user manual

Typhoon

User's Guide v3.0
for Microsoft Windows

um 375-635

Amersham Biosciences

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Preface

About this guide

The *Typhoon User's Guide* provides information on how to use the Typhoon™ system to scan fluorescent samples, chemiluminescent samples, and storage phosphor screens that have been exposed to radioactive samples.

- **Part one: Introduction—**Describes the Typhoon instrument, the safety information, and basic instructions for using the instrument and the Scanner Control software.
- **Part two: Scanning in the storage phosphor mode—**Describes how to use the Typhoon system to scan a storage phosphor screen.
- **Part three: Scanning in the fluorescence mode—**Describes how to use the Typhoon system to scan a fluorescent sample.
- **Part four: Scanning in the chemiluminescence mode—**Describes how to use the Typhoon system to scan a chemiluminescent sample.
- **Part five: Maintaining the Typhoon system hardware—**Describes how to maintain the Typhoon instrument, Image Eraser, exposure cassettes, microarray slide holder, and gel alignment guides.
- **Part six: Appendixes—**Provides troubleshooting and reference information.

Related publications

In addition to the *Typhoon User's Guide* and *Typhoon Installation Instructions*, you should be familiar with the ImageQuant™ image analysis software documentation.

Safety

Chapter 2 in this guide provides important safety information that should be used when operating the Typhoon system. Before using the Typhoon system, 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 serious or fatal injury 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.

Trained operator

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

Assumptions

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

Safety standards

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

Site requirements

This section lists a summary of the site requirements for the Typhoon system. For more information on site requirements, see section 13.8.1.

Electrical

The electrical currents listed below refer to the minimum required circuit breaker size.

Instrument:	15 A 100–120 V~
	10 A 220–240 V~
Blue laser module:	20 A 100–120 V~ (dedicated circuit)
	13 A 220–240 V~ (dedicated circuit)
Line frequency:	50–60 Hz

Environmental

Temperature:	15–30 °C (59–86 ˚F)
Humidity:	Noncondensing 10%–80% relative humidity

Instrument

Weight:	160 kg	(350 lb)
Height:	48.26 cm	(19 in)
Width:	118.11 cm	(46.5 in)
Depth:	77.47 cm	(30.5 in)

Blue laser module (Typhoon 9400 and 9410 only)

Weight:	29.5 kg	(65 lb)
Height:	48.0 cm	(18.9 in)
Width:	30 cm	(11.8 in)
Depth:	78 cm	(30.4 in)

A fan is built into the blue laser module. The fan exhausts the heat from the blue laser module into the instrument room. The blue laser module can dissipate over 2 kW (6 800 BTU/hr) of heat. To reduce heat buildup in the instrument room, you can vent the exhaust into the ceiling space or outside the building if local safety and building codes permit. For additional information on attaching an exhaust hose, contact Technical Support. See Assistance for contact information.

If you vent the exhaust into the ceiling space, make sure that your building ventilation system can handle the heat energy that is vented. You should also take into account that the Typhoon instrument, computer, and monitor can dissipate approximately 900 W (3 000 BTU/hr) of heat energy.

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 Typhoon system

The Typhoon instrument is a variable-mode imager that produces digital images of radioactive, fluorescent, or chemiluminescent samples. The Typhoon system consists of an instrument, instrument control software, and a variety of accessories. Several models of the Typhoon instrument are available. This chapter introduces you to the Typhoon system and contains the following topics:

- Typhoon system hardware components (section 1.1)
- Models of the Typhoon instrument (section 1.2)
- Optional accessories for the Typhoon system (section 1.3)
- Software components (section 1.4)
- How the Typhoon system works (section 1.5)
- Typhoon scan acquisition modes (section 1.6)
- Before you begin (section 1.7)

1.1 Typhoon system hardware components

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

- **Typhoon instrument—**Scans exposed storage phosphor screens, fluorescent samples, and chemiluminescent samples.
- **(Typhoon 9400 and 9410 only) Blue laser module—**Contains the blue laser and the power supply for the laser.

In addition, the Typhoon system includes Ethernet™ cables, power cords, and tools.

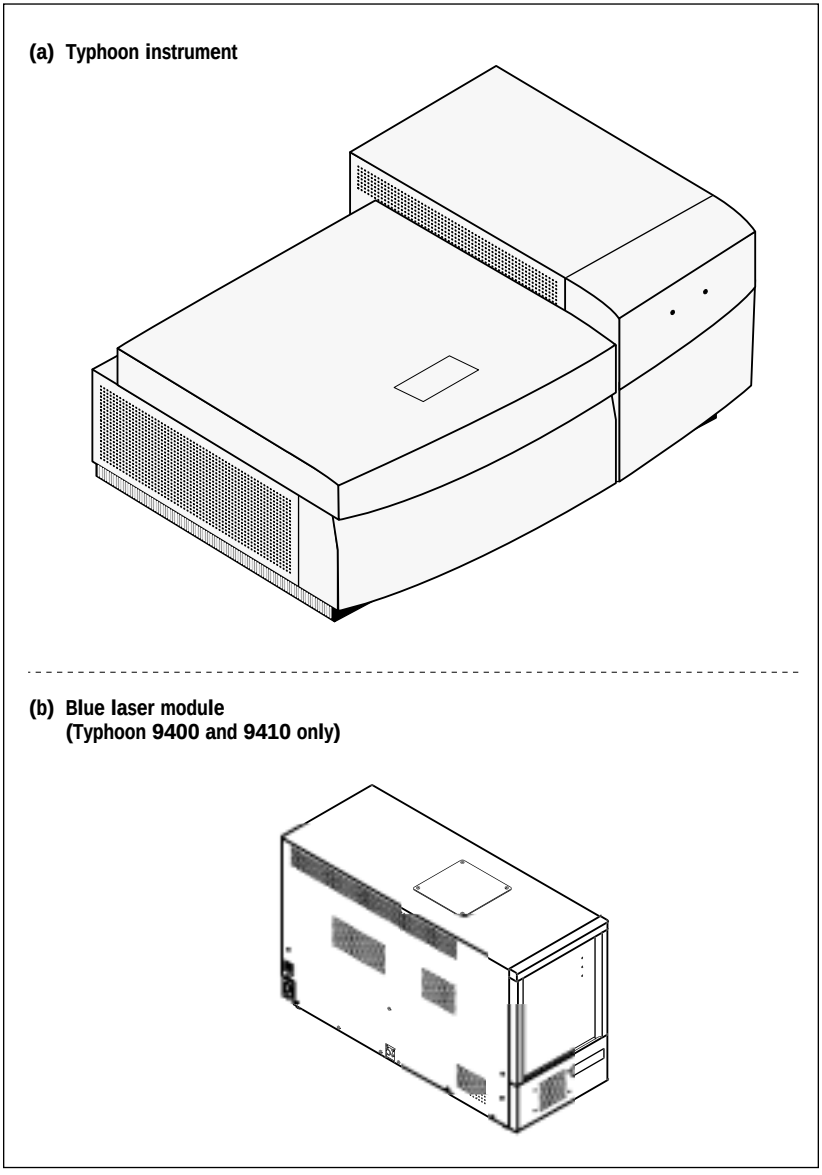


Figure 1-1. Components of the Typhoon system: (a) the Typhoon instrument and (b) the blue laser module (Typhoon 9400 and 9410 only).

1.2 Models of the Typhoon instrument

Table 1-1 describes the various models of the Typhoon instrument.

Table 1-1. Available models of the Typhoon instrument

Model name	Description
Typhoon 9200	Basic instrument model that contains a red and green laser.
Typhoon 9210	Typhoon 9200 with the added capability of scanning using a 10-µm pixel size.
Typhoon 9400	Typhoon 9200 with an external blue laser.
Typhoon 9410	Typhoon 9400 with the added capability of scanning using a 10-µm pixel size.
Typhoon 8610	A Typhoon 8600 instrument that has been updated with the new Scanner Control v2.0 or higher software and has the same features as a Typhoon 9210.

1.3 Optional accessories for the Typhoon system

The following are the optional accessories (figure 1-2) that can be used with the Typhoon system:

- **Image Eraser—**Erases storage phosphor screens for reuse.
- **Storage phosphor screen—**Collects and temporarily stores an image from a radioactive sample. Several types and sizes of storage phosphor screens are available (section 4.3).
- **Exposure cassette—**Holds the storage phosphor screen in a light-tight environment. Several types and sizes of exposure cassettes are available (section 4.3).
- **(Typhoon 8610, 9210, and 9410 only) Microarray slide holder**—Allows you to scan one or two microarray slides (section 8.6).
- **Gel alignment guides**—Allows you to scan gels created using difference gel electrophoresis (DIGE). You can place two large gels (Ettan™ DALT) or four smaller gels (DIGE SE600) so that they can be read in the same scan (section 8.7).

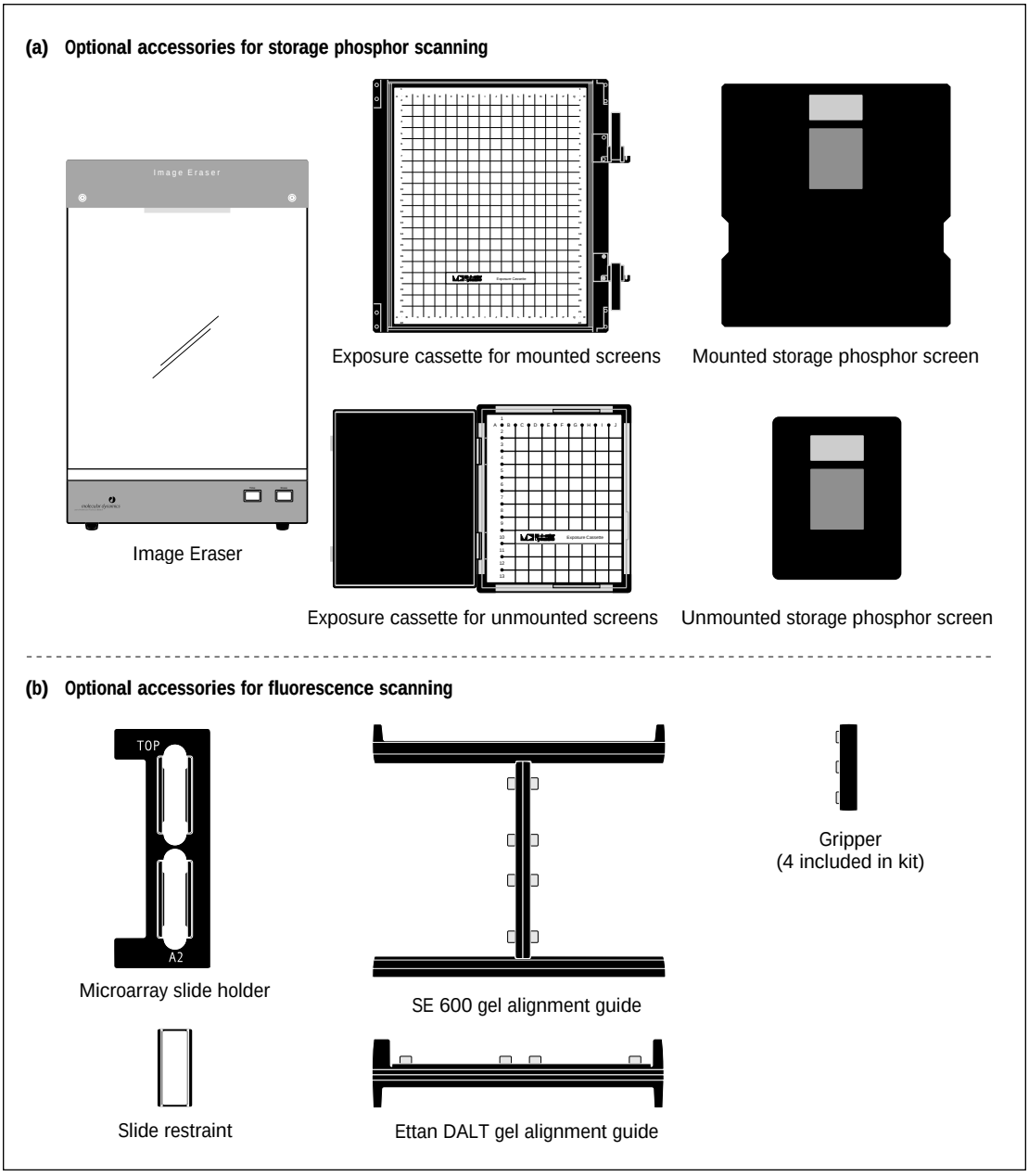


Figure 1-2. Optional accessories for the Typhoon system: (a) storage phosphor accessories and (b) fluorescence accessories.

1.4 Software components

You install the Scanner Control software on a computer running the Microsoft Windows operating system. The computer connects to the Typhoon instrument using an Ethernet connection.

In addition, you must install the Internet Explorer browser software to use the Typhoon Direct Instrument Access software. You use the Typhoon Direct Instrument Access software to perform several maintenance procedures, which are described in chapter 13.

1.5 How the Typhoon system works

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

1. You place an exposed storage phosphor screen, a fluorescent sample, or a chemiluminescent sample onto the glass platen of the instrument.
2. You use the Scanner Control software to select parameters that are appropriate for the type of sample you are scanning and to start the scan.
3. The screen or sample emits light in the following ways:
   - When the red (633 nm) laser illuminates each one-pixel section of an exposed storage phosphor screen, the phosphor emits blue light.
   - When the green laser (532 nm) or the red laser (633 nm) illuminates each one-pixel section of a fluorescent sample, the fluorochrome emits light with a characteristic spectrum.
   - (Typhoon 9400 and 9410 only) When the blue laser (457 nm or 488 nm) illuminates each one-pixel section of a fluorescent sample, the fluorochrome emits light with a characteristic spectrum.
   - The chemiluminescent sample emits light with a characteristic spectrum.
4. The optical system collects the emitted light. If you select one or more emission filters, the optical system directs the light through the selected emission filter(s). Each filter allows only the emitted light within the filter's bandwidth to pass through to the designated photomultiplier tube (PMT).
5. The PMT converts the light to an electric current, which varies with the intensity of the light collected by the PMT. The analog signal from the PMT is then converted into digital information, and the digital information is stored on the hard disk of the computer.

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

1.6 Typhoon scan acquisition modes

The Typhoon instrument scans samples using the following scan acquisition modes:

- **Storage phosphor mode—**Creates images from samples labeled with radioisotopes using storage phosphor technology. Part two of this guide describes how to use the Typhoon system to scan in the storage phosphor mode.
- **Fluorescence mode—**Creates images from samples labeled with fluorescent dyes. The available excitation modes are—
  - **Red-excited mode—**Creates an image from a sample labeled or stained with a fluorescent dye that is excited at 633 nm.
  - **Green-excited mode—**Creates an image from a sample labeled or stained with a fluorescent dye that is excited at 532 nm.
  - **(Typhoon 9400 and 9410 only) Blue-excited mode—**Creates an image from a sample labeled or stained with a fluorescent dye that is excited at either 457 nm or 488 nm. You select the desired wavelength.
  - **Multilabel-excited mode—**Creates an image from a sample labeled with up to four fluorescent dyes. The dyes can be excited by one or more of the four laser wavelengths.

  Part three of this guide describes how to use the Typhoon system to scan using each of the fluorescence modes.
- **Chemiluminescence mode—**Creates images from chemiluminescent samples. Part four of this guide describes how to use the Typhoon system to scan in the chemiluminescence mode.

1.7 Before you begin

Before using the Typhoon system, you should become familiar with—

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

Chapter 2 Safety

The Typhoon system is 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)
- Typhoon system serial numbers and safety labels (section 2.2)
- Electrical safety (section 2.3)
- Laser light safety (section 2.4)
- Pinching and general warnings (section 2.5)
- Hazardous materials precautions (section 2.6)

2.1 General safety precautions

Warnings

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

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

When using the Typhoon system, follow the precautions below—

- Make sure the instrument has at least 5 cm (2 in) of clearance on both sides and behind it. Make sure you have 1.2 m (4 ft) of clearance above the instrument. If the temperature of the room containing the instrument exceeds 30 °C (86 °F), increase the clearance on all sides by 2.5 cm (1 in).

Caution

Blocking the airflow to the Typhoon instrument can cause the instrument to overheat, which can impact the instrument's performance.

- (Typhoon 9400 and 9410 only) Make sure the blue laser module has at least 7.5 cm (3 in) of clearance behind it and 20 cm (8 in) of clearance on both sides. If the blue laser module is located under a laboratory bench, you must provide 30 cm (12 in) of clearance on both sides of the module. If the temperature in the room exceeds 30 °C (86 °F), you should use an external fan to vent the exhaust out of the room.

Caution

(Typhoon 9400 and 9410 only) If you do not use an external exhaust fan, the top of the blue laser module can get very warm. Do not place any temperature-sensitive materials on top of the blue laser module.

