right** corners of the area you want to scan (figure 8-2). You will use this information later in the Scanner Control window (section 9.1.3).
5. Close the sample lid and press it down until the latch clicks. The sample is ready to scan.

Placing a sandwich gel

Important **Make sure the low-fluorescence electrophoresis glass plate that you place on the glass platen is 3 mm thick. The +3 mm focal plane parameter is optimized for 3-mm thick glass.**

To place a sandwich gel on the glass plate—

1. If the sample lid is not already open, grasp the lid release under the center front of the lid and pull the release forward until the lid opens (figure 8-1). Raise the lid all the way.
2. Squirt approximately 0.5–2 ml of deionized water on the glass plate. The water helps to adhere the sandwich gel to the glass plate. (Alternatively, you can place the sandwich gel on the dry glass plate.)
3. Hold the sandwich gel by the edges and place the upper left corner of the gel on the upper left corner of the glass plate (figure 8-3).
4. Lower the sandwich gel diagonally until the lower right corner of the gel rests on the glass plate. Do not trap air bubbles under the sample.

Important **Do not touch the part of the sandwich gel that will be read by the Storm instrument. Do not touch the glass plate. Oil from fingerprints and powder from gloves, even thoroughly washed gloves, can leave a print that can be detected.**

5. Make a note of the coordinates of the **upper left** and **lower right** corners of the area you want to scan (figure 8-2). You will use this information later in the Scanner Control window (section 9.1.3).
6. Close the sample lid and press it down until the latch clicks. The sample is ready to scan.

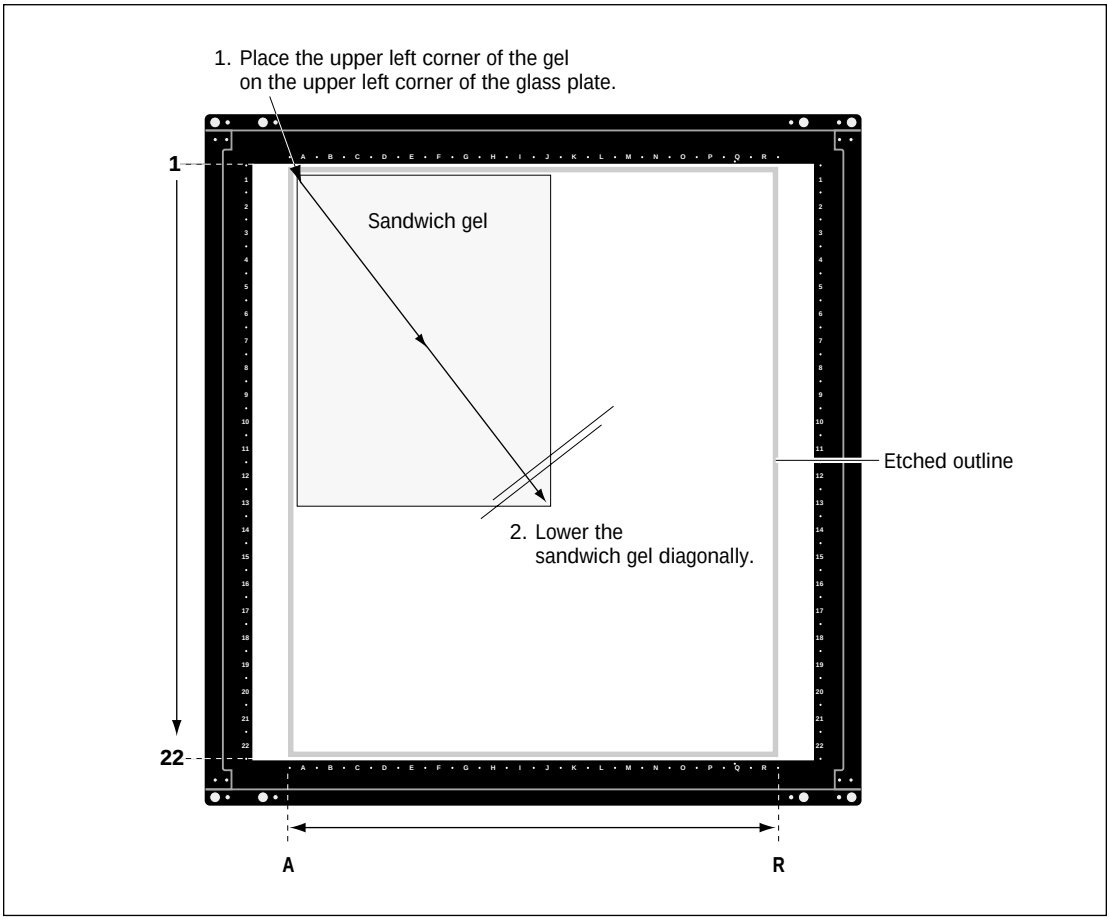

Figure 8-3. Aligning the sandwich gel on the glass plate.

Chapter 9 Scanning the fluorescent sample

This chapter provides step-by-step instructions for scanning a fluorescent sample. The topics in this chapter are—

- Selecting the scan parameters in the Scanner Control window (section 9.1)
- Using the Setup window to select the fluorescence parameters (section 9.2)
- Using templates (section 9.3)
- Starting the scan and checking the progress (section 9.4)
- Removing the sample from the Storm instrument (section 9.5)
- Cleaning the glass platen and sample lid (section 9.6)
- Analyzing or preprocessing the scanned image (section 9.7)

9.1 Selecting the scan parameters in the Scanner Control window

You use the Scanner Control window (figure 9-1) to select the parameters you want to use when you scan the fluorescent sample.

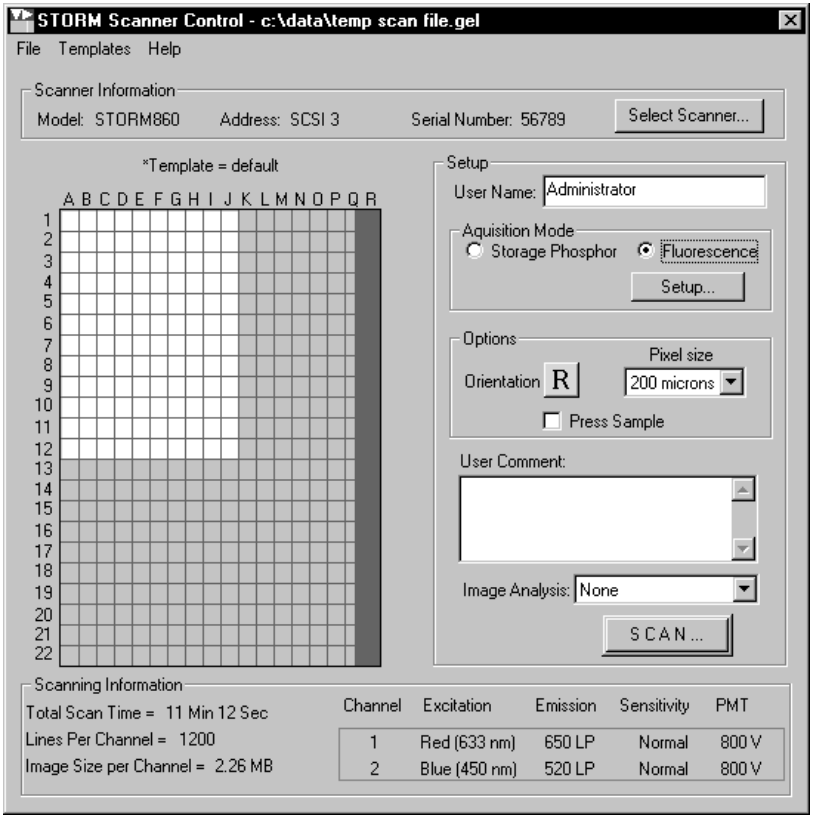

Figure 9-1. The Scanner Control window set for the fluorescence scan acquisition mode.

9.1.1 Selecting a different instrument (optional)

If you have more than one Storm instrument connected to the same computer, make sure you have the correct Storm instrument selected. The Scanner Information area displays the model number, SCSI address, and serial number of the selected instrument.

To change to a different Storm instrument, click the **Select Scanner** button. In the Select Scanner window (figure 9-2), select the instrument you want to use, and then click **OK.** The Select Scanner window closes, and the Scanner Control window changes to the default parameters for the instrument you selected.

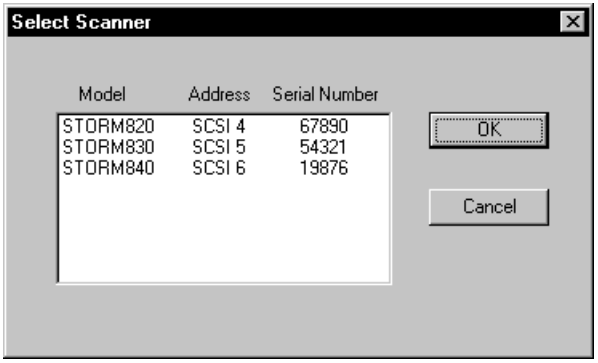

Figure 9-2. The Select Scanner window.

9.1.2 Selecting the Fluorescence scan acquisition mode

Storage phosphor is the default scan acquisition mode when you first start the Scanner Control software. To change to the fluorescence mode, click the **Fluorescence** button in the Acquisition Mode area. The selections for the fluorescence scan acquisition mode appear in the Scanner Control window (figure 9-1) and in the additional Setup window. **Note:** The Fluorescence button is not available on the Storm 820.

9.1.3 Selecting the scan area

The grid in the Scanner Control window allows you to select the area to scan. The letter and number markings correspond to the markings on the glass platen of the Storm instrument.

The white rectangle on the grid designates the area you want to scan. You can either use the current scan area or select a new one.

To select a new scan area—

1. Place the pointer in the grid square corresponding to the upper left corner of the area you want to scan.
2. Drag the pointer to the grid square corresponding to the lower right corner of the area you want to scan (figure 9-3).

   Note: For blue-excited fluorescence, the maximum width of the scan area is from section A to the middle of section Q. You cannot scan a narrow strip along the right side of the grid.

3. Release the mouse button. The scan area you selected appears in white. The Scanning Information area displays the image file size, approximate scan time, and number of lines per channel.

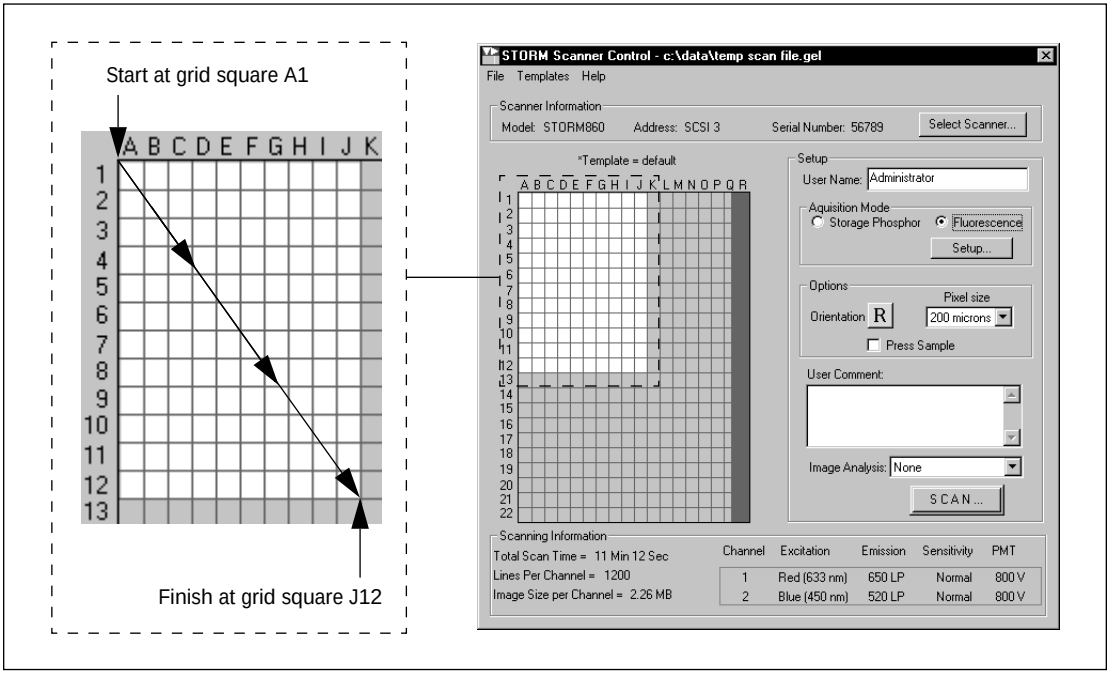

Figure 9-3. Selecting the scan area.

9.1.4 Selecting the sample orientation

You choose the orientation from the Setup Options area in the Scanner Control window (figure 9-1). The orientation buttons allow you to select an icon that represents how you placed the sample on the glass platen of the Storm instrument. Figure 9-4 describes each sample orientation option.

To display the orientation buttons, place the pointer on the button next to Orientation in the Options area and hold down the mouse button. The orientation buttons appear. Move the pointer to the button that represents how you placed the sample on the glass platen and release the mouse button. During the scan, the software maps the pixels to display the image of your sample face up and top-end up.

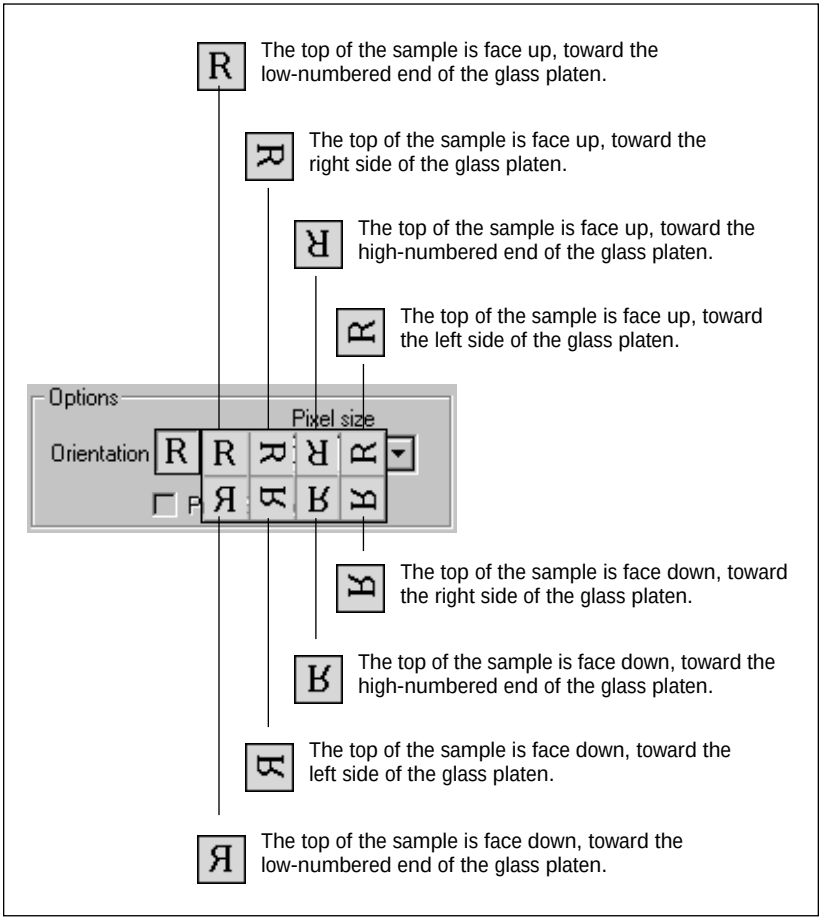

Figure 9-4. The sample orientation buttons.

9.1.5 Selecting the pixel size

Pixel size refers to the size of each individual picture element that is recorded and, together with the thousands of other pixels, forms the image. You choose the pixel size from the Pixel Size list in the Setup Options area of the Scanner Control window (figure 9-1).

- **For most samples,** choose **200 microns,** which provides the fastest scan time and the smallest image file size. The 200-µm pixel size produces 50 data points per cm and 100 data lines per grid square.
- **For samples that require a higher resolution,** choose **100 microns.** The 100-µm pixel size produces 100 data points per cm and 200 data lines per grid square.

- **For red-excited fluorescent samples that require very high resolution,** choose **50 microns.** The 50-µm pixel size produces 200 data points per cm and 400 data lines per grid square. **Note:** Blue-excited fluorescence does not include the 50-µm pixel size.

After you change the pixel size, Scanner Control updates the image size, scan time, and number of lines per channel in the Scanning Information area (figure 9-1).

9.1.6 Selecting the Press Sample parameter

Important **The Press Sample parameter is not saved as part of a template. You must select this parameter each time you scan.**

If you are scanning a sample, such as a dry membrane or filter paper, that does not lie flat against the glass platen of the instrument, you should select the Press Sample check box. When you scan the sample, the inner lid of the Storm instrument lowers to press the sample flat. If you do not select Press Sample, the inner lid remains retracted during the scan.

Caution **Do not select Press Sample if you are scanning wet membranes, wet gels, or soft samples. Pressing wet or soft samples can damage the instrument. In addition, pressing a soft sample can distort the scanned image.**

9.1.7 Selecting the image analysis software

You can analyze the scanned image immediately after the scan is finished. To do this, select the analysis software you want to use at the end of your scan from the Image Analysis list in the Setup area of the Scanner Control window (figure 9-1). After the scan is complete, the selected software starts and displays the image.

In the fluorescence scan acquisition mode, you can choose—

- **ImageQuant Tools—**To view and preprocess the scanned image. See the *ImageQuant Utilities User's Guide* or ImageQuant Tools Help for more information.
- **FluorSep—**To separate an image that was scanned on the Storm 860 using both excitation parameters (multichannel image). See the *ImageQuant Utilities User's Guide* or FluorSep Help for more information.
- **ImageQuant—**To view and analyze the scanned image. See the *ImageQuant User's Guide* or Help for more information.

- **None—**To leave the Scan in Progress window active so that you can perform another scan using the current scan settings. Scanner Control saves the image you just scanned for later viewing and analysis.

9.1.8 Entering User Comments (optional)

In the User Comment box, type any comment (up to 4 000 characters, including spaces) you want to save with the image. The comments are for reference only and do not affect the scan. After the scan, you can view, but not change, the information in ImageQuant, ImageQuant Tools, and FluorSep (multichannel images only).

Note: Scanner Control saves the scan parameters with the image. You can view, but not change, these parameters in ImageQuant, ImageQuant Tools, and FluorSep (multichannel images only). The scan parameters include pixel size, PMT voltage, file size, image type, scan date and time, and the instrument make and model.For more information, see the ImageQuant User Documentation.

9.2 Using the Setup window to select the fluorescence parameters

You use the Setup window to select the fluorescence parameters to use with the sample. To display the Setup window, click the **Setup** button in the Acquisition Mode area. Depending on the model of the Storm instrument, the following parameters will be active:

- **Model 860—**Scan parameters for red-excited (channel 1) and blue-excited (channel 2) fluorescence (figure 9-5). You can scan using one or both excitation parameters. If you scan using both excitation parameters, Scanner Control creates a multichannel image consisting of one image of the blue-excited fluorescence and one image of the red-excited fluorescence.
- **Model 840—**Scan parameters for blue-excited fluorescence only.
- **Model 830—**Scan parameters for red-excited fluorescence only.

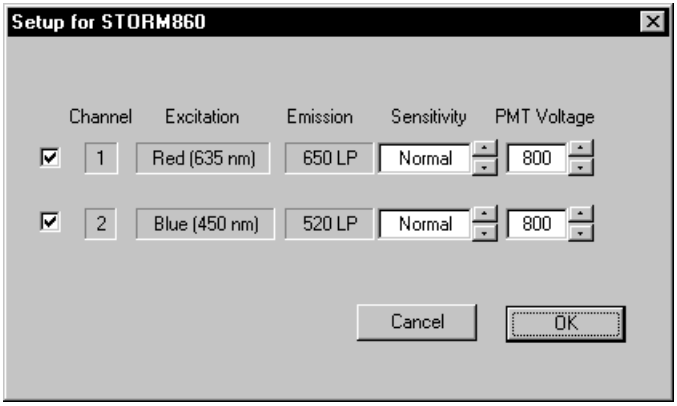

Figure 9-5. The Setup window for the Storm 860, which contains parameters for both red-excited (channel 1) and blue-excited (channel 2) fluorescence.