- If the cover or doors of the Typhoon instrument become damaged, do not continue to use the instrument.
- (Typhoon 9400 and 9410 only) If the fiber-optic cable becomes damaged, do not continue to use the instrument.
- If the Image Eraser becomes damaged, do not continue to use the eraser.
- If you add or remove optical (emission) filters, make sure you follow the procedures in section 13.4. Because the instrument is turned on during the procedures, you might be exposed to moving parts in the instrument.
- To protect your warranty, the Typhoon instrument and Image Eraser should be serviced only by an authorized Technical Support representative.

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

2.2 Typhoon system serial numbers and safety labels

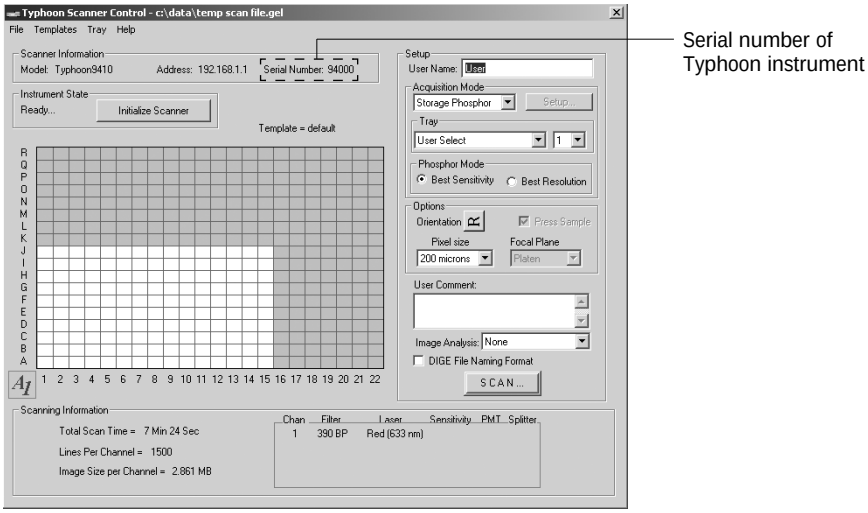
When you call Technical Support, you will be asked for the serial number of your instrument. The serial number of the Typhoon instrument is shown in the Scanner Information area of the Scanner Control window (figure 2-1a) and on the serial number certification label (figure 2-1b). The serial number certification label also displays the model number of the instrument and the Center for Devices and Radiological Health (CDRH) compliance information. The label is located on the lower right side of the Typhoon instrument near the plug. Figure 2-2 shows the locations of all the labels on the Typhoon instrument.

(Typhoon 9400 and 9410 only) If you have a Typhoon 9400 or 9410 instrument, see figure 2-1c for the serial number label for the blue laser module, and figure 2-3 for the locations of all the labels on the blue laser module. The serial number certification label also displays the model number of the blue laser module and the CDRH compliance information.

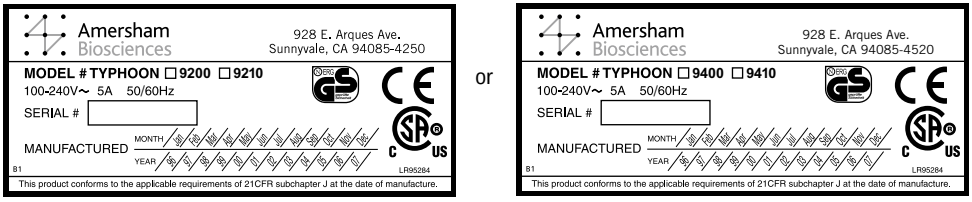
The serial number certification label (figure 2-1d) of the Image Eraser is located on the lower right side of the eraser near the plug. Figure 2-4 shows the locations of all the labels on the Image Eraser.

If any label becomes illegible or is missing for any reason, contact Technical Support for a free replacement label. See Assistance in the preface for contact information. While waiting for the replacement label, copy the figure containing the missing label and attach the copy of the label to the instrument.

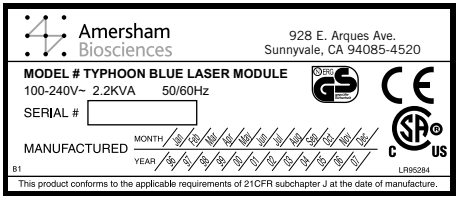
(a) Serial number location in the Scanner Control window



(b) Typhoon instrument



(c) Blue laser module



(d) Image Eraser

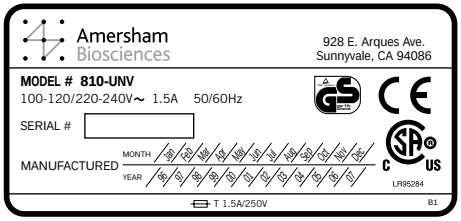


Figure 2-1. The locations of the serial numbers. (a) The instrument serial number location in the Scanner Control window. (b) The serial number labels for the Typhoon instrument. (c) The serial number label for the blue laser module (Typhoon 9400 and 9410 only). (d) The serial number label for the Image Eraser.

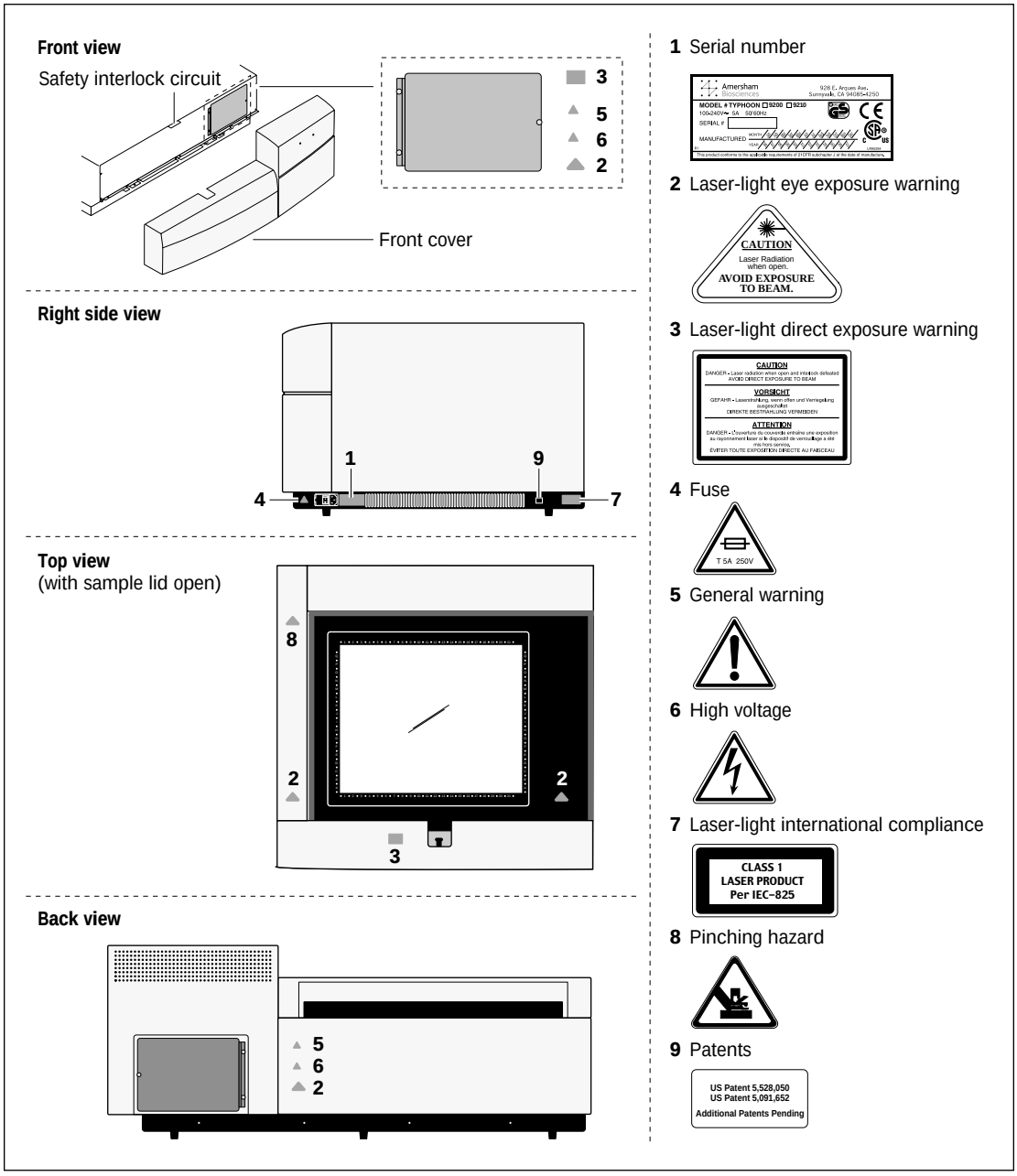


Figure 2-2. Location of important labels on the Typhoon instrument.

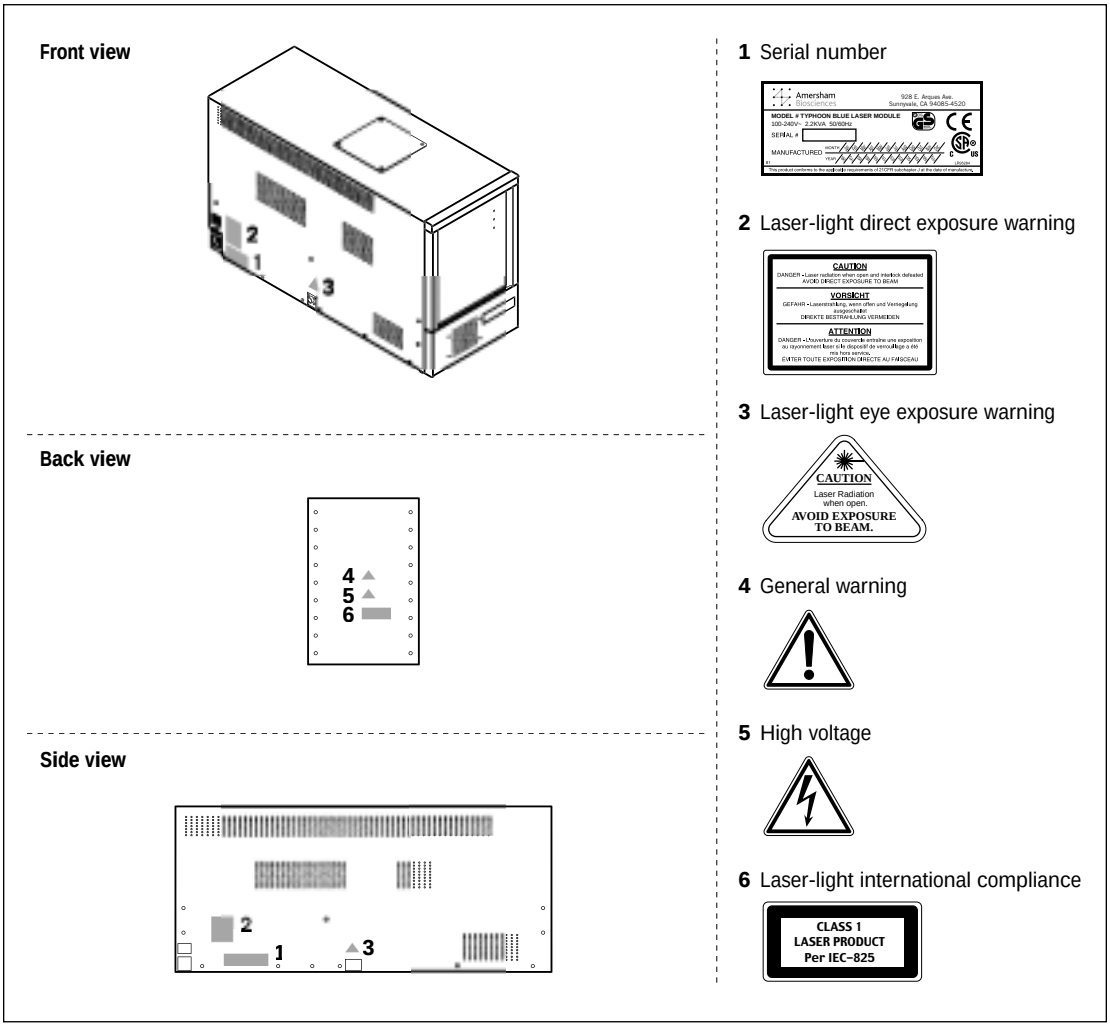


Figure 2-3. Location of important labels on the blue laser module (Typhoon 9400 and 9410 only).

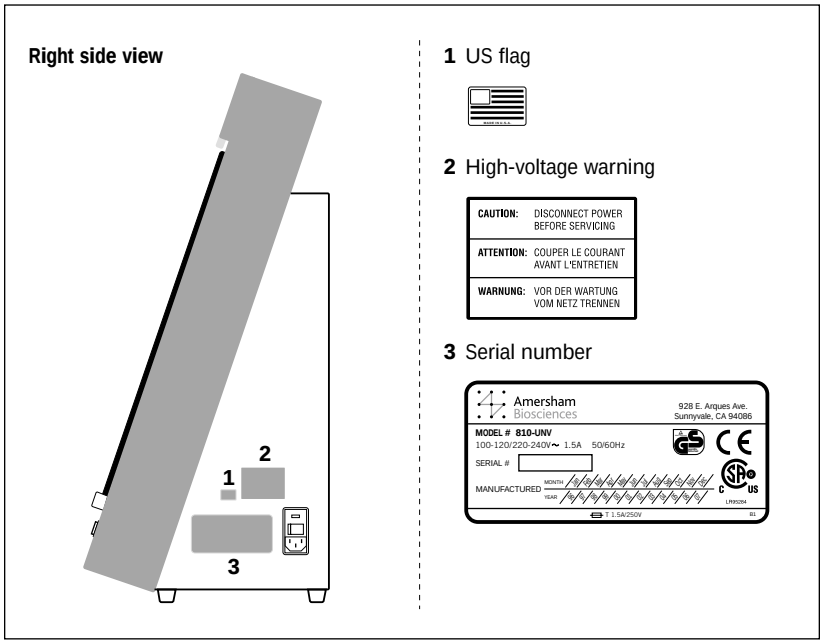


Figure 2-4. Location of important labels on the Image Eraser.

2.3 Electrical safety

You should follow the electrical safety information provided in this section to make sure you are operating the Typhoon instrument and Image Eraser safely.

2.3.1 Electrical connections

Before you turn on the Typhoon instrument, Image Eraser, or computer, check that the instrument, blue laser module (Typhoon 9400 and 9410 only), eraser, computer, and monitor are plugged into surge protectors, and the surge protectors are plugged in and turned on.

Note: The currents listed below refer to the minimum required circuit breaker size.

Plug the Typhoon 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

(Typhoon 9400 and 9410 only) Plug the blue laser module into a grounded circuit capable of delivering at least—

- 20 A for a 100–120 V~ power source (dedicated circuit)
- 13 A for a 200–240 V~ power source (dedicated circuit)

Plug the Image Eraser 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.

2.3.2 Operating voltage

When you turn on the Typhoon instrument, the instrument automatically switches to the correct operating voltage. The Image Eraser does not automatically switch to the correct operating voltage. You must manually select the correct voltage (section 14.3).

Caution **Before turning on the Image Eraser, make sure that the correct operating voltage appears in the fuse window. Using the wrong voltage can severely damage the eraser.**

2.3.3 Fuses

Both the Typhoon instrument and the Image Eraser contain user-replaceable fuses.

- **On the Typhoon instrument—**The fuse holder is on the lower right side of the Typhoon instrument and contains two 5A, 250V (⊟, T5A, 250V) slow-acting fuses. The fuse label contains the fuse specifications (figure 2-5). The fuse holder accepts 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 lower right side of the Image Eraser and contains two 1.5A, 250V (⊟, T1.5A, 250V) slow-acting fuses. The fuse holder accepts both 0.25-in by 1.25-in English fuses (designated 3AG fuses) and 5-mm by 20-mm metric fuses.

(Typhoon 9400 and 9410 only) The blue laser module does not contain any user-replaceable fuses. If the blue laser module does not turn on, check the power cord and the communications cable. If the problem is not the connections, contact Technical Support.

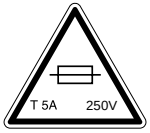


Figure 2-5. The fuse label on the Typhoon instrument.

To change the fuses on the Typhoon instrument, follow the instructions in section 13.7. To change the fuses on the Image Eraser, follow the instructions in section 14.3.

Warning

If you need to change the fuses in the instrument or eraser, 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 a malfunctioning instrument or eraser. You could expose yourself to electrical shock. Contact Technical Support. See Assistance in the preface for contact information.

2.3.4 High-voltage hazard and precautions

Inside the Typhoon instrument, blue laser module (Typhoon 9400 and 9410 only), Image Eraser, computer, and monitor are high-voltage electronics.

Typhoon instrument

In general, you do not need to access the interior of the Typhoon instrument. However, to install or remove an optical (emission) filter, you must remove the front cover while the instrument is turned on. If you need to install or remove emission filters, make sure you follow the procedures in section 13.4.