9.2.1 Setting the sensitivity

The sensitivity parameter controls how long the Storm instrument collects data from each pixel. The Sensitivity list contains two choices—

- **Normal,** the default parameter, samples each pixel once. In general, you should use Normal.
- **High** collects data from each pixel eight times and averages the results. Using High to detect weak signals improves the sensitivity without increasing the noise and background. **Note:** Selecting High increases the scanning time, but does not increase the size of the image file. Check the Scanning Information area in the Scanner Control window for the time required to complete the scan.

(Storm 860 only) If you are performing a multichannel scan, you can set a sensitivity parameter for each channel. If you select High for both channels, the scan can take over 5 hours to complete.

9.2.2 Setting the PMT voltage

For fluorescent samples, you can alter the PMT voltage to improve the signal collection capabilities of the Storm instrument.

Guidelines for changing the PMT voltage

For accurate quantitation of intense samples, pixel intensities in your scanned image should be no greater than 99 978.08 counts.

- If the values are less than 99 978.08 counts, all spots in your image are within the dynamic range of the instrument.

- If some of the values are equal to or greater than 99 978.08 counts, part of your image is at or near saturation. Lower the PMT voltage, rescan, and confirm that the image is not saturated.

If your experiment includes only very weak samples or only very intense samples, you can adjust the voltage to extend the lower or upper end of the sensitivity range.

- For very weak samples, increase the PMT voltage. You might lose quantitation at the upper end of the signal range. If above 900 V, you might lose sensitivity because of increased PMT noise. Note that you cannot exceed 1 000 V.
- For very intense samples that saturate the system, decrease the PMT voltage. This brings high-intensity signals into the linear range of the instrument. You might lose detection and accuracy of quantitation at the lower end of the signal range. If you decrease the voltage below 500, you might lose linearity.

Important **Increasing the PMT voltage can cause the signal-to-noise ratio to deteriorate and lower the sensitivity. For the best sensitivity, use the High sensitivity parameter with a lower PMT voltage.**

If you change your sample matrix, you might need to test several PMT parameters between 500 V and 1 000 V to determine which gives an acceptable background.

As a suggested starting point, use—

- 900 V for all transparent matrices (polyacrylamide or agarose).
- 800 V for opaque matrices (membranes or TLC plates).

Procedure for changing the PMT voltage

To set the PMT voltage box—

- Double-click the box to select it, and then type the value you want (maximum voltage = 1 000 V).
- Use the up or down arrows to change the value in 5-V increments.

(Storm 860 only) If you are performing a multichannel scan, you can select a PMT voltage for each channel.

9.2.3 Deselecting an excitation parameter (Storm 860 only)

The default parameter for the Storm 860 is to scan the sample once with each excitation parameter. Channel 1 displays the parameters for red-excited fluorescence. Channel 2 displays the parameters for blue-excited fluorescence.

If you labeled the sample with a fluorochrome for only one excitation parameter, clear the check box to the left of the channel number for the excitation parameter that you do not want to use. Scanner Control disables the parameters for the deselected channel.

9.3 Using templates

Using a template is a quick way to retrieve the parameters you use frequently. The template contains the scan parameters for the selected instrument, which include the grid area, acquisition mode, sample orientation, pixel size, image analysis software, sensitivity, and PMT voltage. Comments in the User Comment box are not saved with the template.

Important **You cannot use the templates you created with previous versions of the Storm Scanner Control software.**

9.3.1 Creating a new template

To create a template—

1. Select the parameters in the Scanner Control and Setup windows (sections 9.1 and 9.2).
2. Choose **Save As Template** from the Templates menu. The Save As Template window appears (figure 9-6).
3. Type a new name for the template in the box. **Note:** Because the Template list displays all the available templates for the selected instrument, you might want to add a label to identify which templates are for storage phosphor and which templates are for fluorescence. For example, a template for fluorescence can be named TemplateName_Fluorescence. (You should type an underscore instead of a space in template names.)
4. (Optional) If you want the template to become the new default, click the **Set as Default Template** check box. **Note:** You can select the system default template from the Templates menu.
5. Click **OK.** The template name appears above the grid area in the Scanner Control window.

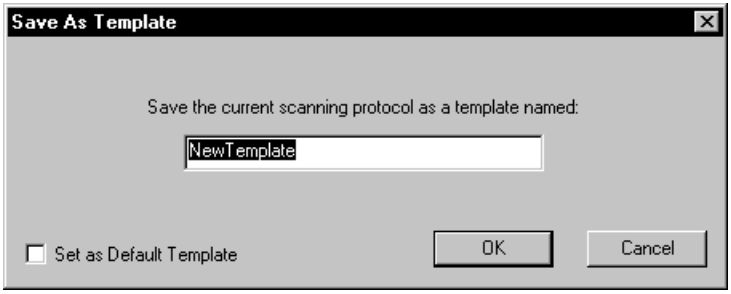

Figure 9-6. The Save As Template window for saving a template.

9.3.2 Selecting a template

If you want to use a different template, choose **Load** from the Templates menu and then select the template name from the list.

Note: The Templates list contains the available templates for the selected Storm instrument. For Storm instruments that scan in both the storage phosphor and the fluorescence mode, selecting a template can change the Scanner Control window to a different scan acquisition mode.

9.3.3 Modifying a template

You can modify a template and use the modified parameters to scan a sample. After you change a parameter, an asterisk appears next to the template name to show that the parameters have been changed. Scanner Control will not allow you to save the modified parameters.

If you want to save a modified template, you must delete the old template. Next, select the new parameters and save the template using the name of the template you deleted.

9.3.4 Selecting a template to use as a default

If you want to select a different template to use as the default, choose **Set Default/Load** from the Templates menu and select the template name from the list.

9.3.5 Deleting a template

If you want to delete a template, choose **Delete** from the Templates menu. The Delete Template(s) window appears (figure 9-7). Select the template name that you want to delete and click **Delete.**

Note: Because you cannot delete the template displayed in the Scanner Control window, the template name does not appear in the list. To delete the template in use, close the Delete Template(s) window, select a different template from the Load list, and then choose **Delete** again. You should see the template name in the list.

To delete all the templates except the template displayed in the Scanner Control window, click **Select All** and then click **Delete.**

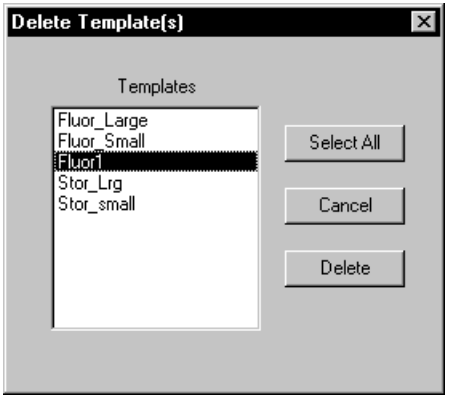

Figure 9-7. The Delete Template(s) window.

9.4 Starting the scan and checking the progress

Warning

Do not attempt to defeat the safety interlocks on the sample lid. These interlocks are designed to protect you from laser light exposure.

Cautions

Never turn off the Storm instrument or disconnect the SCSI cable while scanning. You can severely damage the internal mechanism of the instrument.

Keep the sample lid closed during the scan. Opening the lid shuts off the light source, aborts the scan, and saves the data already collected.

The scanning process, which is rapid and uses low-intensity light, causes little or no photobleaching of most samples. Light hits each position on the sample only while the corresponding pixel is being recorded. The position is only minimally exposed during the rest of the scan.

Scanner Control creates an image file and automatically adds the correct file extension, which is either—

- **Data File (.gel)—**Creates a single image file.
- **Data Set File (.ds)—**Creates a collection of files and folders for multichannel images.

Note: If you select or type the wrong file extension, Scanner Control changes the file type back to the correct extension.

9.4.1 Starting the scan

To start the scan—

1. Make sure that the sample is in place, the sample lid is shut, and all the parameters are correct in the Scanner Control and Setup windows.
2. Click the **Scan** button. The Save As window appears (figure 9-8).

Figure 9-8. The Save As window for entering the dataset name for the image.

3. In the Save As window, type a name in the **File name** box.

Caution **If you type a name that has already been used, a message appears asking you if you want to replace the existing file. Before you click Yes, make sure you do not want the existing image file. Clicking Yes deletes the image and all the associated auxiliary files. If you have analyzed the image using ImageQuant, all the data will be deleted.**

4. (Optional) Change to a different folder. Otherwise, the software saves the image in the Data folder.

Caution **Saving a scan to a folder located on a removable media disk drive can cause a loss of data. For best results, choose a folder on the computer hard drive. After you scan the sample, move the image file(s) to the removable media.**

5. Click **Save** to start the scan. If you selected Press Sample in section 9.1.6, the inner lid of the Storm instrument lowers to hold the sample flat against the glass platen of the instrument.

9.4.2 Monitoring the scan progress

After you start the scan, the Scan in Progress window (figure 9-9) appears, and the green Scan indicator light on the top of the instrument blinks. As the Storm instrument scans the sample, Scanner Control displays the image in the Scan in Progress window. In addition, Scanner Control displays the number of data lines scanned and the total scan time remaining (excluding initialization). Scanner messages can also appear as the scan progresses.

Note: To abort the scan, click **Cancel Scan** in the Scan in Progress window. A message appears asking if you want to delete the data file. If you choose to keep the file, the file size will be larger than the actual data. When you view the file size information in ImageQuant, a large file size indicates that the data has been truncated.

(Storm 860 only) If you are scanning a sample using both the red-excited and blue-excited parameters, the Scan in Progress window displays the red-excited (channel 1) scan, resets the Lines Scanned box to zero and displays the blue-excited (channel 2) scan.