Warning

Do not remove the front cover of the instrument unless you are installing or removing optical (emission) filters. There are no other user-serviceable components inside. When you change the optical filter selection, you must leave the Typhoon instrument turned on. After you remove the front cover of the Typhoon instrument and open the access and filter doors, make sure you follow the procedures in section 13.4 to avoid injury.

When using the Typhoon instrument, follow the precautions below—

- Always plug the power cord into a grounded outlet.
- Before changing a fuse, always turn off the power and unplug the instrument. Follow the instructions in section 13.7.

- If the covers become damaged, do not use the instrument. Contact Technical Support for repair. See Assistance in the preface for contact information.
- If fuses must be replaced repeatedly, do not use the instrument. Contact Technical Support for repair.

Figure 2-6 displays the high-voltage warning label on the Typhoon instrument. See figure 2-2 for the location of the label.

Figure 2-6. High-voltage warning label.

Blue laser module (Typhoon 9400 and 9410 only)

You do not need to access the interior of the blue laser module. The module has no user-serviceable parts or user-replaceable fuses.

Warning

Do not remove the covers of the blue laser module. You could be exposed to high voltages.

When using the Typhoon 9400 or 9410 instrument, follow the precautions below—

- Always plug the power cord of the blue laser module into a grounded outlet (dedicated circuit).
- If the covers of the blue laser module become damaged, do not use the instrument. Contact Technical Support for repair. See Assistance in the preface for contact information.

Figure 2-6 displays the high-voltage warning label on the blue laser module. See figure 2-2 for the location of the label.

Image Eraser

Warning

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

Figure 2-7 displays the high-voltage hazard label on the Image Eraser. See figure 2-4 for the location of the label.

CAUTION:	DISCONNECT POWER BEFORE SERVICING
ATTENTION:	COUPER LE COURANT AVANT L'ENTRETIEN
WARNUNG:	VOR DER WARTUNG VOM NETZ TRENNEN

Figure 2-7. 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—

- Always plug the power cord into a grounded outlet.
- Before cleaning the Image Eraser, always turn off the power and unplug the eraser. Follow the instructions in section 14.1.
- Before changing a bulb, always turn off the power and unplug the eraser. Follow the instructions in section 14.2.
- When changing a bulb, do not remove covers other than the front diffuser panel. There are no user-serviceable parts inside.
- Before changing a fuse, always turn off the power and unplug the eraser. Follow the instructions in section 14.3.
- If the covers become damaged, do not use the eraser. Contact Technical Support for repair. See Assistance in the preface for contact information.
- If fuses must be replaced repeatedly, do not use the eraser. Contact Technical Support for repair.

Computer and monitor

See the manufacturer's computer and monitor precautions before opening the computer or monitor.

2.4 Laser light safety

Warning

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

The Typhoon instrument is a Class I laser instrument that houses two Class IIIB lasers inside the instrument. Under the specified operating procedures, the instrument does not allow operator exposure to laser light. The lasers, with power of 10–20 mW, are accessible in the interior of the instrument.

(Typhoon 9400 and 9410 only) The blue laser module contains one Class IIIB laser. The blue laser, with power at 30 mW, is accessible in the interior of the module.

The safety interlocks (figure 2-2) in the Typhoon instrument are designed to prevent you from being exposed to the laser beams. If you open the sample lid while the scanner is in operation, the laser shutter closes and blocks the light from the lasers.

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.

2.4.1 Laser-light warning labels

The labels shown in figure 2-8 warn of exposure to laser light. The locations of the labels are shown in figures 2-2 and 2-3.

International compliance | Eye exposure | Direct exposure

Figure 2-8. 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.**

2.4.2 Instrument safety precautions

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

The Typhoon instrument has been designed to protect you from the laser beam emissions during normal operation. Nevertheless, laser power of 10–30 mW can be accessed from within the interior of the instrument.

The Typhoon instrument contains a red laser and a green laser. The output of the red visible laser is specified at a maximum of 10 mW at 633 nm in a diverging beam. The output of the green visible laser is specified at a maximum of 20 mW at 532 nm in a diverging beam.

When using the Typhoon instrument, follow the precautions below—

- Do not remove the front cover (figure 2-2) of the Typhoon instrument except to install or remove optical (emission) filters. There are no other user-serviceable components inside, and you can be exposed to laser light.
- Do not defeat the safety interlocks of the sample lid (figure 2-2). The safety interlocks are designed to protect you from exposure to the laser light. If you lift the sample lid during a scan, the safety interlocks signal the laser shutters to close and block the light from the lasers.
- Do not continue to use the instrument—
  - If a cover or the sample lid becomes damaged and the instrument is no longer light tight.
  - If fuses must be replaced repeatedly.

  Contact Technical Support immediately to arrange for repair. See Assistance in the preface for contact information.

  To protect your warranty and for proper operation, your Typhoon instrument should be serviced only by an authorized Technical Support representative. If the instrument is not working correctly, contact Technical Support. See Assistance in the preface for contact information.

2.4.3 Blue laser module safety precautions (Typhoon 9400 and 9410 only)

The blue laser module contains the blue laser and the power supply for the blue laser. The fiber-optic cable that connects the instrument and the blue laser module transmits the output of the blue visible laser at a maximum of 30 mW at wavelengths from 457 nm to 488 nm in a diverging beam. The cable must be connected by an authorized Service Support engineer. See Assistance in the preface for contact information.

Caution **The core of the fiber-optic cable is protected by a flexible metal sheath, but you still must take precautions to protect the cable from damage.**

To protect the cable from damage—

- Position the fiber-optic cable away from the high-traffic areas in the laboratory.
- Do not coil the cable into a circle with a diameter smaller than 15.24 cm (6 in).
- Make sure you do not stretch the cable.
- If the fiber-optic cable becomes damaged, do not continue to use the instrument. Contact Technical Support. See Assistance in the preface for contact information.

2.5 Pinching and general warnings

A pinching hazard label (figure 2-9a) appears near the support bars for the sample lid. This label warns you that your fingers could be pinched if the sample lid closes unexpectedly.

A general warning label (figure 2-9b) appears on the back of the Typhoon instrument near the power supply fan exhaust. This label warns you that you could be exposed to the moving parts when the instrument is running.

A general warning label appears near the access door to the optical filter changer. This label warns you that you could be exposed to moving parts if you open the access door while the instrument is running.

(Typhoon 9400 and 9410 only) A general warning label appears on the back of the blue laser module near the power supply fan exhaust. This label warns you that you could be exposed to the moving parts when the module is running.

Figure 2-2 shows the locations of pinching hazard and general warning labels.

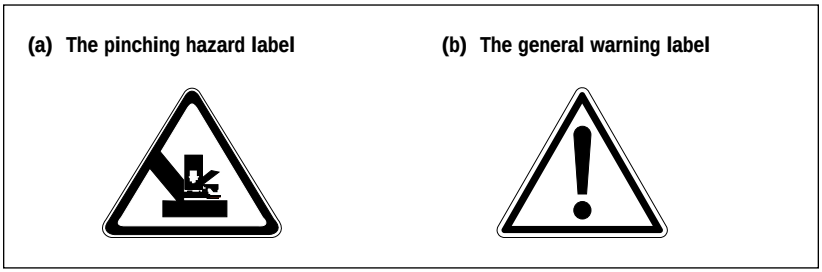


Figure 2-9. (a) The pinching hazard label. (b) The general warning label.

2.6 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 Typhoon system, become familiar with your laboratory's hazardous materials procedures.

Warning

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

Cautions

Do not expose the Typhoon instrument and storage phosphor screen to 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.

Chapter 3 Getting started

This chapter describes the procedures for using the Typhoon system. The topics in this chapter are—

- Turning on and warming up the Typhoon instrument (section 3.1)
- Turning on the computer (section 3.2)
- Starting the Scanner Control software (section 3.3)
- About the Typhoon instrument states (section 3.4)
- Workflows for scanning (section 3.5)
- Customizing the scan area for multiple samples (section 3.6)
- Saving the scan parameters as a template (section 3.7)
- Closing the Scanner Control software (section 3.8)
- Turning off the Typhoon instrument (section 3.9)
- Turning off the computer (section 3.10)

3.1 Turning on and warming up the Typhoon instrument

To turn on the Typhoon instrument, press the on/off switch on the lower right side of the instrument (figure 3-1). The power indicator light on the front of the instrument turns on and remains red during the self-test sequence, which takes a few seconds. During the initialization, the instrument flashes the green Power light for approximately 45 seconds. After initialization, the flashing green light turns solid green.

Important **After you turn on the Typhoon instrument, wait approximately 30 minutes for the instrument to warm up before you start the first scan. Scanning before the instrument is warmed up can affect the accuracy of the collected data.**

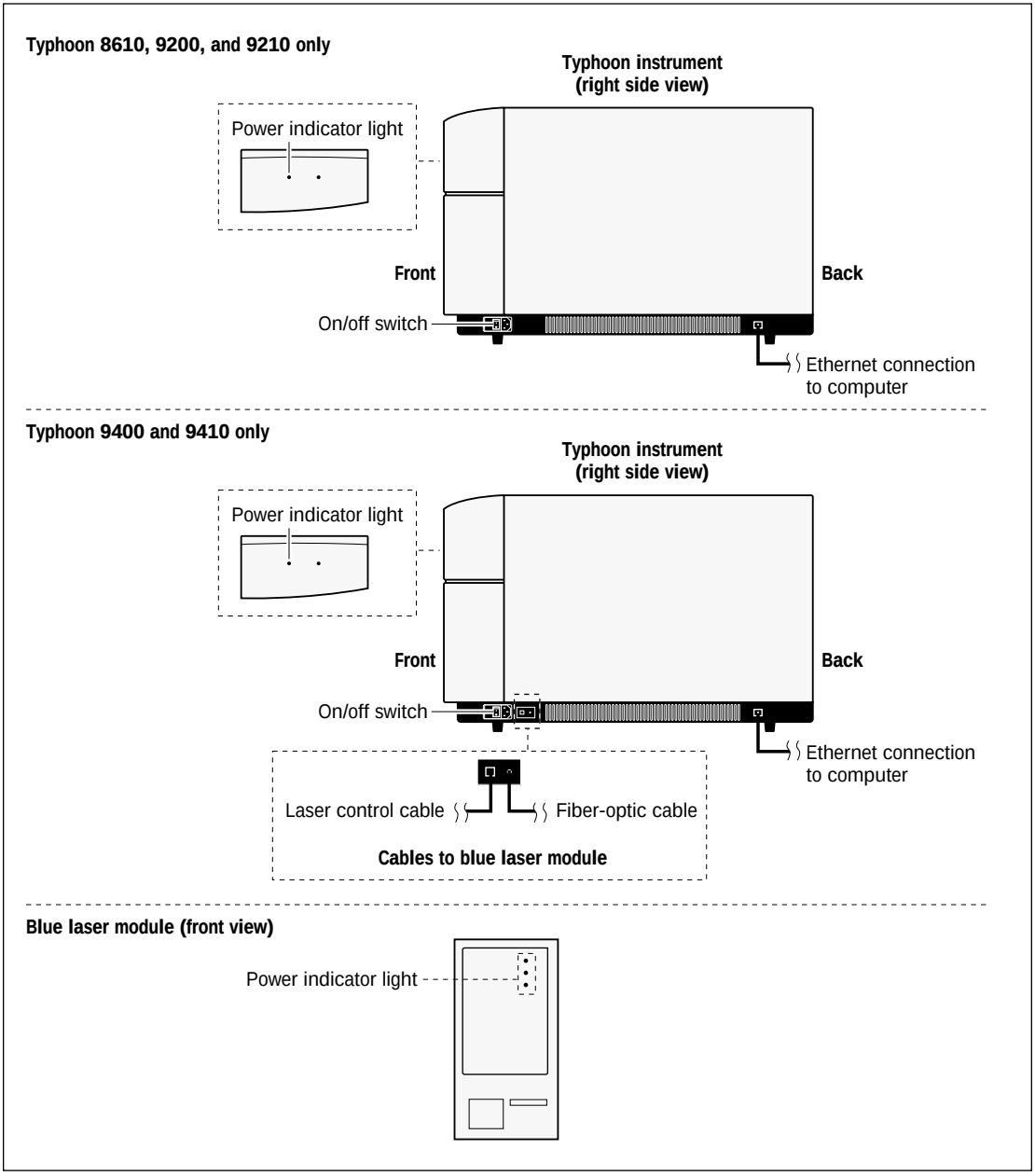


Figure 3-1. Right side views of the Typhoon instruments and the front of the blue laser module. **Note:** The laser control cable and the fiber-optic cable are used on the Typhoon 9400 and 9410 only.

(Typhoon 9400 and 9410 only) The blue laser module does not have a power switch. When you start the Typhoon instrument, the blue laser module turns on automatically. The Power light on the front of the blue laser module turns green, and then the Connection light turns green, which indicates the instrument is communicating with the blue laser module. The blue laser starts running after the Typhoon instrument completes the self-test (approximately 1 minute), and then the Laser On light turns green.

3.2 Turning on the computer

So that the computer can communicate with the Typhoon instrument, the instrument must be turned on first, and the power indicator light must be solid green. Use the computer manufacturer's instructions for turning on 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 start the Scanner Control software using the Start menu.) The Scanner Control window appears (figure 3-2).

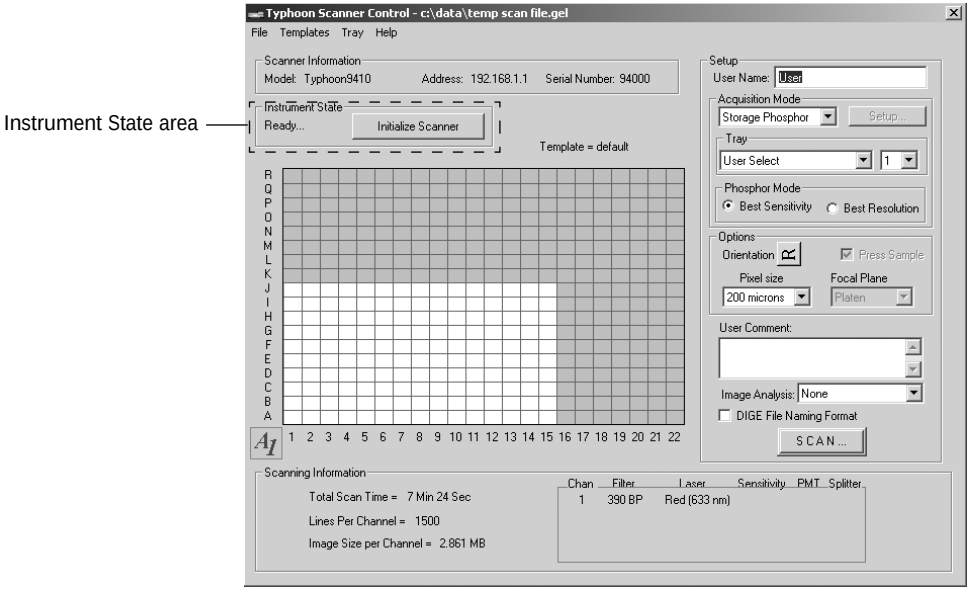


Figure 3-2. The Scanner Control window.

3.4 About the Typhoon instrument states

The Typhoon instrument can be in one of five states. The Scanner Control window displays the current instrument state and an Initialize Scanner button (figure 3-2).

The instrument states are—

- **Warming Up—**Scanner Control displays the **Warming Up** message for 5 minutes after you turn on the system or click the Initialize Scanner button. During the 5 minutes, the Typhoon system stabilizes the lasers and system components. However, complete warm-up takes 30 minutes when you first turn on the instrument.
- **Ready—**The instrument is ready to scan. If you just turned on the instrument, the **Ready** message appears after approximately 5 minutes. However, for best performance, you should let the instrument warm up for the full 30 minutes before scanning.
- **Sleep—**The instrument has not been used for the amount of time you set using the Typhoon Direct Instrument Access software (section 13.2). (**Note:** The default setting is 4 hours.) After the selected time elapses, the lasers shut off and the **Sleep** message appears in the Scanner Control window.
- **Initialization**—After you click the Scan button, the **Initialization** message appears. The **Initialization** message remains displayed until the instrument begins scanning. If the prior instrument state was Ready, the initialization takes approximately 1 minute. If the prior instrument state was Sleep or Warming Up, the initialization takes approximately 5 minutes.
- **Scanning**—The **Scanning** message appears while the instrument is scanning.

Note: If the **Service** message appears, the instrument requires servicing. You should call Technical Support. See Assistance in the preface for contact information.

While you are preparing the sample, you can click the **Initialize Scanner** button to reset the sleep clock and bring the instrument to the Ready state. When your sample is ready to scan, the instrument scans without delay. If the sample is already in position on the glass platen, you can start the scan even if the instrument is in the Sleep state. If this is the case, 5 minutes are added to the initialization time to allow the lasers and other system components to stabilize before the scan starts.

3.5 Workflows for scanning

You can scan three types of samples on the Typhoon instrument:

- A storage phosphor screen that has been exposed to a radioactive sample (section 3.5.1)
- A fluorescent sample (section 3.5.2)
- A chemiluminescent sample (section 3.5.3)

This section provides an overview of the workflow for each sample type and indicates where in this user's guide you should look for detailed information.