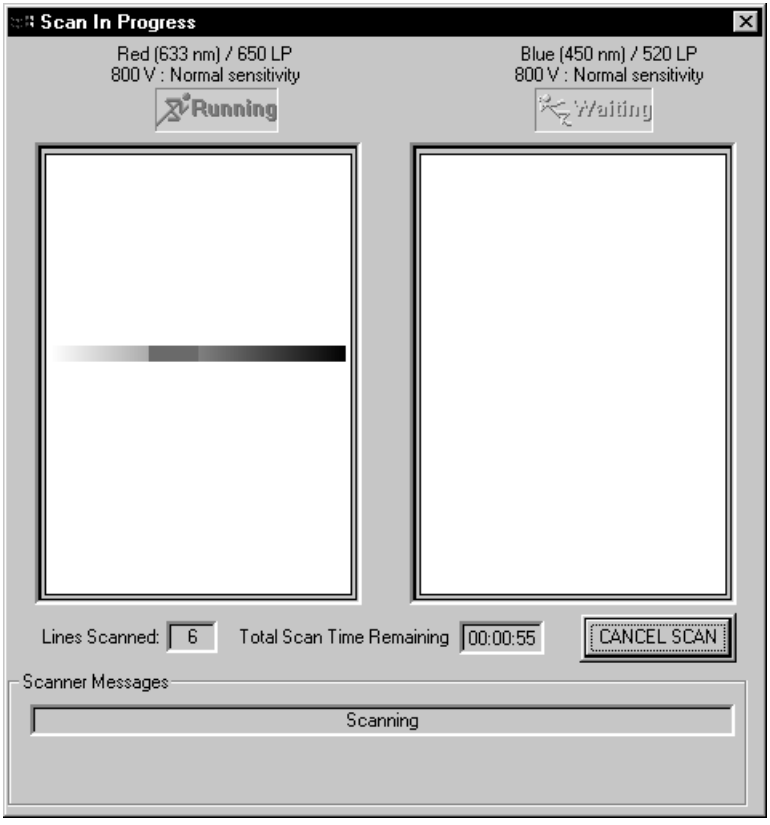

Figure 9-9. The Scan In Progress window for the Storm 860 showing the red-excited fluorescence scan in progress and the blue-excited fluorescence scan waiting.

9.4.3 Completing the scan

Scanner Control saves the image or images using the file name you selected in the Save As window. The Scan in Progress window displays a Complete message, and the Scan indicator light on the Storm instrument turns off. At the end of the scan, an image of the sample appears automatically in the software you selected from the Image Analysis list. If you selected None, the Scan in Progress window remains active for more scanning.

9.4.4 Viewing saturated data

Saturated data appear in red in the Scan in Progress window. If the image appears too saturated, you might not be able to analyze the image correctly. To avoid saturation of the data, lower the PMT voltage you selected in the Setup window (section 9.2.2).

Important **Before repeating the scan, verify in ImageQuant that the image is not too saturated to analyze. See the Gray/Color Adjust feature in the *ImageQuant User's Guide* for instructions on removing saturated data from the image display.**

9.5 Removing the sample from the Storm instrument

After you finish scanning, you should remove the sample from the Storm instrument and dispose of the sample using the established procedures in your laboratory. Make sure the Scan indicator light turns off before you open the sample lid and remove the sample.

9.6 Cleaning the glass platen and sample lid

To remove fluorescent contamination, you should clean the glass platen and sample lid after you finish each scan. See section 8.2 for details.

9.7 Analyzing or preprocessing the scanned image

You should display the image of the sample to determine the quality of the scan. If you selected ImageQuant for analyzing, FluorSep for multichannel separation, or ImageQuant Tools for preprocessing in the Scanner Control window, the software starts and displays the image. Refer to the applicable user's guide or Help for the selected software for detailed information.

Part four

Maintaining the Storm system

Chapter 10 Maintaining the Storm instrument

This chapter provides information about maintaining the Storm instrument. The topics in this chapter are—

- Changing the fuses (section 10.1)
- Attaching peripheral devices using a SCSI connection (section 10.2)
- Moving the Storm instrument (section 10.3)

10.1 Changing the fuses

Caution **If fuses must be replaced repeatedly, the Storm instrument might have an electrical problem. Do not use the instrument. Contact Amersham Biosciences Technical Support.**

You can use the Storm instrument with either a 220–240 V (230 V) power source or a 110–120 V (115 V) power source. The power supply in the instrument switches to the correct voltage automatically. The fuse box is located to the right of the power switch (figure 10-1).

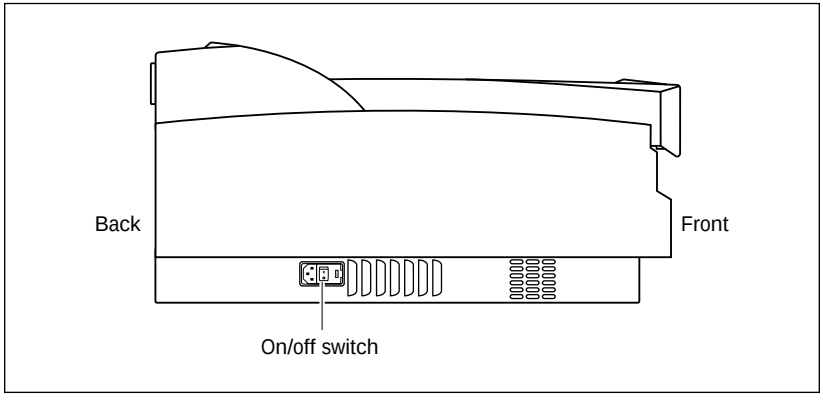

Figure 10-1. Left side view of the Storm instrument.

The Storm instrument takes two 5A, 250V (⊟ , F5A, 250V) fast-blow fuses. The fuse holder is designed to accept both 0.25-in by 1.25-in English fuses (designated 3AG fuses) and 5-mm by 20-mm metric fuses.

To change a fuse—

1. Turn off the Storm instrument (section 3.5) and disconnect the power cord.
2. Look for the small groove to the right of the fuse window.
3. Place the end of the small flathead screwdriver into the groove and pry open the fuse box (figure 10-2).

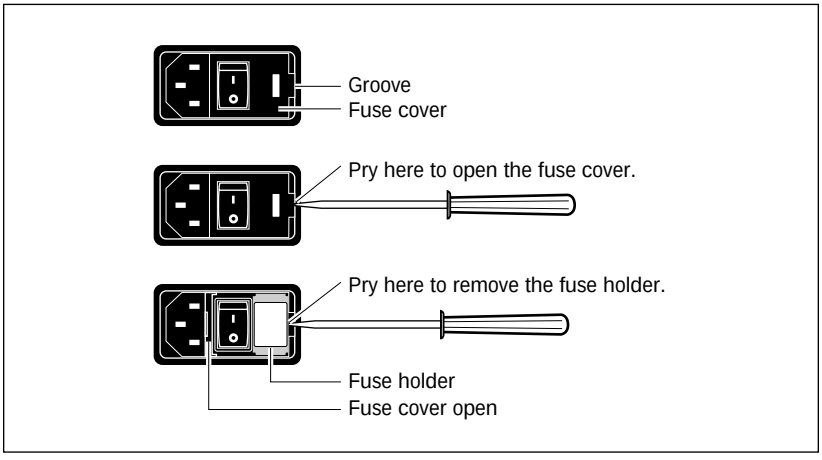

Figure 10-2. Opening the fuse box.

4. Place the screwdriver in the groove on the side of the fuse holder and pry out the fuse holder. When the holder is loose, pull it out of the fuse box.
5. One or both fuses might be blown. Replace the blown fuse(s) with new fuse(s) of the same type and rating. Fuse specifications are listed at the beginning of this section and on the label located on the underside of the sample lid.
6. Insert the fuse holder into the fuse box, making sure the correct voltage rating is to the right.
7. Snap the cover of the fuse box back into place. Check that the correct voltage rating appears in the voltage window. If the voltage is incorrect for your power source, remove the fuse holder, rotate the holder 180° so that the correct voltage rating is to the right, and reinsert the fuse holder.

Warning

Before you turn on the Storm instrument after you change the fuse, make sure that the correct operating voltage appears in the fuse window. Selecting the wrong voltage can severely damage the instrument.

8. Reconnect the power cord and turn on the Storm instrument (section 3.1).

10.2 Attaching peripheral devices using a SCSI connection

This section describes the use of SCSI connections and how to attach peripheral devices, such as a removable media disk drive.

10.2.1 Overview of SCSI connections

The Storm instrument has two Centronics™ 50 SCSI (small computer systems interface) ports. One of these ports connects the instrument to the computer. You can use the other port to connect to additional peripheral devices.

SCSI chain terminations

Each end of the SCSI chain must be terminated. The computer is at one end of the SCSI chain, and the SCSI adapter in the computer has a built-in terminator. You must add a terminator to the other end of the SCSI chain. Figure 10.3 shows sample connection configurations.

Important **Make sure that the total length of all SCSI cables (including internal cables) does not exceed 6 m (19.7 ft). (The cable length inside the Storm instrument is 3 cm (1.2 in) meter. For the internal cable length of other scanners, refer to the user's guide for the specific scanner.**

SCSI IDs

A SCSI ID number identifies each device connected to the SCSI bus. The Storm instrument must use a SCSI ID different from those occupied on the internal and external SCSI buses. SCSI IDs 0 and 7 are reserved for the computer. The Storm instrument has been preset to SCSI ID 5 in most cases. The SCSI ID for a peripheral device or additional scanner must use a number that is different from those of the computer and the Storm instrument.

Your Storm instrument uses a dial or push buttons to set the SCSI ID. The location of the dial or push buttons appears in figure 10-3.