3.5.1 Workflow for storage phosphor scanning

Table 3-1 describes the workflow for storage phosphor scanning and lists the sections in this user's guide that contain the procedural steps for each task.

Table 3-1. Storage phosphor scanning workflow

Task	Description	Section
Preparing for storage phosphor screen autoradiography		
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 before exposing it to the GP screen. Do not use the LE or TR screens with a wet gel.	5.1
2. Prepare the storage phosphor screen.	Decontaminate (optional), clean, and erase the GP or LE screen. Erase the TR screen. Protect the Image Eraser from contamination when erasing the TR screen.	5.2
3. Prepare the exposure cassette.	Decontaminate (optional) and clean the exposure cassette.	14.4
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. If the sample is thick or wet, do not use the exposure cassette. Instead, expose the sample to the screen in a light-tight environment, such as a light-tight drawer.	5.5
5. Place the storage phosphor screen on the sample.	Place the screen on the sample. To avoid a double image of the sample, do not reposition the screen after it has touched the sample.	5.6
6. Expose the storage phosphor screen to the sample.	Leave the screen in the exposure cassette (or light-tight drawer) until the screen is exposed. To determine the length of exposure, consider a 2-hour exposure to the screen to be equal to an overnight exposure to basic x-ray film.	5.7

Table 3-1. Storage phosphor scanning workflow (continued)

Task	Description	Section
Scanning the storage phosphor screen		
1. Prepare the Typhoon instrument.	Decontaminate (optional) and clean the glass platen and sample lid of the Typhoon instrument.	6.1
2. Place the storage phosphor screen in the Typhoon instrument.	In subdued light, remove the storage phosphor screen from the exposure cassette (or light-tight drawer). Keep the screen face down while you move it from the cassette to the instrument. With the screen still face down, position the screen on the glass platen so that the A1 corner of the screen aligns with the A1 corner of the glass platen. Close the sample lid.	6.2, 6.3
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. Choose whether you want best sensitivity or best resolution. Select the grid area or tray template, pixel size, sample orientation, and image analysis software. **Note:** In the storage phosphor scan acquisition mode, selecting DIGE File Naming Format has no effect on the way the software saves the image file. Type comments you want saved with the image.	6.5
4. Start the scan.	Click **Scan.** Type a file name and click **Save.** (If you are creating multiple images, type a base file name and click **Set,** or click **Edit Sample File Name,** type separate image file names, and click **OK.** Optionally, click the Folder **Browse** button, select a new storage location, and click **Set.** Click **Scan** to start the scan.) The ImageQuant Preview window appears. While the Typhoon instrument scans the screen, an image of the screen appears in the window.	6.6
5. Evaluate the results.	During scanning, check the image in the ImageQuant Preview window for saturation. Saturated pixels appear in red. If the image appears saturated, you might need to expose a clean screen to the sample for a shorter amount of time. If the image appears faint, you might need to expose a clean screen for a longer amount of time. If the image appears usable, 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.	6.6.6
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 Typhoon instrument, exposure cassette, and Image Eraser. Decontaminate, clean, and erase a GP or LE screen. Erase a TR screen. Store the screen in the exposure cassette or another dark place.	6.7, 6.8

3.5.2 Workflow for fluorescence scanning

Table 3-2 describes the workflow for fluorescence scanning and lists the sections in this user's guide that contain the procedural steps for each task.

Table 3-2. Fluorescence scanning workflow

Task	Description	Section
Preparing for fluorescence scanning		
1. Prepare the sample.	Prepare the sample using fluorescent dyes that are compatible with the green (532 nm) or the red (633 nm) excitation mode. (Typhoon 9400 and 9410 only) Prepare the sample using fluorescent dyes that are compatible with the blue (457 nm or 488 nm) excitation modes.	8.1
2. Prepare the Typhoon instrument.	Decontaminate (optional) and clean the glass platen and sample lid of the Typhoon instrument.	8.2
Scanning the fluorescent sample		
1. Place the sample in the Typhoon instrument.	In subdued light, position the sample on the glass platen. Make a note of the grid coordinates on the glass platen. Close the sample lid.	8.3
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 or tray template, pixel size, sample orientation, press sample (optional), focal plane, DIGE file format naming for multichannel images (optional), and image analysis software from the Scanner Control window. **Note:** For a single-channel scan, selecting DIGE File Naming Format has no effect on the way the software saves the image file. Then click **Setup.** In the Fluorescence Setup window, select the emission filter, PMT voltage, laser, and sensitivity for each scan. Optionally, you can select scans that can be linked. Click **OK.** In the Scanner Control window, type comments you want saved with the image.	9.2, 9.3
3. Start the scan	Click **Scan.** Type a file name and click **Save.** (If you are creating multiple images, type a base file name and click **Set,** or click **Edit Sample File Name,** type separate image file names, and click **OK.** Optionally, click the Folder **Browse** button, select a new storage location, and click **Set.** Click **Scan** to start the scan.) The ImageQuant Preview window appears. While the Typhoon instrument scans the sample, an image of the sample appears in the window.	9.4

Table 3-2. Fluorescence scanning workflow (continued)

Task	Description	Section
4. Evaluate the results.	During scanning, check the image in the ImageQuant Preview window for saturation. Saturated pixels appear in red. If the image appears saturated, you might need to repeat the scan. If the image appears usable, 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.	9.4.7
5. Clean up after the scan.	Remove the sample from the Typhoon instrument and dispose of the sample using the appropriate laboratory procedures. Clean the Typhoon instrument.	9.5, 9.6

3.5.3 Workflow for chemiluminescence scanning

Table 3-3 describes the workflow for chemiluminescence scanning and lists the sections in this user's guide that contain the procedural steps for each task.

Table 3-3. Chemiluminescence scanning workflow

Task	Description	Section
Preparing for chemiluminescence scanning		
1. Prepare the sample.	Prepare the sample using chemiluminescent enzyme substrate systems. To create two images with a single scan, use substrates that can be collected and separated using a beamsplitter.	11.1
2. Prepare the Typhoon instrument.	Decontaminate (optional) and clean the glass platen and sample lid of the Typhoon instrument.	11.2
Scanning the chemiluminescent sample		
1. Place the sample in the Typhoon instrument.	In subdued light, position the sample on the glass platen. Make a note of the grid coordinates on the glass platen. Close the sample lid.	11.3

Table 3-3. Chemiluminescence scanning workflow (continued)

Task	Description	Section
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 Chemiluminescence is the selected scan acquisition mode. Then select the grid area, pixel size, sample orientation, press sample (optional), focal plane, and image analysis software from the Scanner Control window. **Note:** For a single-channel scan, selecting the DIGE File Naming Format has no effect on the way the software saves the image file. Then click **Setup**. In the Chemiluminescence Setup window, select the sensitivity and PMT voltage for the scan. To create two images with a single scan, select a beamsplitter and a second PMT voltage. Click **OK.** In the Scanner Control window, type comments you want saved with the image.	12.2, 12.3
3. Start the scan.	Click **Scan.** Type a file name and click **Save.** (If you are creating multiple images, type a base file name and click **Set,** or click **Edit Sample File Name,** type separate image file names, and click **OK.** Optionally, click the Folder **Browse** button, select a new storage location, and cllck **Set.** Click **Scan** to start the scan.) The ImageQuant Preview window appears. While the Typhoon instrument scans the sample, an image of the sample appears in the window.	12.4
4. Evaluate the results.	During scanning, check the image in the ImageQuant Preview window for saturation. Saturated pixels appear in red. If the image appears saturated, you might need to repeat the scan. If the image appears usable, 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.	12.4.7
5. Clean up after the scan.	Remove the sample from the Typhoon instrument and dispose of the sample using the appropriate laboratory procedures. Clean the Typhoon instrument.	12.5, 12.6

3.6 Customizing the scan area for multiple samples

If you are scanning multiple samples (for example, DIGE gels), you can use the Tray Editor window (figure 3-3) to create a separate scan area for each sample. You provide a name for each scan area. When you select the scan parameters, you choose the tray definition from the Tray list. When you scan the samples, Scanner Control creates a separate image for each scan area defined in the selected tray definition.

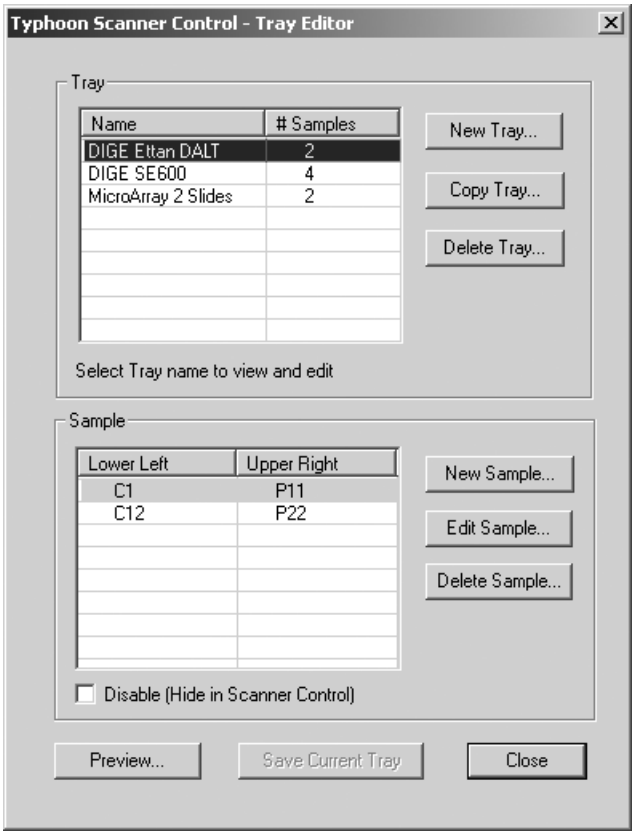


Figure 3-3. The Tray Editor window.

Scanner Control includes four predefined tray definitions. They are—

- **User Select**—Allows you to define one scan area by selecting the area using the grid in the Scanner Control window.
- **DIGE Ettan DALT**—Contains two predefined scan areas for use with the DIGE Ettan DALT gel alignment guide. In the Tray area in the Scanner Control window, you can choose to scan one scan area or both.
- **DIGE SE600**—Contains four predefined scan areas for use with the DIGE SE600 gel alignment guide. In the Tray area in the Scanner Control window, you can choose to scan one through four scan areas.
- **MicroArray 2 Slides**—Contains two predefined scan areas for use with the microarray slide holder. In the Tray area in the Scanner Control window, you can choose to scan one scan area or both.

3.6.1 Creating a new tray definition

You define the new tray by selecting the sample dimensions and the sample placement of each sample you want included in the tray definition.

To create a tray definition—

1. Choose **Tray Editor** from the Tray menu. The Tray Editor window appears and displays the current list of defined trays (figure 3-3).
2. Click **New Tray.** The New Tray window appears (figure 3-4).

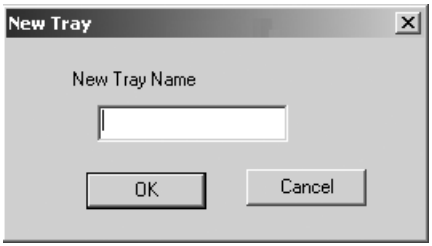


Figure 3-4. The New Tray window.

3. Type a name for the tray definition in the New Tray Name box and click **OK.** A window appears and tells you to select New Sample to enter the sample dimensions.
4. Click **OK.** The name you typed appears in the Tray list.
5. Click **Save Current Tray** to save the tray definition name.

6. Click the new tray definition name in the Tray list.
7. Click **New Sample.** The New Sample Tray window appears (figure 3-5).

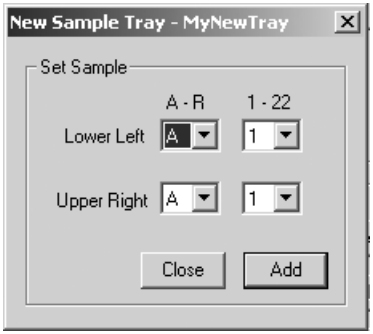


Figure 3-5. The New Sample Tray window.

8. Using the grid coordinates on the glass platen of the Typhoon instrument, determine where the lower left and upper right corners will be for the first sample.
9. Select the coordinates in the New Sample Tray window and click **Add.**
10. Repeat steps 7 through 9 for each sample you will be placing on the glass platen. After you enter the last set of coordinates, click **Close** instead of Add to close the New Sample Tray window and return to the Tray Editor window. The Tray area displays the name of the tray you defined and the number of samples you defined for the tray.
11. (Optional) If you want to change the coordinates for a sample, click **Edit Sample.** The Edit Sample Tray window appears (figure 3-6). Change the grid coordinates for the sample and click **OK.**

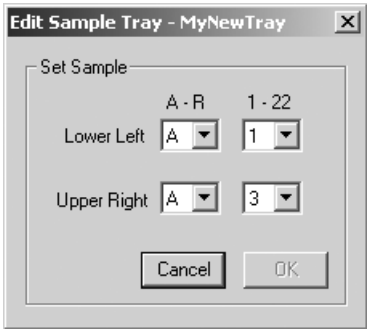


Figure 3-6. The Edit Sample Tray window.

12. (Optional) If you want to delete the coordinates for a sample, click the coordinates in the Sample list and then click **Delete Sample.** The sample coordinates disappear from the list.

13. Click **Preview.** The Preview Sample Tray window appears and displays the scan areas for the samples you defined (figure 3-7).

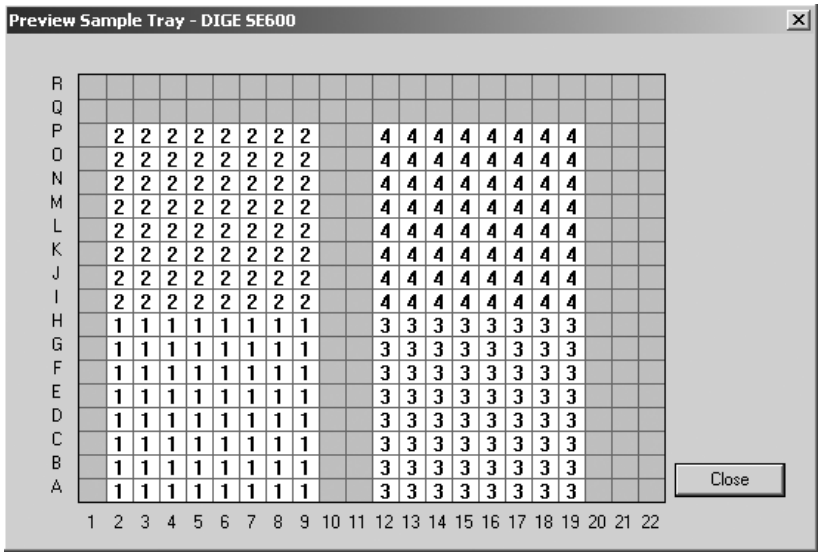


Figure 3-7. The Preview Sample Tray window.

14. Click **Close** to close the Preview Sample Tray window and return to the Tray Editor window.

15. (Optional) To modify the grid coordinates for any scan area that you incorrectly defined, repeat step 11.

16. Click **Save Current Tray** to save the tray configuration.

3.6.2 Copying a tray definition

You can copy a tray definition so that you can create a new tray definition. To do this—

1. Choose **Tray Editor** from the Tray menu. The Tray Editor window appears and displays the current list of defined trays (figure 3-3).

2. Click the tray definition that you want to copy.

3. Click **Copy Tray.** The Copy Tray window appears.

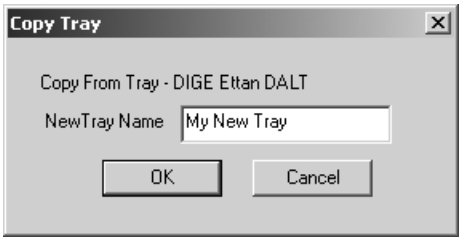


Figure 3-8. The Copy Tray window.

4. Type a name for the new tray definition in the New Tray Name box and click **OK.**
5. Click **Save Current Tray** to save the tray definition name.
6. Click the new tray definition name in the Tray list.
7. Modify the grid coordinates to match how you will place the samples on the glass platen of the Typhoon instrument. You can—
   - **Change the coordinates for an existing sample**—Click **Edit Sample.** The Edit Sample Tray window appears (figure 3-6). Change the grid coordinates for the sample and click **OK.**
   - **Delete the coordinates for an existing sample**—Click the coordinates in the Sample list, and then click **Delete Sample.** A window appears asking you to confirm that you want to delete the coordinates. Click **Yes.** The sample coordinates disappear from the list.
   - **Add a new set of coordinates**—Click **New Sample.** The New Sample Tray window appears (figure 3-5). Select the coordinates in the New Sample Tray window and click **Close.**
8. Click **Preview.** The Preview Sample Tray window appears and displays the scan areas for the samples you defined (figure 3-7).
9. Click **Close** to close the Preview Sample Tray window and return to the Tray Editor.
10. (Optional) To modify the grid coordinates for any scan area that you incorrectly defined, repeat step 7.
11. Click **Save Current Tray** to save the tray configuration.