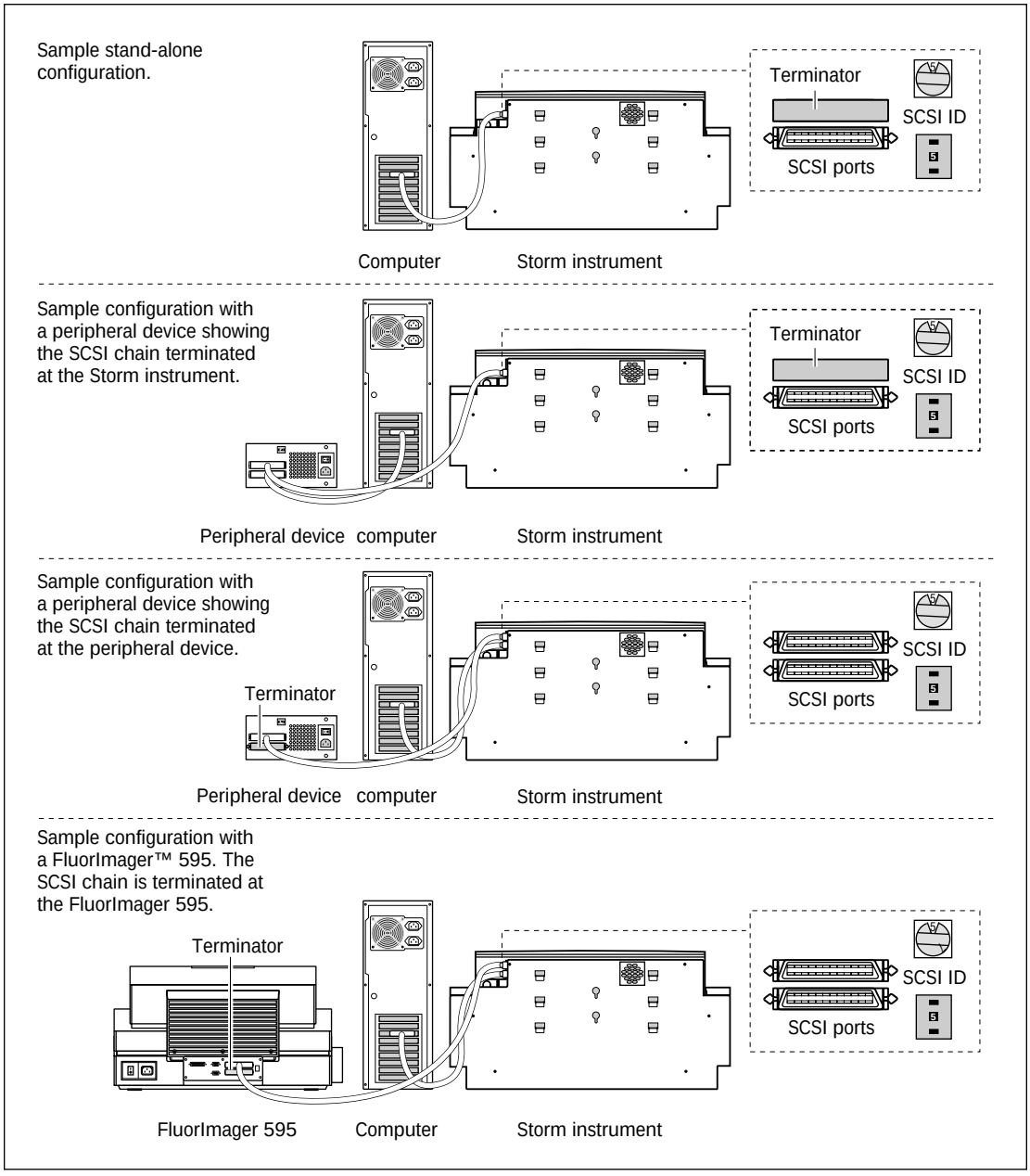

Figure 10-3. Sample configurations for connecting the Storm instrument, computer, and peripheral device or additional scanner. Each device is shown as viewed from the back.

10.2.2 Connecting a peripheral device

See the Amersham Biosciences Web site (www.amershambiosciences.com) for a list of the supported peripheral devices.

To connect a peripheral device—

1. Turn off the Storm instrument, computer, and any other peripheral device and disconnect them from the power source.
2. Grasp the two forward extensions of the SCSI cover (figure 10-4) and pull upward to remove the cover. The SCSI ports are located near the back of the Storm instrument (figure 10-3).

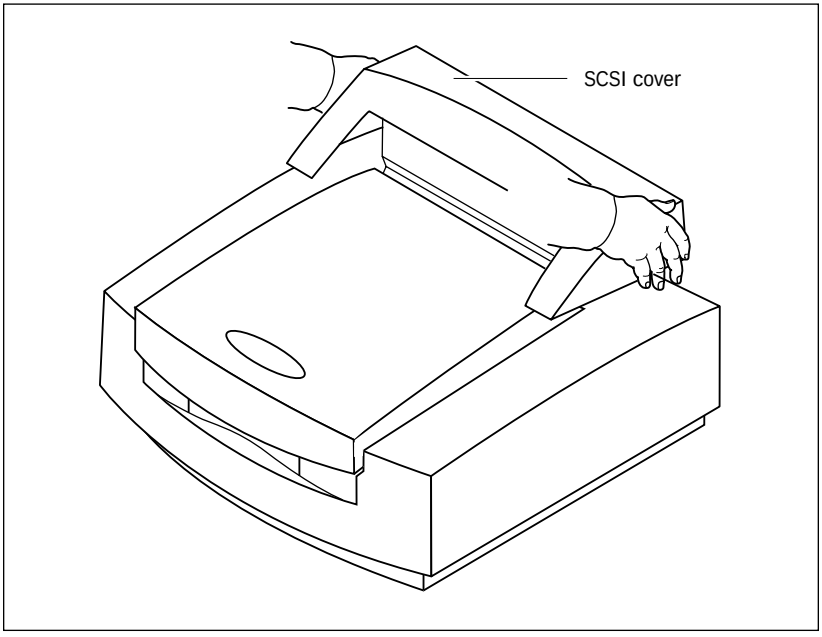

Figure 10-4. Removing the SCSI cover over the SCSI ports.

Important **Make sure that the total length of all the SCSI cables does not exceed 6 m (19.7 ft). The cable length inside the instrument is 3 cm (1.2 in). If you are connecting multiple devices, use the shortest cables possible. Not all SCSI cables are constructed the same. To avoid problems, contact Amersham Biosciences to purchase additional cables.**

3. Make sure the SCSI ID used by the peripheral device is different from those of the computer and the instrument (figure 10-3 and section 10.2.1).

   If the number is not different, change the SCSI ID of either the peripheral device or the Storm instrument. On the Storm instrument, turn the dial or press one of the push buttons to change the SCSI ID number (figure 10-5).

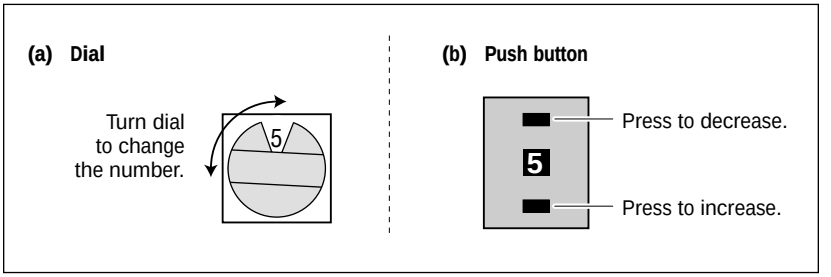

Figure 10-5. Setting the SCSI ID: (a) Dial on newer Storm instruments and (b) Push button on older Storm instruments.

4. Connect the SCSI cables and terminators as appropriate for your configuration (figure 10.3).

5. Reconnect the power cords and turn on the instrument, peripheral device, and computer (sections 3.1 and 3.2).

For information on installing device drivers, see the manufacturers' instructions.

10.3 Moving the Storm instrument

If you need to move the instrument, use this section for information regarding site requirements and cable and power connections.

Warnings

Do not connect or disconnect cables with the power on. Instead, turn off the instrument and computer by following the instructions in sections 3.5 and 3.6.

The Storm instrument is very heavy. Use safe lifting practices and move the instrument carefully.

When you move the instrument, make sure you place the instrument away from strong air currents. The airflow can carry dust or dirt particles into sensitive parts of the instrument. In addition, place the instrument away from direct sunlight or other very bright light. Bright light may cause excessive heat or compromise the light-tight operation of the instrument.

To move the Storm system—

1. In the Scanner Control window, choose **Park Head and Exit** from the File menu. The software positions the scan head so that it is protected during the move.
2. Turn off the instrument, computer, and peripheral devices using the instructions in sections 3.5 and 3.6.
3. Disconnect the power cords and the SCSI connection(s).
4. Move the instrument, computer, and peripheral devices to the new location.
5. Reconnect each device (section 10.2 explains how to attach the SCSI devices).

   Note: If you connect the Storm instrument to a different computer than the one used previously, you must make sure that a SCSI adapter card is installed in the computer and the device drivers are loaded. See the instructions provided with the adapter card and in the Windows documentation.
6. Make sure you plug the instrument, computer, and peripherals into a surge protector, which is plugged into a properly grounded outlet.
7. Turn on the instrument, computer, and peripheral devices using the instructions in sections 3.1 and 3.2.

Chapter 11 Maintaining the Image Eraser and exposure cassettes

This chapter provides information on the care and maintenance of the Image Eraser and the exposure cassettes. The topics in this chapter are—

- Cleaning the Image Eraser (section 11.1)
- Changing the bulbs on the Image Eraser (section 11.2)
- Changing the fuses in the Image Eraser (section 11.3)
- Cleaning and protecting the exposure cassettes (section 11.4)

11.1 Cleaning the Image Eraser

The surface of the eraser must be clean and free of radioactive contamination when you erase screens. Otherwise, the screens will be exposed to radioactive contamination during the erasure process.

Warning ⚠ **Because the Image Eraser uses high voltage, always turn off and unplug the eraser before cleaning the surface.**

1. Turn off the Image Eraser and disconnect the power cord (figure 11-1).
2. Clean the surface of the eraser with a damp cloth moistened with a solution of totally rinsable laboratory detergent. Do not use powdered detergents. Any undissolved particles can scratch the surface of the eraser.

Caution **Do not allow liquid to seep into the Image Eraser. Use a damp cloth only. Do not pour or spray liquid over the surface.**

3. Verify that the eraser surface is free from radioactive contamination.
4. Reconnect the power cord and turn on the Image Eraser.

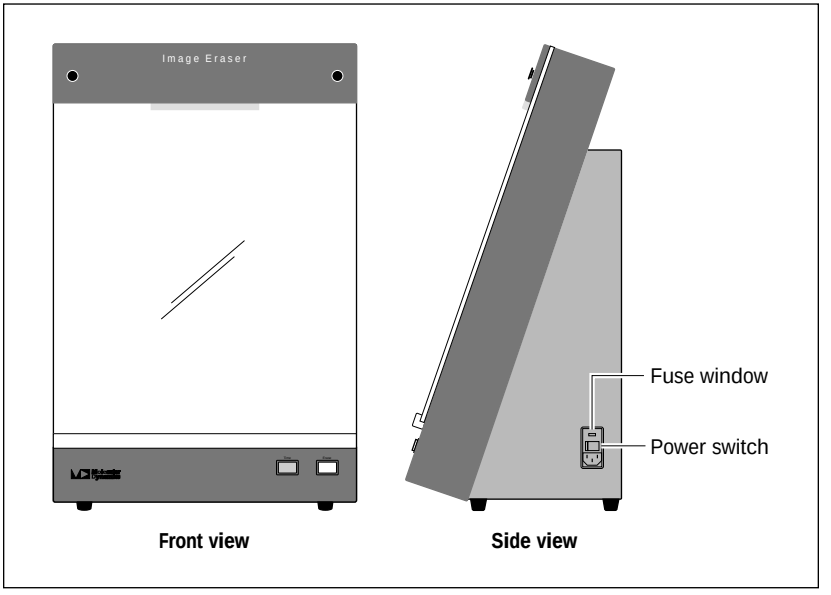

Figure 11-1. The Image Eraser.

11.2 Changing the bulbs on the Image Eraser

The Image Eraser contains four warm-white compact fluorescent bulbs. When any one of the bulbs fails, replace all four bulbs to provide uniform light intensity across the screen. To order replacement bulbs, contact Amersham Biosciences Technical Support or your local distributor (see the manufacturer part number on the bulb). See Assistance in the preface for contact information.

Important **If you are replacing the bulbs shipped with the Image Eraser, you must order replacement bulbs and fixtures from Amersham Biosciences Technical Support. The bulbs shipped with the Image Eraser are epoxied to the fixture to avoid damage during shipment. The replacement fixture and bulbs are not epoxied.**

To replace the bulbs—

1. Turn off the Image Eraser and disconnect the power cord (figure 11-1).
2. Remove the two thumbscrews that hold the clip support and diffuser screen in place (figure 11-2).
3. Lift off the clip support and diffuser screen.
4. To replace the bulb and fixture, unscrew the fixture from each receptacle.

5. Screw a bulb and fixture into each receptacle.
6. Replace the front diffuser screen and clip support, and reinsert the two screws.
7. Verify the fuse voltage is correct. If the fuse voltage is not correct, refer to your instrument user's guide for instructions.
8. Connect the power cord and turn on the Image Eraser.

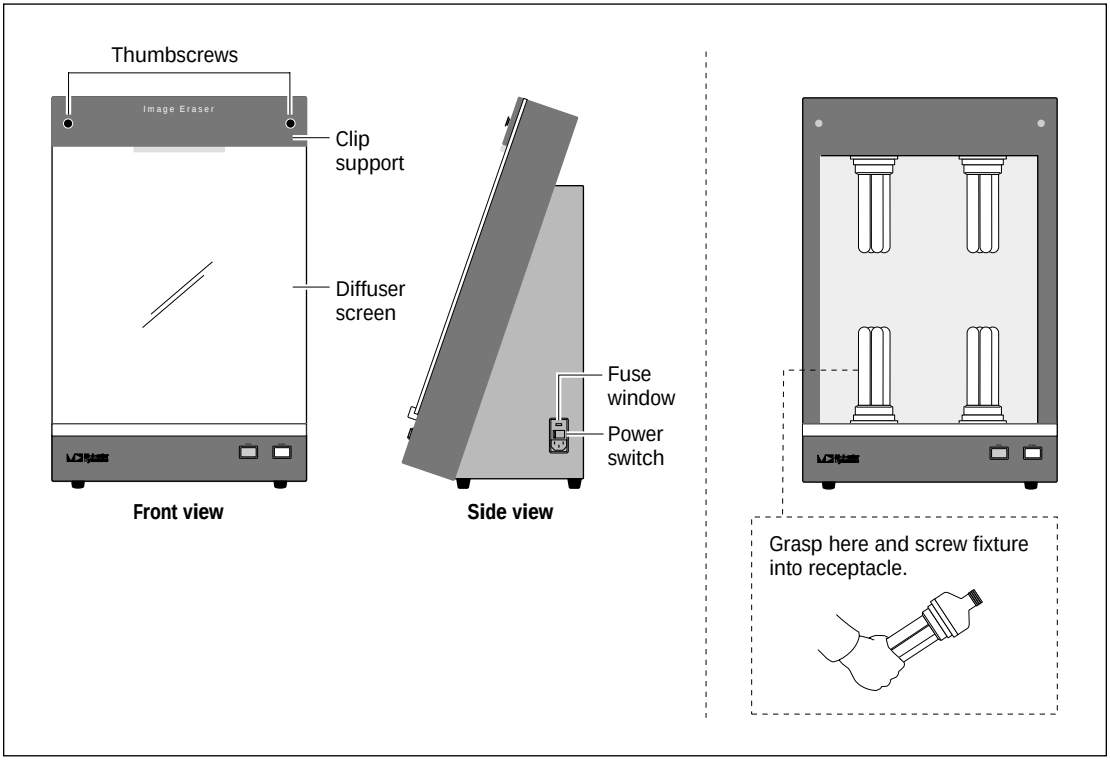

Figure 11-2. Changing the Image Eraser bulb.

11.3 Changing the fuses in the Image Eraser

Caution **If the fuses must be replaced repeatedly, the Image Eraser might have an electrical problem. Do not use the Image Eraser. Contact Amersham Biosciences Technical Support. See Assistance in the preface for contact information.**

You can use the Image Eraser with either a 220–240 V (230 V) power source or a 110–120 V (115 V) power source. The selected operating voltage of the Image Eraser appears in the fuse window on the right side of the eraser, next to the power switch (figure 11-1).

The fuse holder of the Image Eraser is identical to the fuse holder of the Storm instrument. The Image Eraser takes two 1.5A, 250V (⊟ , T1.5A, 250V) slow-acting fuses. The fuse holder is designed to accept both 0.25-in by 1.25-in English fuses (designated 3AG fuses) and 5-mm by 20-mm metric fuses.

To change a fuse—

1. Turn off the Image Eraser and disconnect the power cord (figure 11-1).
2. Note the voltage (115 V or 230 V) showing in the fuse window (figure 11-3).

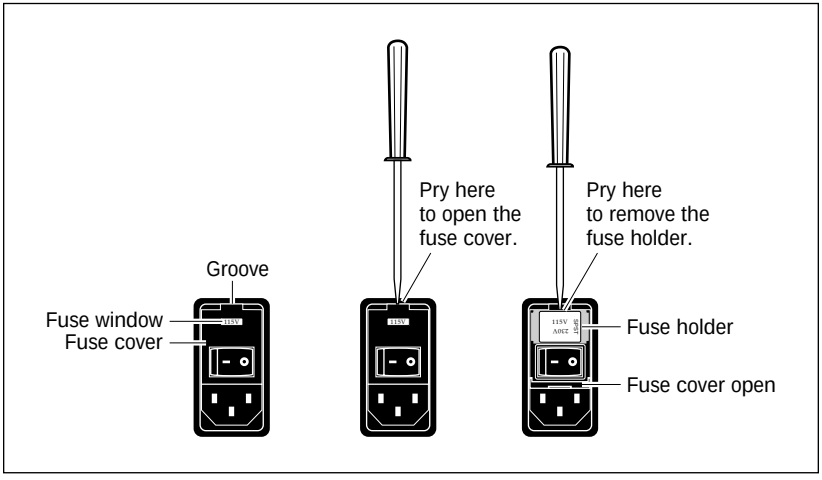

Figure 11-3. Opening the fuse box.

3. Look for the small groove above the fuse window.
4. Place the end of a small flathead screwdriver in the groove and pry open the fuse box (figure 11-3).

5. Place the screwdriver in the groove on the upper edge of the fuse holder and pry out the fuse holder. When the holder is loose, pull it out of the fuse box.
6. One or both fuses might be blown. Replace the blown fuse(s) with new fuse(s) of the same type and rating. Fuse specifications are listed on the label for the unit.
7. Insert the fuse holder into the fuse box making sure the correct voltage rating for your power supply is right side up.
8. Snap the cover of the fuse box back into place. Check that the correct voltage rating for your power source appears in the voltage window. If the voltage is incorrect for your power source, remove the fuse holder, rotate the holder 180° so that the correct voltage rating is right side up, and reinsert the fuse holder.

Warning ⚠ **Before you turn on the Image Eraser after you change the fuse, make sure that the correct operating voltage appears in the fuse window. Selecting the wrong voltage can severely damage the eraser.**

9. Reconnect the power cord and turn on the Image Eraser (figure 11-1).

11.4 Cleaning and protecting the exposure cassettes

To protect the exposure cassettes from contamination and damage, observe the following precautions and cleaning procedure:

- Do not place uncovered wet gels in the exposure cassette. Wet gels can permanently contaminate the cassette. For information on using wet gels, see section 5.1.2.
- Do not place sharp or heavy objects inside the cassette. A crease or dent in the cassette lining causes uneven pressure on the sample.
- Keep the foam inside the cassette dry.
- Immediately before placing the sample in the cassette, clean the grid surface of the cassette with a damp cloth moistened with a solution of totally rinsable laboratory detergent.

Caution **Do not allow liquid to seep into the exposure cassette. Use a damp cloth only. Do not pour or spray liquid in the cassette.**

Part five

Appendixes

Appendix A Troubleshooting

If you are having problems with your Storm instrument, use the troubleshooting sections below to locate the description that matches your problem. If you cannot find a solution, call Amersham Biosciences Technical Support (see Assistance in the preface for contact information).

Problems, in bold print, are followed by possible causes and solutions. The topics in this appendix are—

- Power and communication (section A.1)
- Scanning (section A.2)
- Image (section A.3)

A.1 Power and communication

The Power indicator light on the Storm instrument will not turn on.