3.6.3 Deleting a tray definition

You can delete a tray definition that you no longer need from the list in the Tray Editor window (figure 3-3).

Important **If you created a template that includes a tray definition as one of the scan parameters, the software will not delete the tray definition until you modify the template to remove the tray definition. For more information on modifying templates, see section 3.7.3.**

To delete a tray definition—

1. Click the name of the tray definition that you want to delete.
2. Click **Delete Tray.** A window appears asking you to confirm that you want to delete the definition.
3. Click **Yes.** The software removes the definition from the Tray list.

3.7 Saving the scan parameters as a template

The Scanner Control software allows you to save the scan parameters you use frequently as a uniquely named template. You use the template name to retrieve the parameters and display them in the Scanner Control and Setup windows.

Important **When you create a template for the fluorescence acquisition mode, you select an emission filter or filters from the filters installed in the Typhoon instrument and, for a multichannel scan, a beamsplitter from the beamsplitters installed in the Typhoon instrument. If you remove an emission filter or a beamsplitter from the instrument, Scanner Control removes all the templates from the template list that used the filter or beamsplitter. If you reinstall the filter or beamsplitter, the templates reappear in the list.**

3.7.1 Creating a new template

To create a template—

1. In the Setup area of the Scanner Control window (figure 3-9), select the parameters you want saved with the template. Note that comments in the User Comment box are not saved with the template.

   For a description of the setup parameters, see section 3.6 (scan area definitions for multiple samples), section 6.5 (storage phosphor), section 9.2 (fluorescence), or section 12.2 (chemiluminescence).

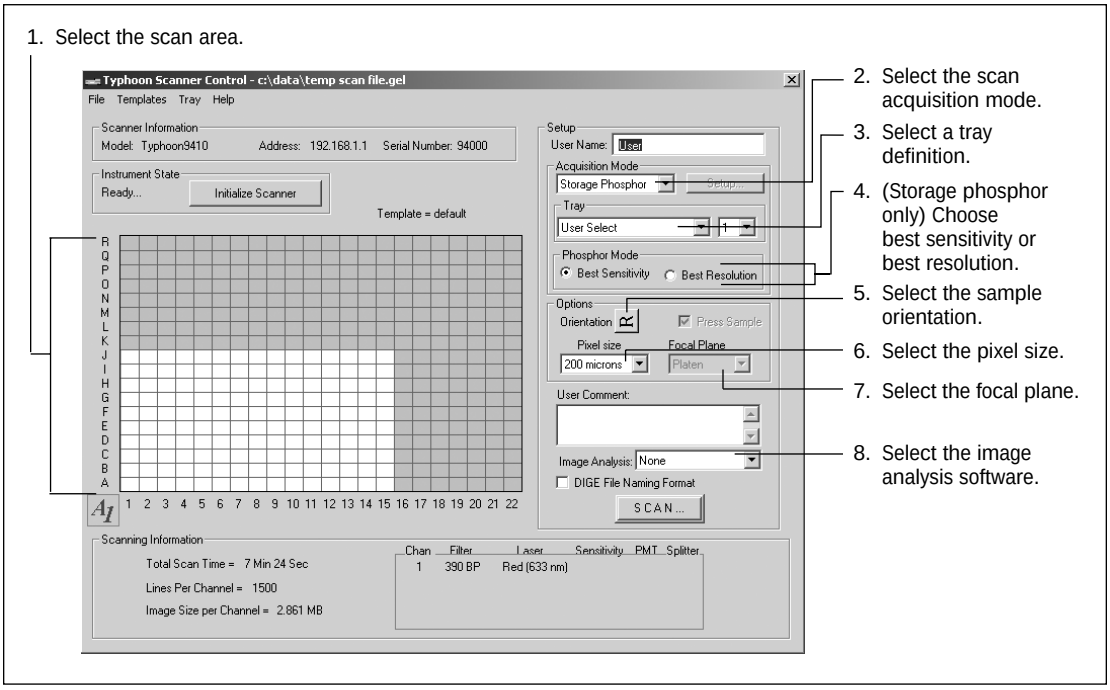
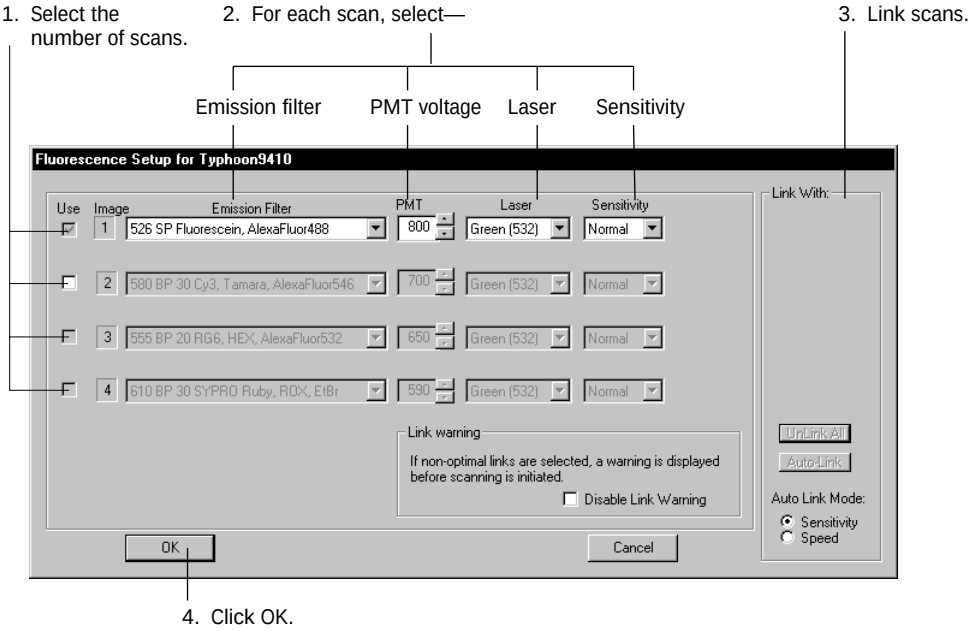


Figure 3-9. Selecting parameters in the Setup area of the Scanner Control window.

2. (Storage phosphor only) Select whether you want to scan using the **Best Resolution** or the **Best Sensitivity.** For more information on these parameters, see section 6.5.5.

3. (Fluorescence and chemiluminescence only) Click the **Setup** button and select the parameters in the Setup window (figure 3-10). For more information on selecting parameters, see section 9.3 (fluorescence) or section 12.3 (chemiluminescence).

(a) Fluorescence Setup window



(b) Chemiluminescence Setup window

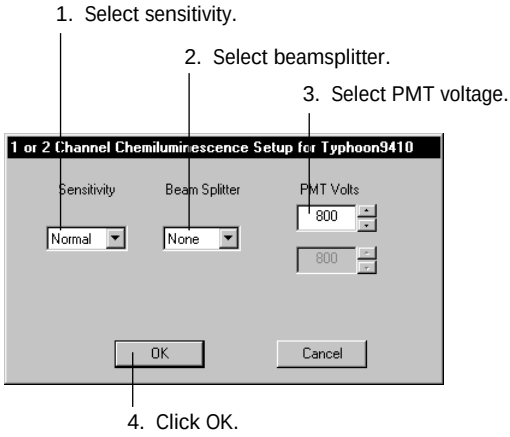


Figure 3-10. Selecting parameters in the (a) Fluorescence Setup window or (b) Chemiluminescence Setup window.

4. Choose **Save As Template** from the Templates menu. The Save As Template window appears (figure 3-11).

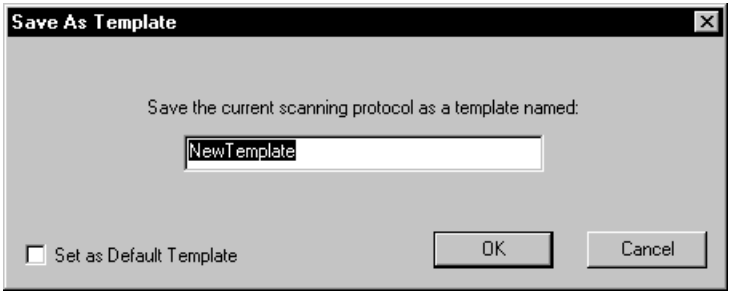


Figure 3-11. The Save As Template window.

5. Type a new name for the template in the box. **Note:** Because the Template list displays all the available templates, you might want to label the templates by scan acquisition mode. For example, a template for storage phosphor can be named TemplateName_Phos.

Important **A template name can be a maximum of 32 characters, and you should type an underscore instead of a space.**

6. (Optional) If you want the template to become the new default, select the **Set as Default Template** check box. **Note:** You can select the original default template (system) supplied with the instrument from the Templates menu.

7. Click **OK.** The template name appears above the grid area in the Scanner Control window.

3.7.2 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.

3.7.3 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. If you want to use the original unmodified parameters, select the template from the Load menu again. The original parameters appear in the Scanner Control window.

Scanner Control will not allow you to overwrite the existing parameter values in the original template. If you want to save a modified template using the same name, you must delete the old template (section 3.7.5). Next, select the new parameters and save the template using the name of the template you deleted.

3.7.4 Selecting a template to use as a default

If you use the same scan acquisition mode when scanning samples, you can create a template using a standard set of parameters for that mode. You can select the template as the default template to load when you start the Scanner Control software. For example, if you typically scan fluorescently labeled samples, set up the scan parameters for a fluorescent scan and save the parameters as the default template. The next time you start Scanner Control, the parameters appear in the Scanner Control window and the Fluorescence Setup window.

To select a template to use as the default, choose **Set Default/Load** from the Templates menu and select the template name from the list. The template name and parameters appear in the Scanner Control window. Each time you start the Scanner Control software, the parameters for the template you selected as the default appear in the Scanner Control window. If the default template uses the fluorescence or chemiluminescence scan acquisition mode, the parameters also appear in the Setup window.

3.7.5 Deleting a template

If you want to delete a template, choose **Delete** from the Templates menu. The Delete Template(s) window appears (figure 3-12). 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, this 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** from the Templates menu 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.**

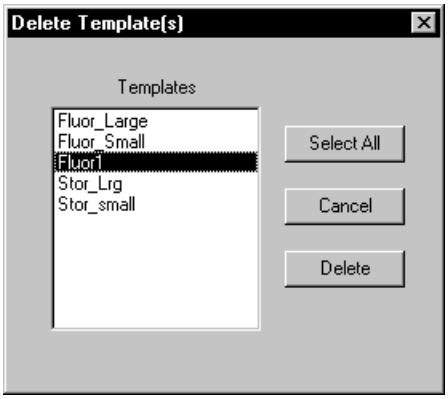


Figure 3-12. The Delete Template(s) window. **Note:** The Templates list does not include the template currently displayed in the Scanner Control window.

3.8 Closing the Scanner Control software

Although it is not necessary, you can close the Scanner Control software after you finish scanning.

Important **Before installing or removing an emission filter, you must close the Scanner Control software. See section 13.4 for more information on changing an emission filter.**

To close the Scanner Control software, choose **Exit** from the File menu.

3.9 Turning off the Typhoon instrument

Caution **Do not turn off the Typhoon instrument while the instrument is scanning. Doing so can damage the internal mechanism of the instrument.**

To turn off the Typhoon instrument, press the on/off switch on the lower right side of the Typhoon instrument (figure 3-1) to the off position. The power indicator light turns off. You can continue to use the computer after you turn off the Typhoon instrument.

(Typhoon 9400 and 9410 only) The blue laser module does not have a power switch. After you turn off the Typhoon instrument, the Connection light on the blue laser module turns off. After 10 seconds, the Laser On light turns off. The laser cooling fan continues to run for approximately 3 minutes to allow the blue laser to cool down. Approximately 5 minutes after you turn off the Typhoon instrument, the blue laser module turns off, and the Power light turns off.

3.10 Turning off the computer

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

Part two

Scanning in the storage phosphor mode

Chapter 4 About storage phosphor screen autoradiography

This chapter describes storage phosphor screen autoradiography. The topics in this chapter are—

- How the Typhoon instrument generates an image (section 4.1)
- Advantages of storage phosphor screen autoradiography (section 4.2)
- Types of storage phosphor screens (section 4.3)

4.1 How the Typhoon instrument generates an image

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

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

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 the electrons 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 Typhoon instrument scans the screen using a red light at 633 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 Typhoon 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.

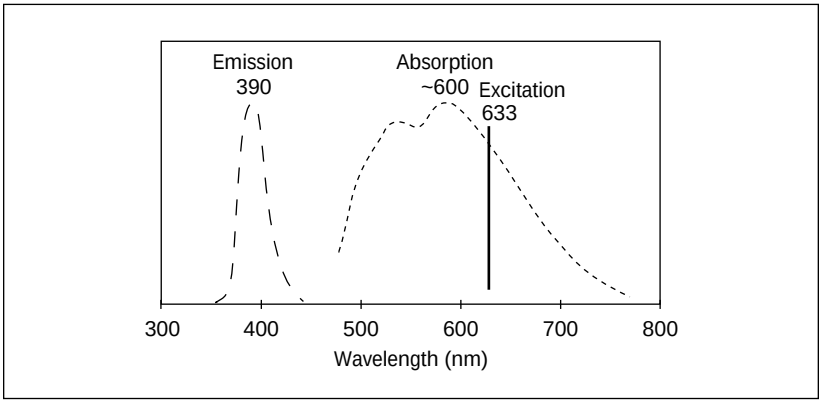


Figure 4-1. Emission (— —) and absorption (---) spectra of the activated storage phosphor screen. The wavelength with maximum stimulation or emission is shown above the curves. (These curves are approximations based on data presented in an article by Sonoda et al., 1983. See appendix C for a reference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

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). The linear dynamic range of the storage phosphor screen allows both weak and strong signals to be visualized and quantitated in a single exposure, 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 are required.
- Results are in a digital format 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 Typhoon 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 Typhoon 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 the storage phosphor screen (section 5.3)
- Guidelines for placing the sample in the exposure cassette (section 5.4)
- Placing the sample in the exposure cassette (section 5.5)
- Placing the screen in the exposure cassette (section 5.6)
- Exposing the storage phosphor screen (section 5.7)

5.1 Guidelines for preparing the samples

This section provides guidelines for preparing samples for use with the storage phosphor screen.

5.1.1 General

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™.
- If you also use the Typhoon instrument for fluorescence scanning, make sure you wear powder-free gloves when handling the samples. Always rinse the gloves thoroughly with distilled or tap water before handling the samples. Dust and powder fluoresce and scatter light, which can affect the quality of images created from fluorescent samples.

- 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 polyester film on wet samples.
- Do not use scintillants or enhancers, such as PPO or Amplify™. These products interfere with the proper function of the screen.

5.1.2 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 Using radioactive standards

By exposing your sample in conjunction with a set of radioactive standards, you can convert Typhoon 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 the 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 screens consist 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.
- Do not fold, roll, or gouge an unmounted screen.

5.2.2 Verifying that the storage phosphor screen is clean

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 14.1.**

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 screen:

- 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. Hold the screen by its edges 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.

Caution **Do not use the clip on the TR screen. The clip can damage the surface of the 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 14.2.

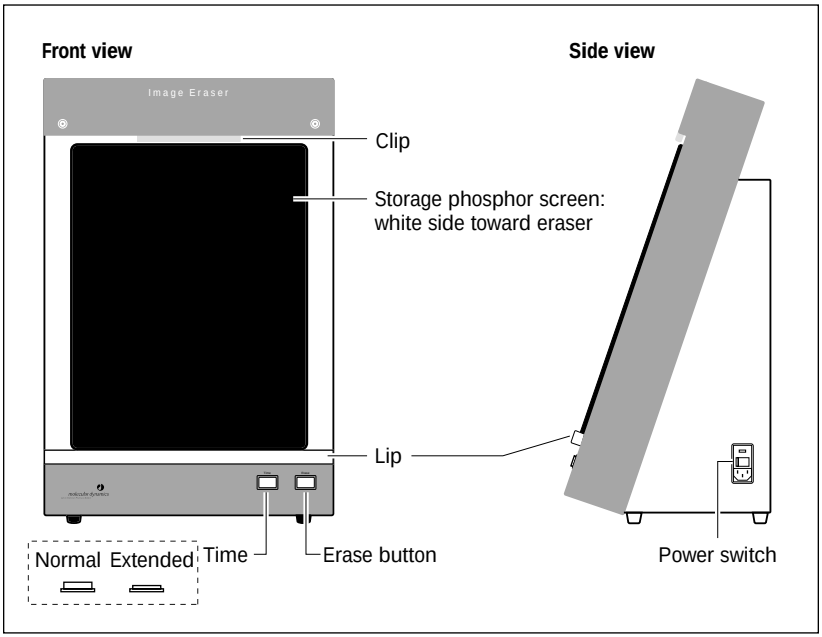


Figure 5-1. Erasing the storage phosphor screen.