- The instrument might not be plugged in, or the surge protector switch might be turned off. Plug in the instrument or turn on the surge protector.
- The wall outlet might be faulty. Test the outlet or try another one.
- The fuse(s) might have blown. To change the fuse(s), see section 10.1.

The Storm instrument is on but cannot communicate with the computer.

- The instrument might be set to an incorrect SCSI address, or another SCSI device could have the same address as the instrument. Make sure that the SCSI address on the instrument is the same as the SCSI address of the instrument selected in the Scanner Control window. Make sure that no other device uses the same SCSI address (section 10.2).
- The computer might have been turned on before the instrument. Make sure the instrument is turned on and then restart the computer.
- The SCSI cable might not be properly plugged into all the peripherals. Check the connections to make sure they are plugged in and are securely fastened.
- The SCSI cables might be incorrect or too long. SCSI cables are the most frequent cause of communication problems.
  - The combined length of the cables (including any internal cables) cannot exceed 6 m (19.7 ft). The cable inside the instrument is 3 cm (1.2 in) long. For the internal cable lengths of the peripheral devices, see the documentation for the device.

- Try another SCSI cable that you know is good. Not all SCSI cables are constructed the same. We recommend that you use only SCSI cables from Amersham Biosciences.

- The last peripheral on the SCSI bus might not be properly terminated. Add the terminator as discussed in section 10.2.

A.2 Scanning

The instrument will not scan, and a warning message tells you that the sample lid is up.

The sample lid might not be completely closed. Make sure you press the lid all the way down until the latch clicks.

You start a scan, the Scan in Progress window remains unchanged for at least three minutes, and the pointer no longer responds when you move the mouse.

The computer has stopped functioning. Restart the computer using the manufacturer's instructions. Then open the Scanner Control software, reselect the scanner parameters, and start the scan again.

You start a scan, and the software aborts the scan and displays the following message: "Scan aborted—hardware error, please try to scan again."

The first time this happens, try starting the scan again. If it happens repeatedly, contact Amersham Biosciences Technical Support.

You start a scan, the software aborts the scan, and either—

- **The software displays a message that does not give instructions for resolving the problem (note the number of the message).**
- **The red Scan indicator light blinks.**

Restart the scan as follows:

1. Close the Scanner Control window. Leave the computer on.
2. Turn off the power switch on the back of the Storm instrument. Wait a few seconds and turn it on again.
3. Open the Scanner Control software, reselect the parameters, and rescan. Because the Scanner Control software was closed while the instrument was off, the computer continues to recognize the instrument.

If the problem occurs repeatedly, contact Amersham Biosciences Technical Support.

A.3 Image

The image has a high background or inaccurate readings.

- The instrument might not have been warmed up before the sample was scanned. If the Storm instrument has been turned off, allow 15 minutes warmup time.
- The instrument might be damaged and is no longer light-tight. If so, do not continue to use the instrument. Contact Amersham Biosciences Technical Support to arrange for repair.
- The screen, sample, or glass plate could have dust, fingerprints, or other dirt on it. Clean the glass plate. If necessary, for fluorescence scans, filter the liquid samples, reagents, and components used to make the gels. For more information on removing contaminants, see sections 5.2 and 8.2.
- The storage phosphor screen might not have been erased immediately before you exposed it to the sample, or the residual image from an intense sample might not have been erased completely.
- The sample support might have high autofluorescence. Use a low-fluorescence material (section 8.1.3).

The image is incomplete.

The selected scan area in the Scanner Control window might be incorrect. See sections 6.4.3 and 9.1.3.

The edges of the image created from a storage phosphor screen show a loss of signal.

A light leak might have occurred during exposure. Make sure you expose screens in the exposure cassette (or, with wet gels or thick samples, in a light-tight drawer or other light-tight place). Check that your exposure cassette closes properly.

The image created from a storage phosphor screen shows a loss of resolution.

The sample might not be placed directly against the screen. Use the exposure cassette whenever possible. Do not expose wet gels or thick samples in exposure cassettes. Cover a wet gel with plastic wrap or polyester film to protect the general purpose (GP) screen.

The image created from either a storage phosphor screen or fluorescent sample contains streaks or other artifacts.

- The instrument might not have been warmed up before the sample was scanned. If the Storm instrument has been turned off, allow 15 minutes warmup time.
- Diagonal streaks might indicate a light leak during scanning. Check for damaged panels on the instrument. Contact Amersham Biosciences Technical Support.
- The glass platen might be scratched. If possible, scan the sample on another portion of the glass. Contact Amersham Biosciences Technical Support to order replacement glass and arrange for a service call.

The image created from a storage phosphor screen contains streaks or other artifacts.

- Static electricity might have accumulated on the screen. Clean the GP and low-energy (LE) screens with an intensifying screen cleaner. Do not attempt to clean the tritium (TR) screen.
- The screen might be contaminated with radioactive material. The source of this contamination could be the surfaces of the eraser or the exposure cassette. Clean the GP or LE screen, exposure cassette, and eraser surfaces (section 5.2).
- The screen might have been exposed to too much light between the time you removed it from the exposure cassette and the time you put it in the instrument for scanning. Keep the screen face down in subdued light until you place it in the instrument.
- The screen might not have been erased immediately before exposure. Cosmic radiation generates a background signal on screens left unused for long periods of time.
- The screen might not have been completely erased. Select a higher setting and erase again (section 5.2.4).
- The screen might be scratched. If possible, expose the sample on another portion of the screen. For large samples, you will need to use a different screen.
- Fingerprints might be on the screen. Clean the GP and LE screens with an intensifying screen cleaner. Do not clean the TR screen. Instead, use a new TR screen.

The image created from a fluorescent sample contains streaks or other artifacts.

- Fingerprints appear in the scan. Clean the glass plate. If the fingerprints are on the gel, rinse the gel briefly in 0.1% Tween™ or SDS. Rinse the gel in water and then scan again. If the fingerprints persist, you might need to prepare a new gel and handle the new gel more carefully.
- Dust specks appear on the scan. Rinse wet gels in filtered distilled water to remove surface dust prior to scanning. Filter liquid reagents used in gels and buffers. Be sure to dissolve agarose completely before pouring the gel. Clean the glass of the Storm instrument with a damp, lint-free cloth.
- The tracking dye is fluorescing. Place the tracking dye in only one well, or dilute the tracking dye with sample buffer.
- The sample might have stained unevenly. Make sure you mix staining solutions thoroughly, use a large excess of staining solution, and rock or shake the gels during staining, if possible.

The image created from a storage phosphor screen contains a double image.

- The screen might have been moved after the initial placement. Always position the screen correctly on the first try and do not readjust the placement. If readjustment is absolutely necessary, make sure you erase the screen completely before placing it in the exposure cassette again.
- The sample might have shifted when the screen was inserted into the cassette. Erase the screen and re-expose it to the sample. Do not tip the cassette upright during exposure.

The image created from a fluorescent sample contains a double image.

- The sample might have moved after the initial placement. If fluorescent material has come off onto the glass plate, remove the sample and clean the glass. Place the sample correctly on the first try and do not readjust the placement.
- Remove excess liquid from gels so that the gels do not float on the liquid film.

Appendix B Quick reference for menus and windows

The following is a quick reference for the Scanner Control software. The topics in this appendix are—

- Menus (section B.1)
- Windows (section B.2)

B.1 Menus

The following menus and commands are used in the Scanner Control software:

File menu

Command	Description
Name Dataset	Displays the Save As window so that you can type a name for the image you are about to create.
Park Head and Exit	Parks the scan head and closes the Scanner Control software. Protects the scan head from damage while you move the instrument.
Exit	Use when you want to quit the Scanner Control software.

Templates menu

Command	Description
Load	Displays a list of all the templates for the selected Storm instrument. After you select a template from the list, the parameters for that template appear in the Scanner Control window and, if the template is a fluorescence template, in the Setup window.
Set Default/Load	Displays a list of all the templates. After you select a template from the list, the parameters for that template become the default and appear when you open the Scanner Control software.
Save as Template	Displays the Save As Template window so that you can type a name for the template and save the parameters.
Delete Template	Displays the Delete window, which lists all the templates, except the selected template, and allows you to select and delete a template or templates.

Help menu

Command	Description
Contents	Displays a Help window that contains topics about how to use the Scanner Control software.
About	Displays the About Storm Scanner Control window, which contains the copyright, version number of the Scanner Control software, and contact information.

B.2 Windows

The following windows and window options are used in the Scanner Control software:

Scanner Control window

Option	Description
Scanner Information area	Displays the model number, SCSI ID number, and serial number of the selected Storm instrument. The Select Scanner button allows you to change to a different instrument.
Template name	Displays the template name for the parameters displayed in the Scanner Control window and Setup window (fluorescence only). If no template is selected, the default parameters appear in the windows.
Scan area (grid)	Displays the selected scan area (in white) and allows you to change the selected area by redrawing the white rectangle.
Setup area: User Name	Displays the current user name.
Setup area: Acquisition Mode	Allows you to select the scan acquisition mode. The choices are Storage Phosphor (default) and Fluorescence (not available on Storm 820).
Setup area: Setup button	Displays the Setup window, which allows you to select additional fluorescence parameters. The Setup button is disabled when Storage Phosphor is the selected scan acquisition mode.
Setup area: Options area: Orientation button	Click to display the six orientation options. Allows you to select the option that matches how you placed the sample in the exposure cassette or on the glass platen of the instrument.

Option	Description
Setup area: Options area: Pixel Size list	Displays a list of pixel size options. The choices are 200 microns (for normal samples), 100 microns (when a higher resolution is required), and 50 microns (when very high resolution is required for storage phosphor and red-excited fluorescence, not available for blue-excited fluorescence).
Setup area: Options area: Press Sample check box	Available only in the fluorescence mode. Allows you to select to press the sample before scanning. (Pressing is automatic in the storage phosphor mode).
Setup area: User Comment box	Allows you to save comments (maximum 4 000 characters) with the image file. You can view the comments in ImageQuant, ImageQuant Tools, and FluorSep (multichannel only).
Setup area: Image Analysis list	Allows you to select the image analysis software to open after scanning. Choose among ImageQuant, ImageQuant Tools, FluorSep (multichannel only), or None (leaves Scanner Control active). Note that not all the selections are available in both scan acquisition modes.
Setup area: Scan button	Click to start the scan. If you have not provided a name for the scanned image, you are prompted to provide one before the scan begins.
Scanning Information area	Displays the parameters selected for the scan and the approximate scan time and image file size required for the scan.