5.3 Guidelines for exposing the storage phosphor screen

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 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 image. If you must adjust the position, remove the screen and erase it. Then place the screen on the sample again.**

5.4 Guidelines for placing the sample in the exposure cassette

Use the following guidelines when placing the sample in the exposure cassette:

- 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).
- To avoid possible edge effects on the image, you should place the sample at least 1 cm (0.4 in) 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 scan time—
  - Place the sample near the upper left corner of the grid (for example, start in grid square B2).
  - Position a rectangular sample so that the shortest edge is along the numbered side of the exposure cassette as shown in figure 5-2. Because each pass of the scan head moves the entire length of the glass platen (from front to back), placing the longest edge of the sample along the scan head path minimizes the scan time. The scan head path is from A to R.

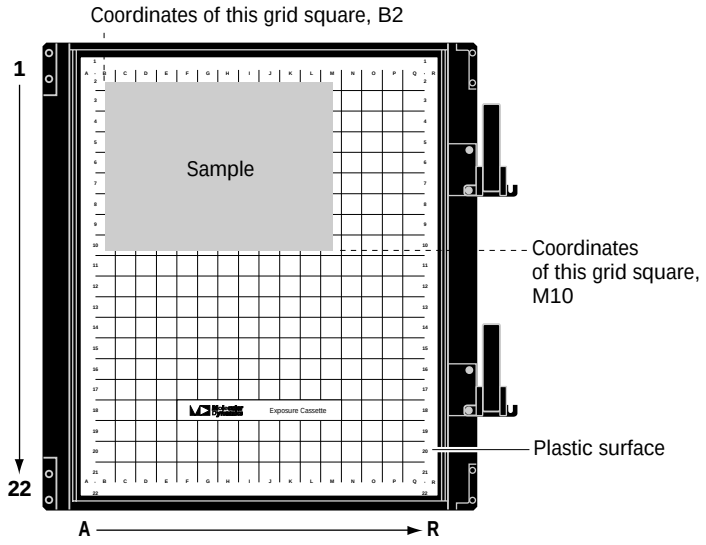


Figure 5-2. Placing the sample in the exposure cassette.

- 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.
  - (Typhoon 8610, 9210, and 9410 only) Use the 10-µm pixel size to scan smaller samples only. Because the 10-µm pixel size creates a large file, you can only scan approximately two-thirds of a large screen before you exceed the 1-GB file size limit. Scanner Control warns you if you try to select a scan area that exceeds the two-thirds limit.
- 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.

5.5 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 contamination (section 14.4).
2. Place the sample on the grid using the guidelines in section 5.4.
3. Make a note of the grid coordinates of the area you want to scan (as shown in figure 5-2). The grid coordinates in the exposure cassette also appear in the Typhoon instrument (section 6.3) and in the Scanner Control window (section 6.5.3).

5.6 Placing the screen in the exposure cassette

You should perform storage phosphor screen autoradiography at room temperature. Exposing the screen at subzero temperatures provides no 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 of the screen can result in a double image.

5.6.1 Positioning a mounted screen

To position 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 or clamps. Place the edge of the screen into the groove. The white side of the screen should be face down, toward the sample. The top of the label on the screen should be toward the top of the cassette (figure 5-3).
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 of the screen can result in a double image.

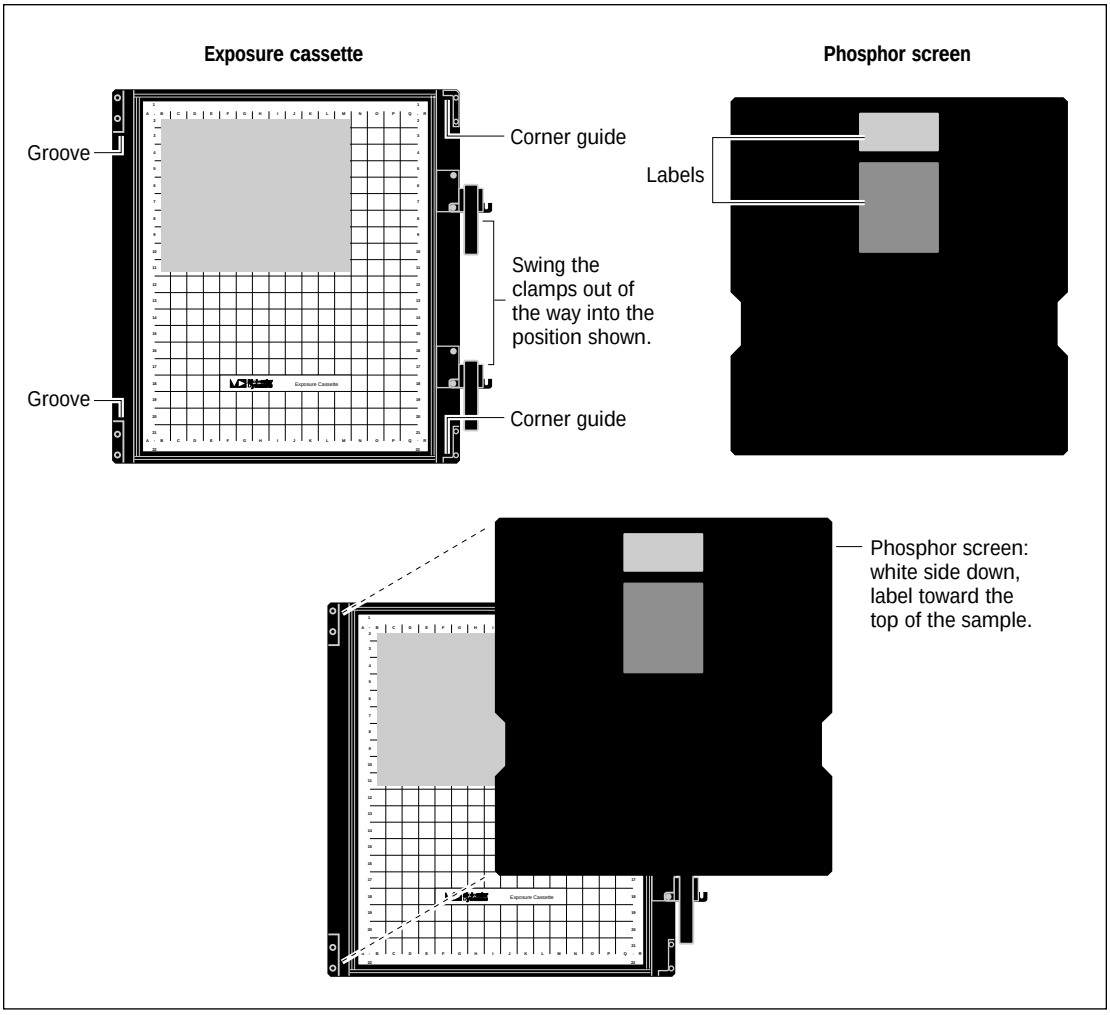
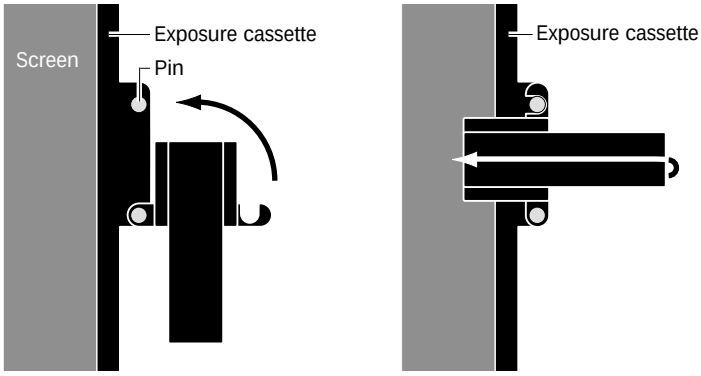


Figure 5-3. Loading the large-format mounted screen into the exposure cassette.

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.



1. Rotate the lever 90 degrees.
2. Flip the lever down over the screen to lock it into place.

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

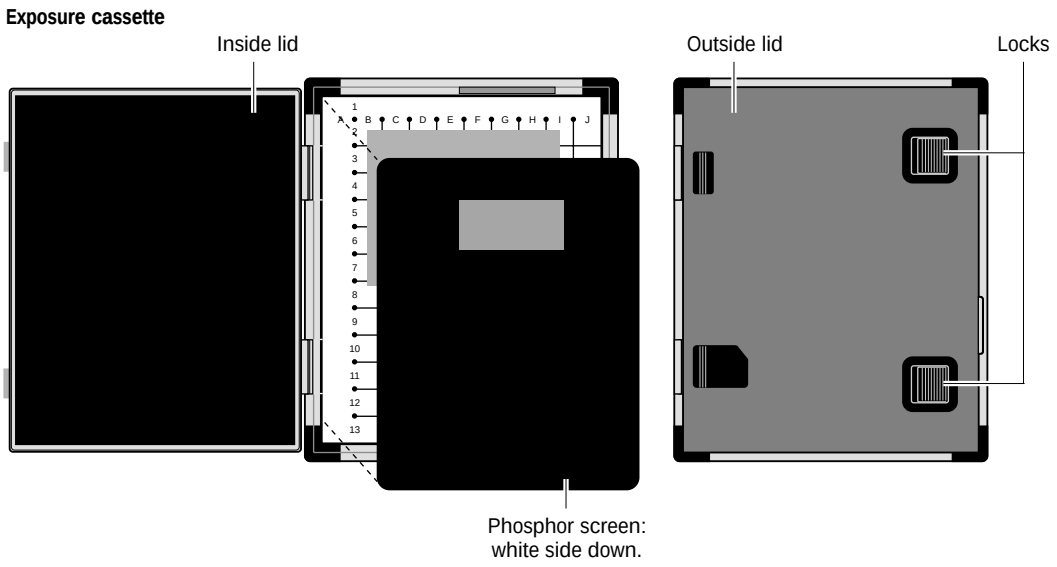
5.6.2 Positioning an unmounted screen

To position an unmounted screen in the exposure cassette—

1. Remove the screen from the Image 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 of the screen can result in a double image.**

4. Close the exposure cassette and press the lid shut until the lock or locks click.



1. Place the screen in the exposure cassette.
2. Press the lid shut until both locks click.

Figure 5-5. Loading a large unmounted screen into the exposure cassette.

5.7 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 2-hour exposure to the storage phosphor screen to be equal to an overnight exposure to basic 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 a storage phosphor screen

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

- Verifying the Typhoon instrument is clean (section 6.1)
- Removing the exposed screen from the exposure cassette (section 6.2)
- Loading the storage phosphor screen into the instrument (section 6.3)
- Checking the instrument state (section 6.4)
- Selecting the scan parameters in the Scanner Control window (section 6.5)
- Starting a 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 Typhoon instrument is clean

You should check the Typhoon 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 ImageQuant Preview 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 ImageQuant Preview 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 Typhoon instrument.
4. Leave the screen in the instrument overnight.
5. Scan the screen. If an image appears in the ImageQuant Preview 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 Typhoon 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 Typhoon instrument, make sure the gloves are powder free.**

To clean the glass platen—

1. Push up on the lid release under the center front of the sample lid until the lid opens (figure 6-1).
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.**

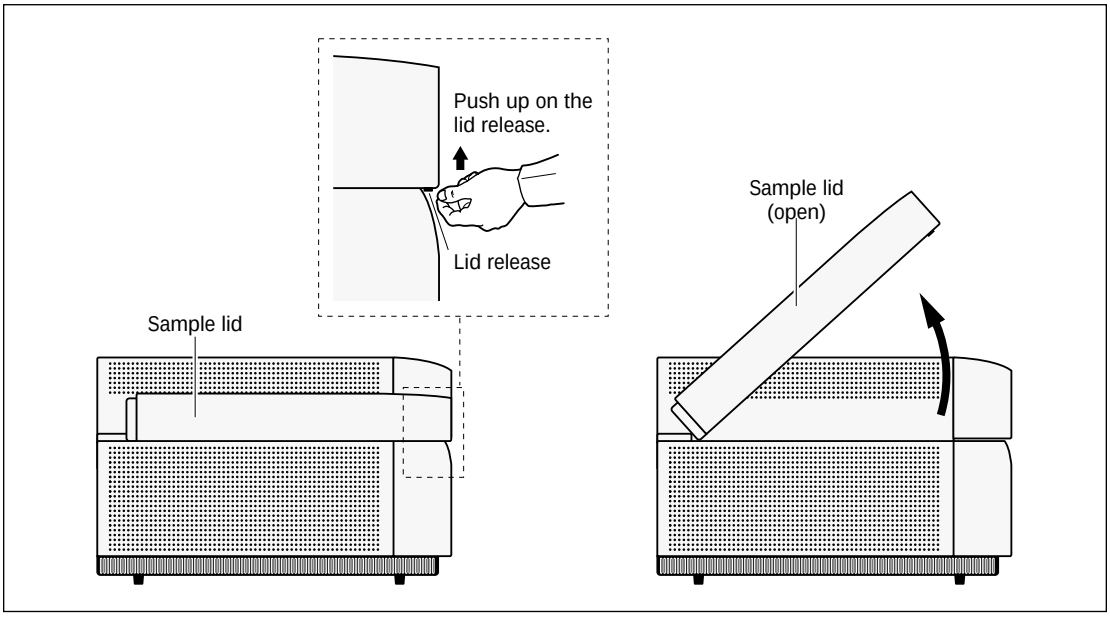


Figure 6-1. Opening the sample lid.

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.

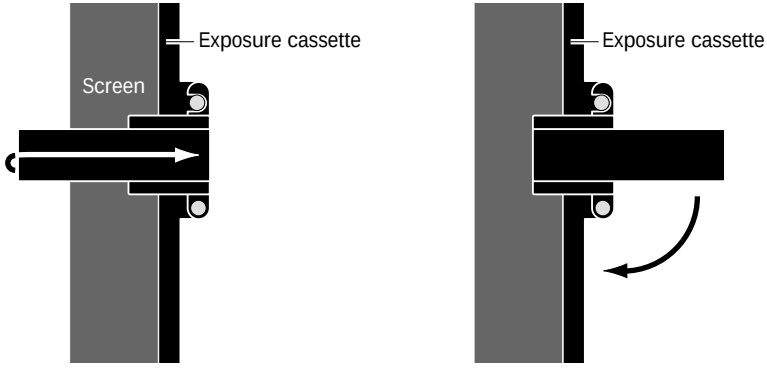
The surface of the inner lid should be clean and free of contamination from radioactive samples. If necessary, clean the surface with a damp (not saturated) cloth moistened with a small amount of distilled water. If visible spots remain, clean the surface first with 75% ethanol and then with distilled 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 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 or levers on the cassette (figure 6-2), and then rotate the clamp or clamps clockwise so that they no longer cover the screen.
   - **For unmounted screens—**Open the latch or latches and raise the lid of the cassette.



1. Flip the lever up away from the screen.
2. Rotate the lever 90 degrees.

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

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.

6.3 Loading the storage phosphor screen into the instrument

To load the storage phosphor screen into the Typhoon instrument—

1. If the sample lid is not already open, push up on the lid release under the center front of the sample lid until the lid opens (figure 6-1).
2. Make sure the white phosphor side of the screen is face down.
3. Use the grid markings that surround the glass platen to align the A1 corner of the screen with the A1 corner of the glass platen (figure 6-3).

Important

If you place a small screen so that the J1 corner of the screen aligns with the A1 corner of the glass platen, make sure you use the orientation buttons (section 6.5.4) in the Scanner Control window to rotate the scanned image.

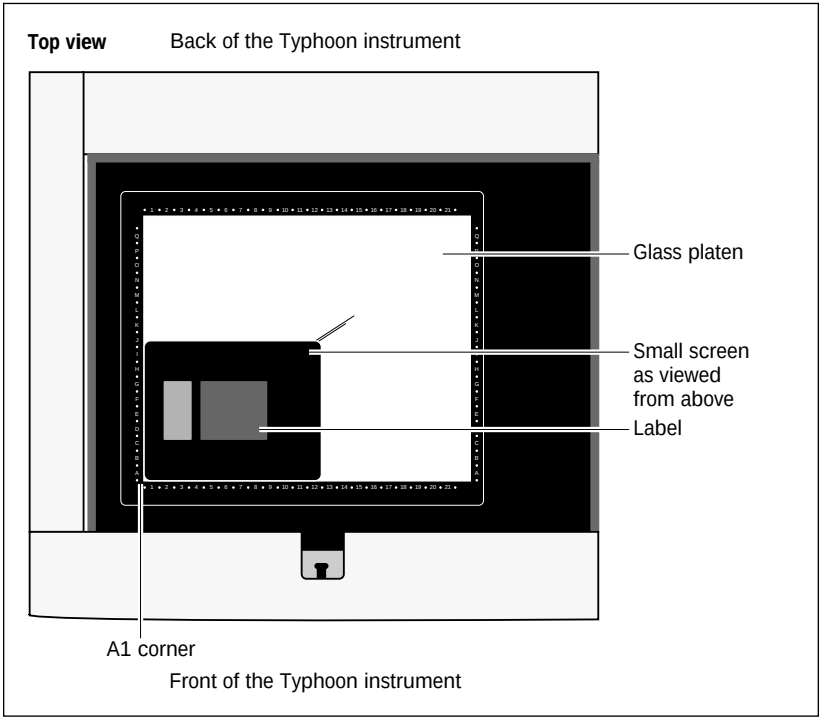


Figure 6-3. Load the screen onto the glass platen of the Typhoon instrument.