Select Scanner window

Option	Description
List of available instruments	Displays a list of the instruments available for use by the Scanner Control software. The information includes the model number, SCSI ID number, and serial number for each instrument. Select an instrument from the list. **Note:** The selected instrument is not listed.
OK button	Click to change to the selected instrument.
Cancel button	Click to close the window without changing the selected instrument.

Setup window (Fluorescence mode only)

Option	Description
Channel check box	(Storm 860 only) Allows you to select the number of scans you want to perform. If both boxes are selected, the instrument scans the image using both red-excited and blue-excited fluorescence. If one box is selected, the instrument scans the image with the selected excitation only.
Channel Number box	Displays how many channels are available for scanning. One channel is used on Storm 860, 840, and 830. Two channels are used on Storm 860.
Excitation box	Displays the excitation modes available for scanning. Storm 830 uses red-excited (635-nm) fluorescence, Storm 840 uses blue-excited (450-nm) fluorescence, and Storm 860 uses both red-excited and blue-excited fluorescence.
Emission box	Displays the emission filter available for scanning. Storm 830 uses the 650 long-pass filter, Storm 840 uses the 520 long-pass filter, and Storm 860 uses both filters.
Sensitivity list	Allows you to select the sensitivity for the scan. Normal collects one data point from each pixel. High collects eight data points from each pixel and averages the results. On the Storm 860 only, you choose a sensitivity selection for each channel.
PMT Voltage list	Allows you to select the PMT voltage for the scan. Values range from 0 to 1 000 and are in 5-V increments. On the Storm 860 only, you choose a PMT voltage for each channel.
Cancel button	Closes the Setup window without saving any changes.
OK button	Saves the changes and closes the Setup window.

Scan in Progress window

Option	Description
Instrument Settings	Displays the instrument parameters being used to create the image. After the scan finishes, the window displays a Complete message.
Preview Image	Displays a preview image of the sample as the instrument scans the sample. If a multichannel image is being created on the Storm 860, first the channel 1 image appears and then the channel 2 image appears.
Lines Scanned	Displays the number of lines currently scanned. If a multichannel image is being created on the Storm 860, the box resets to zero at the start of the channel 2 scan.
Total Scan Time Remaining	Displays the amount of time remaining before the scan is complete. The timer stops during initialization and runs only while data are being acquired.
Cancel Scan/ Start Scan button	During the scan, aborts the scan. After the scan, starts the scan.
More Error Information button	Appears when an error occurs and allows you to view a window containing information about the error.
Scanner Messages	Displays a series of messages during the scan.

About Storm Scanner Control window

Option	Description
Version	Displays the version number of the Scanner Control software.
Copyright	Displays the copyright information for the Scanner Control software.
Technical Support	Displays the Amersham Biosciences Technical Support information for the Scanner Control software.
Close button	Click to close the About Storm Scanner Control window.

Save As Template window

Option	Description
Save the current scanning protocol as a template named box	Allows you to provide a name for the template.
Set as Default Template check box	Allows you to specify the template to use when Scanner Control starts.
OK button	Saves the changes in the Save As Template window.
Cancel button	Closes the Save As Template window without saving the changes.

Error Report window

Option	Description
Description of error	Describes the type of error and provides a possible solution.
Error code	Displays an error code for the error. The error code can be used by the Amersham Biosciences Technical Support to help determine the problem.
View About Box button	Displays the About Storm Scanner Control window, which includes the Amersham Biosciences Technical Support information.
OK button	Closes the Error Report window.

Appendix C Workflow overview for scanning using storage phosphor

This appendix is an overview of the workflow for storage phosphor screen autoradiography. The topics in this appendix are—

- Preparing for storage phosphor screen autoradiography (section C.1)
- Scanning the storage phosphor screen (section C.2)

C.1 Preparing for storage phosphor screen autoradiography

The following table lists the main tasks required to expose the sample to the storage phosphor screen.

Important **These tasks are described in detail in chapter 5.**

Task	Description
1. Prepare the sample.	Prepare a sample using a radioactive isotope that can be collected by the general purpose (GP), low-energy (LE) or tritium (TR) storage phosphor screen. Use the laboratory procedures established for the type of sample you are preparing. If you are preparing a wet gel, wrap the gel in plastic wrap or polyester film.
2. Prepare the storage phosphor screen.	Decontaminate, clean, and erase the GP or LE storage phosphor screen, or erase the TR storage phosphor screen. Protect the Image Eraser from contamination when erasing the TR screen.
3. Prepare the exposure cassette.	Decontaminate and clean the exposure cassette.
4. Place the sample in the exposure cassette.	Place the sample in the exposure cassette and note the grid coordinates where you placed the sample. Make sure you do not touch any part of the sample that you want to scan. (If the sample is a wet gel or a thick sample, expose the sample to the screen in a light-tight drawer or other light-tight place.)

Task	Description
5. Place the storage phosphor screen on the sample.	Place the storage phosphor screen on the sample. Do not reposition the screen after it has touched the sample.
6. Expose the storage phosphor screen to the sample.	Leave the storage phosphor screen in the exposure cassette (or light-tight drawer) until the screen is exposed. To determine the length of exposure, consider a one-hour exposure to the screen to be equal to an overnight exposure to x-ray film.

C.2 Scanning the storage phosphor screen

The following table lists the main tasks required to scan the storage phosphor screen.

Important **These tasks are described in detail in chapter 6.**

Task	Description
1. Prepare the Storm instrument.	Decontaminate and clean the glass platen and sample lid of the Storm instrument.
2. Place the storage phosphor screen in the Storm instrument.	If you are scanning a small screen, place the L-shaped adapter on the glass platen. In subdued light, remove the storage phosphor screen from the exposure cassette (or drawer). Position the screen on the glass platen. Do not reposition the screen after it has touched the glass. Close the sample lid.
3. Select scan parameters using the Scanner Control software.	In the Scanner Control window, select an existing template that contains the scan parameters you want to use, or manually select the parameters. To manually select the parameters, make sure Storage Phosphor is the selected scan acquisition mode. Then select the grid area, pixel size, sample orientation, and image analysis software. Type comments you want saved with the image.
4. Start the scan.	Click Scan. Type a file name and click Save. The Scan in Progress window appears. While the Storm instrument scans the storage phosphor screen, a preview image of the screen appears in the window. When the instrument has finished scanning, the Complete message appears at the top of the Scan in Progress window.

Task	Description
5. Evaluate the results.	Check the image in the Scan in Progress window for saturation. Saturated pixels appear in red. If the image is too saturated, you might need to repeat the experiment. View the image in ImageQuant to make sure the image is not too saturated to use. If the image appears usable, close the Scan in Progress window or continue with the next scan. (If you are scanning another screen, make sure you clean the glass platen and sample lid before you place the next screen in the instrument.)
6. Clean up after the scan.	Remove the sample from the exposure cassette and dispose of the sample using the appropriate laboratory procedures. Decontaminate and clean the Storm instrument, exposure cassette, and Image Eraser. Decontaminate, clean, and erase the GP and LE screen, or erase the TR screen. Store the screen in the exposure cassette or other light-tight place.

Appendix D Workflow overview for scanning using fluorescence

This appendix is an overview of the workflow for scanning in the fluorescence mode. The topics in this appendix are—

- Preparing for fluorescence scanning (section D.1)
- Scanning the fluorescent sample (section D.2)

D.1 Preparing for fluorescence scanning

The following table lists the main tasks required to prepare a fluorescent sample.

Important **These tasks are described in detail in chapter 8.**

Task	Description
1. Prepare the sample.	Prepare the sample using fluorescent dyes that can be collected using the blue-excited (450 nm) fluorescence mode on the Storm 860 and 840, or the red-excited (635 nm) fluorescence mode on the Storm 860 or 830. Note that on the Storm 860 you can use two fluorescent dyes, one appropriate for each excitation mode.
2. Prepare the Storm instrument.	Decontaminate and clean the glass platen and sample lid of the Storm instrument.

D.2 Scanning the fluorescent sample

The following table lists the main tasks required to scan the fluorescent sample.

Important **These tasks are described in detail in chapter 8.**

Task	Description
1. Place the sample in the Storm instrument.	In subdued light, position the sample on the glass platen so that the sample is close to the upper left corner of the glass platen. Do not reposition the sample after it has touched the glass. Make a note of the scan coordinates on the glass platen. Close the sample lid.
2. Select scan parameters using the Scanner Control software.	In the Scanner Control window, select an existing template that contains the scan parameters you want to use, or manually select the parameters. To manually select the parameters, make sure Fluorescence is the selected scan acquisition mode. Then select the grid area, pixel size, sample orientation, press sample, and image analysis software from the Scanner Control window. Then click Setup and select the sensitivity and PMT voltage for one or both channels. Type comments you want saved with the image.
3. Start the scan.	Click Scan. Type a file name and click Save. The Scan in Progress window appears. While the Storm instrument scans the sample, a preview image of the sample appears in the window. On the Storm 860, two preview images appear if the instrument scans the sample using both excitation modes. When the instrument has finished scanning, the Complete message appears at the top of the Scan in Progress window.
4. Evaluate the results.	Check the image in the Scan in Progress window for saturation. Saturated pixels appear in red. If the image is too saturated, you might need to repeat the experiment. View the image in ImageQuant to make sure the image is not too saturated to use. If the image appears usable, close the Scan in Progress window or continue with the next scan. (If you are scanning another sample, make sure you clean the glass platen and sample lid before you place the next sample in the instrument.)
5. Clean up after the scan.	Remove the sample from the Storm instrument and dispose of the sample using the appropriate laboratory procedures. Decontaminate and clean the Storm instrument.

Appendix E Literature references

The following references are cited in this manual:

Fluorescence Imaging principles and methods. *Amersham Biosciences Technical Manual #63-0035-28*.

Sonada, M., M. Takano, J. Mayahara, H. Kato. 1983. Computed radiography utilizing scanning laser stimulated luminescence. *Radiology* 148:833-838.

Index

D

E

F

G

H

I

L

S