4. Gently lower the screen on the glass platen.

   Note: The unmounted screen might 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 the lid down until the latch clicks. The screen is ready to scan.

6.4 Checking the instrument state

If time is critical for your experiment, you can make sure the instrument is ready to scan the storage phosphor screen by checking the instrument state, which is displayed at the top left of the Scanner Control window (figure 6-4). If the instrument state is Ready, you can select the scan parameters, place the screen on the glass platen, and start the scan.

If the instrument is in the Sleep state, click the **Initialize Scanner** button to bring the instrument to the Ready state. The Instrument State area displays a **Warming Up** message. The warm-up period takes approximately 5 minutes. During the warm-up period, you can select the scan parameters in the Scanner Control window.

If time is not critical for your experiment, you can select the scan parameters, place the screen on the glass platen, and start the scan while the instrument is in the Sleep state. The instrument takes approximately 4 minutes during the initialization to stabilize the lasers and system components before starting the scan. The instrument takes approximately 1 additional minute to position the scan head, select the correct emission filter (if the filter is not in place), and lower the sample lid.

For more information on the instrument states, see section 3.4.

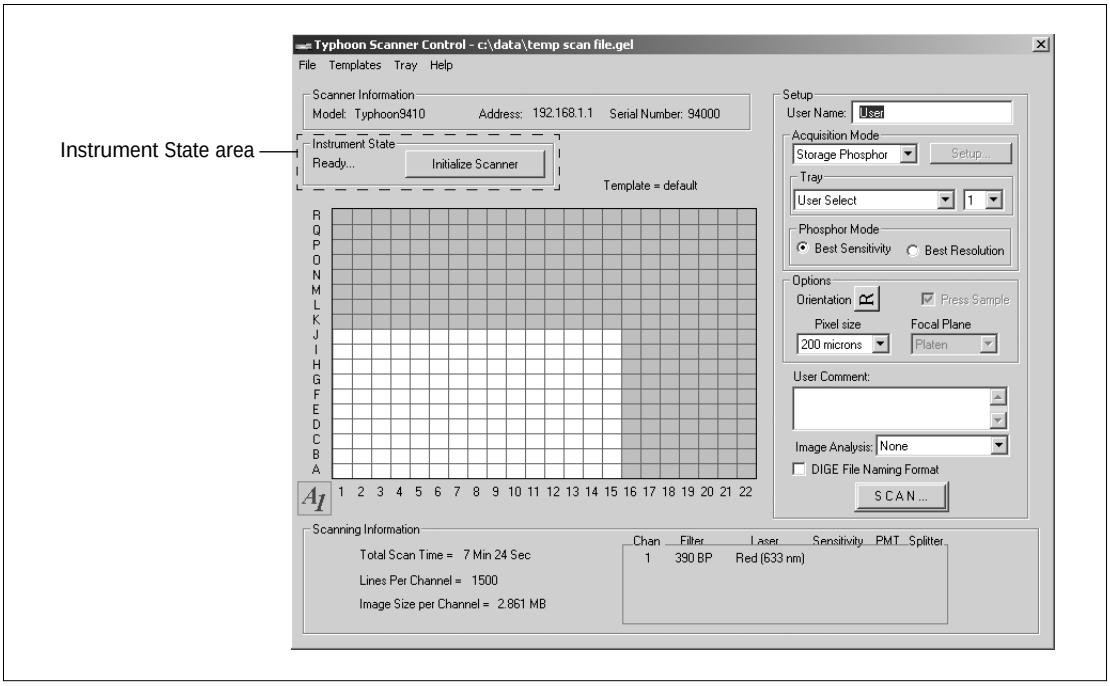


Figure 6-4. The Scanner Control window set for a storage phosphor scan.

6.5 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.

In the storage phosphor scan acquisition mode, two parameters cannot be changed. The Press Sample parameter is selected so that the screen is pressed tightly against the glass platen for scanning. The Platen parameter is selected as the Focal Plane so that the Typhoon instrument scans the area just above the glass platen.

6.5.1 Selecting a template

If a template exists with the parameters you want to use, you can select the template, review the parameters, add user comments (section 6.5.8), and then start the scan (section 6.6). For more information on creating and using templates, see section 3.7.

To select a template, choose **Load** from the Templates menu and then select the template name from the list.

If a template does not exist, select the parameters using the instructions in sections 6.5.2 through 6.5.8.

6.5.2 Selecting the storage phosphor scan acquisition mode

Make sure Storage Phosphor is the selected acquisition mode. If it is not, select **Storage Phosphor** from the Acquisition Mode list. The parameters for the storage phosphor acquisition mode appear in the Scanner Control window (figure 6-4).

6.5.3 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 Typhoon instrument. The white rectangle on the grid designates the area you want to scan. You can either use the current scan area, select a predefined scan area from the Tray list, or select a new scan area using the grid.

If you are selecting a predefined scan area from the Tray list, select the tray definition that matches how you are placing the samples on the storage phosphor screen and how you are placing the storage phosphor screen on the glass platen. The grid changes to the parameters for the tray definition you selected. If you are scanning fewer samples than defined in the tray definition, select the number in the Tray number list that corresponds to the number of samples you are scanning. The grid changes to reflect the number of scan areas you selected.

Important

In the Tray area, the User Select parameter allows you to select the scan area using the procedure below. The 1 parameter indicates that you can select only one scan area. However, if you exposed the storage phosphor screen to multiple samples, you can customize the scan area using the Tray Editor to define a separate scan area for each sample (section 3.6).

To select a new scan area using the grid—

1. In the Tray list, choose **User Select.**
2. Place the pointer in the grid square that corresponds to the lowest lettered and numbered grid square of the area you want to scan.

   For example, if you placed the upper left corner of the sample on the B2 grid square in the exposure cassette, and then aligned the A1 corner of the screen with the A1 corner of the glass platen, place the pointer on the B2 grid square.
3. Drag the pointer to the grid square that corresponds to the highest lettered and numbered grid square of the area you want to scan (figure 6-5).
4. 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.

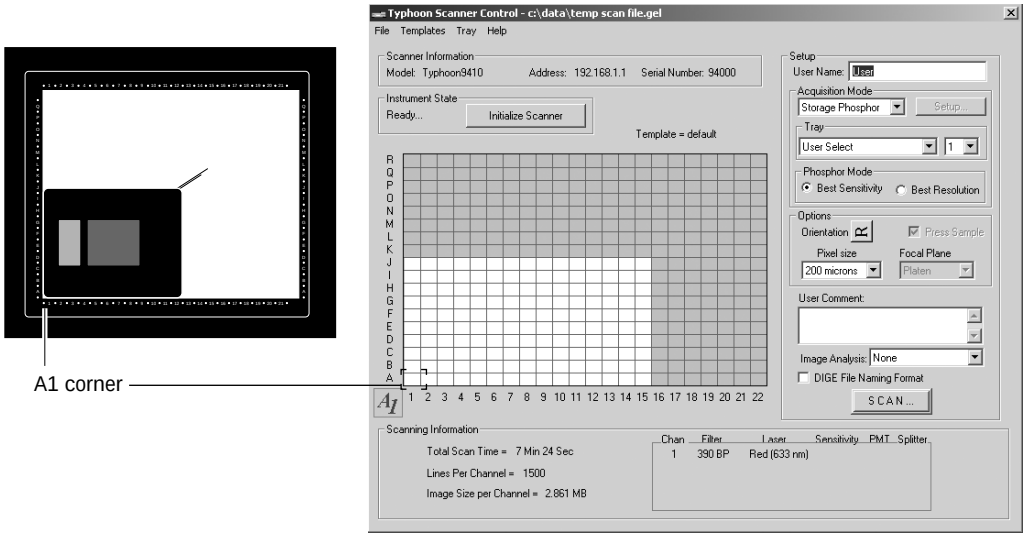


Figure 6-5. Selecting the scan area.

6.5.4 Selecting the sample orientation

You select the sample orientation from the Options area in the Scanner Control window (figure 6-4). The orientation buttons allow you to change how the scanned image will be displayed in ImageQuant. To do this, select an icon that represents how you aligned the upper left corner of the sample with the grid markings on the exposure cassette and how you aligned the A1 corner of the screen with the grid markings on the glass platen. Figure 6-6 describes each sample orientation parameter.

Important

Make sure you accurately match the orientation of the sample on the screen and the orientation of the screen on the glass platen before you select an orientation button. Depending on how you positioned the sample on the screen and how you positioned the screen on the glass platen, the top of the sample may be rotated several times.

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. Using figure 6-6 as a guide, select the orientation button that you want to use, and then release the mouse button. During the scan, the software maps the pixels to permit display of the sample image face up and top-end up in ImageQuant.

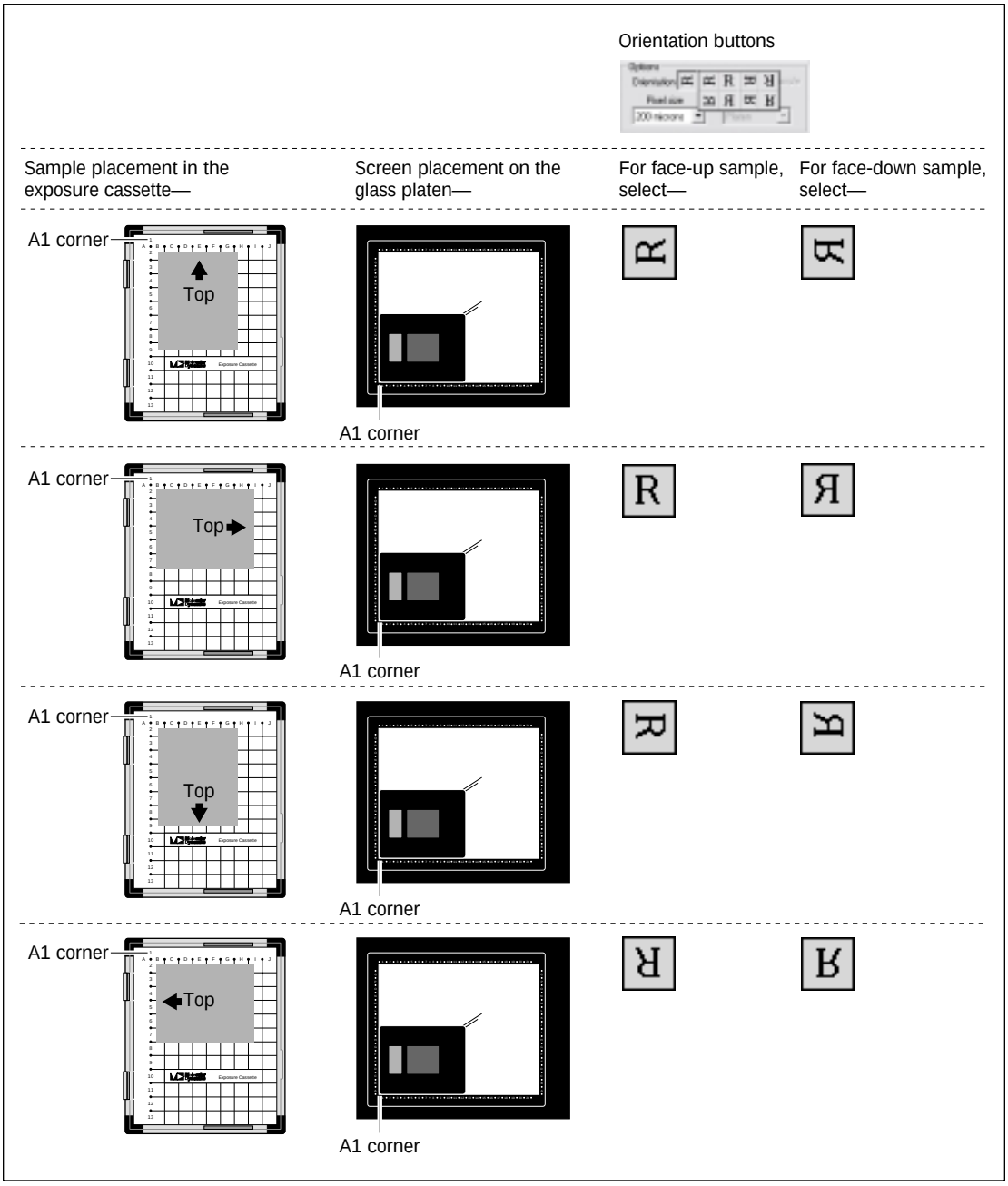


Figure 6-6. Determining which orientation button to use.

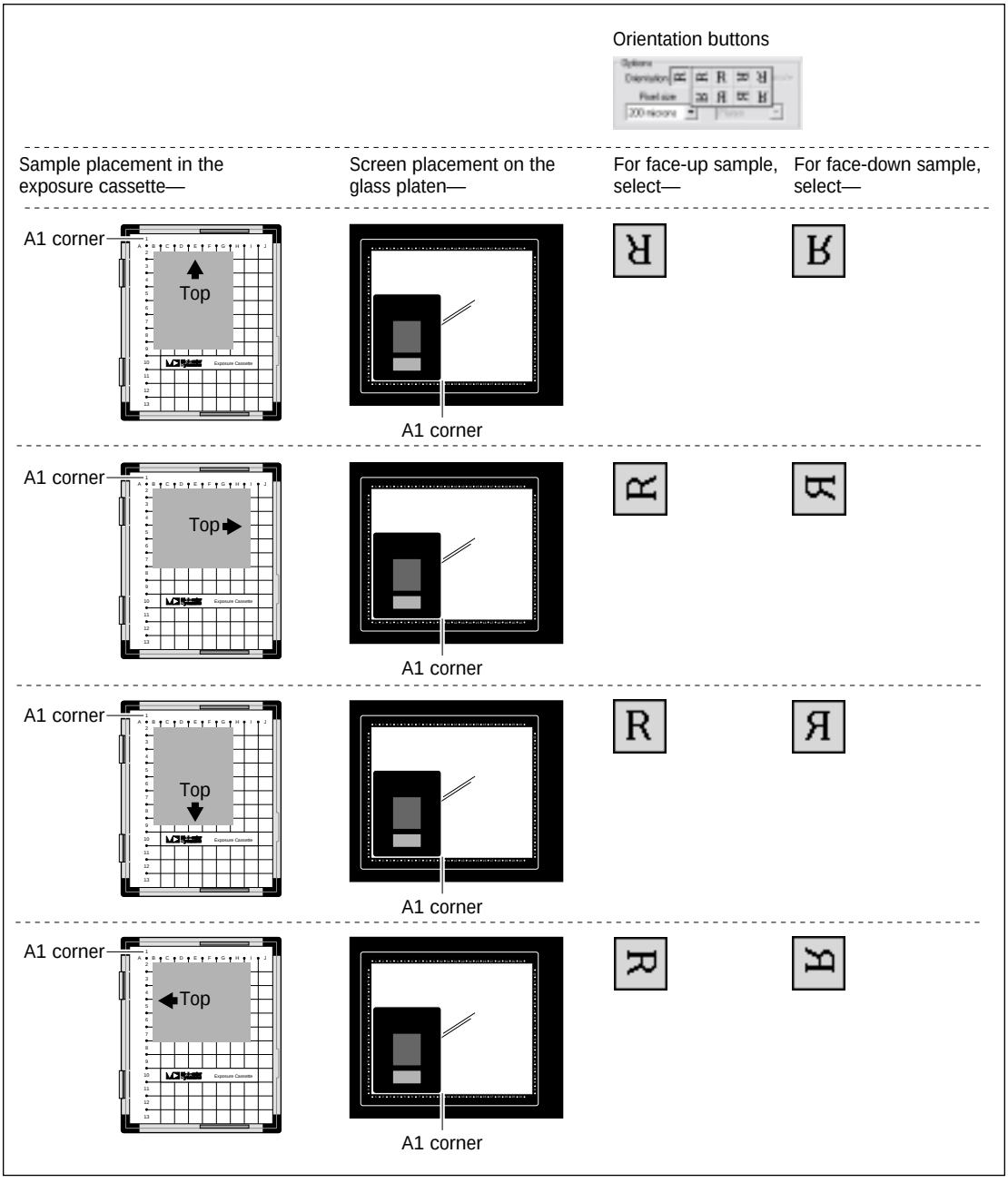


Figure 6-6. Determining which orientation button to use. (continued)

6.5.5 Selecting how to scan the sample

You select whether you want to scan the storage phosphor screen using the best resolution or the best sensitivity. You select one of the parameters from the Phosphor Mode area in the Scanner Control window (figure 6-4).

- **Best Resolution—Use this parameter if you require higher resolution than sensitivity.** The instrument lowers the laser light on the screen to resolve smaller sample features. The increase in resolution is achieved by significantly reducing the amount of laser light that is scattered by the screen. Use Best Resolution with the 50- and 25-µm pixel sizes.
- **Best Sensitivity—Use this parameter if you require higher sensitivity (limit of detection) than resolution.** The instrument increases the laser power to improve the data collection. The higher laser power releases all the stored energy in the storage phosphor screen. Use Best Sensitivity with the larger pixel sizes (above 100 µm).

6.5.6 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 select the pixel size from the Pixel Size list in the Setup area of the Scanner Control window (figure 6-4). After you change the pixel size, Scanner Control updates the image size, the scan time, and the number of data lines per channel in the Scanning Information area.

Table 6-1 lists the available pixel sizes.

Caution **You should not use the 1 000- and 500-µm pixel sizes to perform a prescan of a storage phosphor screen. Because the scanning process destroys the signal on the screen, an exposed storage phosphor screen can be scanned once per exposure.**

Table 6-1. Pixel sizes

Pixel size	Description
1 000 µm	For a very low resolution scan of the screen, select **1 000 microns.** The 1 000-µm pixel size produces 10 data points per centimeter and 20 data lines per grid square. **Note:** The 1 000-µm pixel size produces very rough data that is not recommended for quantitative analysis.
500 µm	For a low resolution scan of the screen, select **500 microns.** The 500-µm pixel size produces 20 data points per centimeter and 40 data lines per grid square.
200 µm	For most standard electrophoresis samples, select **200 microns.** The 200-µm pixel size produces 50 data points per centimeter and 100 data lines per grid square.
100 µm	For samples requiring high resolution (such as DNA sequences), select **100 microns.** The 100-µm pixel size produces 100 data points per centimeter and 200 data lines per grid square.
50 µm	For samples requiring a higher resolution (such as whole body autoradiography), select **50 microns.** The 50-µm pixel size produces 200 data points per centimeter and 400 data lines per grid square.
25 µm	For samples requiring an even higher resolution (such as high-density macroarrays), select **25 microns.** The 25-µm pixel size produces 400 data points per centimeter and 800 data lines per grid square.
10 µm (Typhoon 8610, 9210, and 9410 only)	For samples requiring the highest resolution (such as microarrays), select **10 microns.** The 10-µm pixel size produces 1 000 data points per centimeter and 2 000 data lines per grid square. **Note:** Because of file size limitation, you can only scan approximately two-thirds of the large storage phosphor screen. For more information on minimizing the file size, see section 5.4.

Important **(Typhoon 8610, 9210, and 9410 only) The resolution of a storage phosphor screen is limited by the crystalline structure of the screen. Depending on the screen, scanning at 25 µm or 10 µm might not improve the resolution of the image. If you select 25 microns or 10 microns from the Pixel Size list, make sure you select Best Resolution so that the instrument can make the best use of the smaller pixel sizes.**

6.5.7 Selecting the image analysis software

If you want to analyze the scanned image after you finish scanning, you can select the analysis software you want to use from the Image Analysis list in the Setup area of the Scanner Control window (figure 6-4). After the instrument finishes scanning, the selected software starts and displays the image.

If you select None from the Image Analysis list, the Scanner Control window remains active so that you can perform another scan.

6.5.8 Entering user comments (optional)

Important **The user comments are not saved as part of a template. If you want to include comments, you must type the comments each time you scan.**

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

6.6 Starting a 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.**

Caution **Never turn off the Typhoon instrument or disconnect the Ethernet cable while scanning. You can damage the internal mechanism of the instrument.**

Important **Keep the sample lid closed during the scan. Opening the lid causes the instrument to close the laser shutter and block the laser light. Then Scanner Control aborts the scan and saves the data already collected.**

During the scanning process, light illuminates 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) extension type.

6.6.1 Starting a scan of a single sample or scan area

If you exposed the storage phosphor screen to a single sample or included multiple samples in a single scan area, you create one image file.

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. Make sure the DIGE File Naming Format check box is not selected.
3. In the Scanner Control window, click the **Scan** button. The Save as window appears (figure 6-7).
4. In the Save as window, type a **name** in the File name box. The file type is automatically set to Data File (.gel).

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

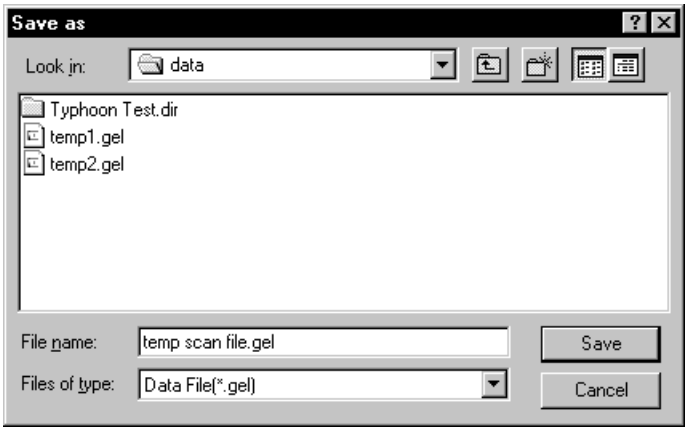


Figure 6-7. The Save as window.

5. (Optional) Select a different folder. Otherwise, the software saves the image file in the Data folder.

Caution **Using a network to save the scanned data to a folder on a remote computer can slow or delay the scan. For the best results, select a folder on the local hard drive. After you scan the screen, move the image file to the remote computer.**

6. To start the scan, click **Save.**

6.6.2 Starting a scan of multiple samples and scan areas

If you exposed the storage phosphor scan to multiple samples and you created separate scan areas for each sample, you can create a separate image file for each scan area.

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.

Important **Make sure the tray definition and number of samples in the Tray area match the positions of the samples on the storage phosphor screen.**

2. In the Scanner Control window, click the **Scan** button. The Multiple Sample Naming window appears (figure 6-8). Default file names appear for each sample.

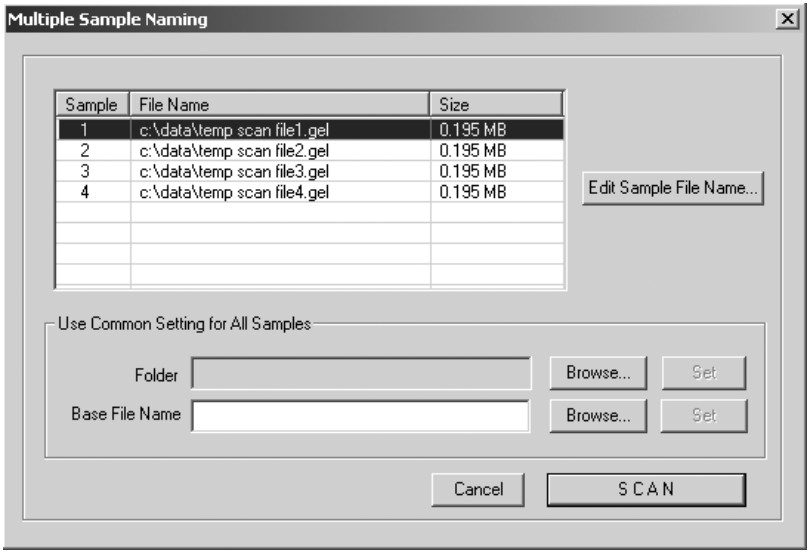


Figure 6-8. The Multiple Sample Naming window.

3. In the Use Common Setting for All Samples area, click **Browse** and locate an existing folder in the Save Folder to window. Click **OK** to close the Save Folder to window, and then click **Set** in the Multiple Sample Naming window.

Caution **Using a network to save the scanned data to a folder on a remote computer can slow or delay the scan. For the best results, select a folder on the local hard drive. After you scan the screen, move the image file to the remote computer.**

4. To name—

   - **Each file individually**—In the File Name list, click the file name that you want to change and then click **Edit Sample File Name.** The Save As window appears. Type the name you want to use in the File Name box, and click **Save** to close the window and return to the Multiple Sample Naming window. Click the next file name that you want to change and repeat the steps in this bullet.

   - **Each file using the same base file name**—In the Use Common Setting for All Samples area, type a name in the Base File Name box, and click **Set.** The software displays the base file name in the File Name list.

- **Each file using an existing base file name**—In the Use Common Setting for All Samples Area, click **Browse,** and locate the file name you want to use in the Name File window. Click **OK** to close the window, and then click **Set** in the Multiple Sample Naming window. The software displays the base file name in the File Name list.

5. Click **Scan** to start the scan.

After the scan, the software saves each image in the folder you selected.

- If you used unique names, the folder contains an image file for each file name.
- If you used a base file name, the software appends a number to each file name to make the names unique. For example, the file name for sample one will be basefilename1, where basefilename is the name you entered in the Base File Name box. All the files have the .gel file extension.

6.6.3 The initialization process

Before the Typhoon instrument begins the scan, an initialization process occurs. Depending on the state of the instrument and the scan parameters you selected in Scanner Control, the initialization can take as long as 5 minutes. A timer displays the time estimated for initialization. The timer counts down as the initialization proceeds.

The initialization procedures include—

- Moving the scan head into position
- Changing the filters to match the scan parameters
- Stabilizing the laser if the laser has been idle, shut down, or recently turned on
- Lowering the inner lid of the Typhoon instrument to hold the screen flat against the glass platen

Note: To avoid the extra 5 minutes of initialization time used to stabilize the lasers when the instrument has been in the Sleep state, click the **Initialize Scanner** button in the Scanner Control window several minutes before you plan to start the scan.

6.6.4 Canceling the scan

If you decide you need to cancel the scan, click **Cancel Scan** in the ImageQuant Preview window. Scanner Control saves the data collected before you canceled the scan.

6.6.5 Monitoring the scan progress

After you start the scan, the ImageQuant Preview window appears (figure 6-9), and the green Scan indicator light on the top of the instrument blinks. As the Typhoon instrument scans the storage phosphor screen, Scanner Control displays an image in the ImageQuant Preview 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.

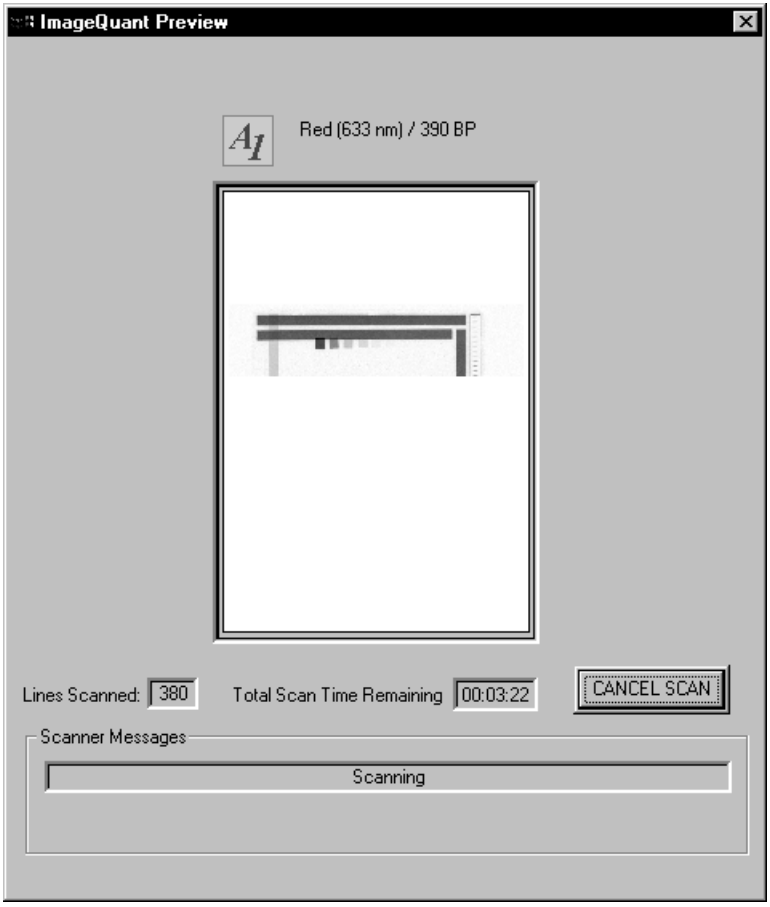


Figure 6-9. The ImageQuant Preview window during a scan of a storage phosphor screen that has one scan area defined.

A More Info button appears in the ImageQuant Preview window when the Typhoon instrument reports errors. Click the **More Info** button to view the error message.

- If the error is a nonfatal error, the instrument continues to scan, and Scanner Control saves the file when the scan is finished. You should check the image in ImageQuant to make sure the data are not corrupted.
- If the error is a fatal error, the instrument aborts the scan, and Scanner Control saves the data collected before the fatal error occurred. You should check the image in ImageQuant. Depending on where the fatal error occurred in the scan, you might be able to use the data. If you cannot use the image for quantitation, you must repeat the exposure and scan again.

If the instrument is scanning multiple scan areas, you see the Preview Sample box at the top of the window, which lists the number of scan areas. To view the progress of a different scan area, select the scan area from the list.

6.6.6 Viewing the preview image for saturated data

While the instrument is scanning, an image appears in the ImageQuant Preview window. The image shows the part of the sample that has been scanned. Saturated data appear in red in the image. If the data portions of the image appear saturated, you will not be able to perform quantitative analysis on the portions of the image that are saturated. Because saturation causes a total loss of the ability to distinguish the signal intensity fluctuations, a saturated image can be used only for viewing the shapes and positions of the spots on the sample.

Because the scanning process destroys the signal on the storage phosphor screen, you should finish the scan. If the image appears unusable, expose a clean, erased storage phosphor screen to the original sample and expose the screen for a shorter time. Alternatively, you can prepare a new sample and expose a clean, erased storage phosphor screen to the new sample.

Important **Before repeating the exposure and the scan, verify in ImageQuant that the image is saturated and that you cannot perform accurate quantitation.**

6.6.7 Completing the scan

Scanner Control saves the image using the file name you typed in the Save as or Multiple Sample Naming window. The ImageQuant Preview window displays a **Complete** message and then closes. The Scan indicator light on the Typhoon 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 Scanner Control window remains active for more scanning.

6.7 Cleaning up after the scan

After the Typhoon 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. If your screen does not have tabs or the tabs are damaged, you should replace the tabs. Contact Technical Support. See Assistance in the preface for contact information.

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 14.1).
- Check the glass platen and sample lid for contamination and clean the glass platen and sample lid of the Typhoon instrument (section 6.1).
- Check the exposure cassette for contamination and clean the exposure cassette (section 14.4).

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 an image analysis software in the Scanner Control window, the software starts and displays the image. For information about how to use the selected software, see the Help within the software or the software documentation.

Part three

Scanning in the fluorescence mode

Chapter 7 About fluorescence scanning

This chapter describes the theory behind fluorescence. The topics in this chapter are—

- How fluorescence works (section 7.1)
- Advantages of direct fluorescence (section 7.2)
- Emission filters (section 7.3)
- Beamsplitters (section 7.4)
- Linking fluorescent scans (section 7.5)
- Multilabel (multichannel) experiments (section 7.6)
- Common fluorochromes (section 7.7)

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 bulk of the fluorescent emission spectrum always shifts toward a longer wavelength (lower energy) relative to the excitation spectrum, and the small tail of the emission spectrum sometimes overlaps 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, the Typhoon instrument uses optical filtration to separate the excitation light from the longer-wavelength emitted light. Because the leading edge of the emission spectra overlaps with the tail of the excitation spectra for some dyes, the Typhoon instrument can excite dyes, such as FAM with 532 nm, and collect emitted light at 526 nm and below.

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

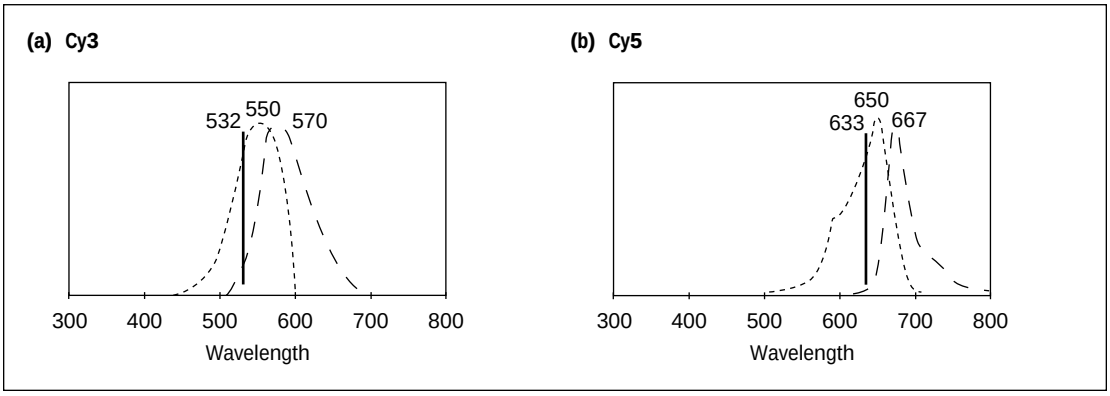


Figure 7-1. Absorption (---) and emission (— —) spectra of the fluorescent product of (a) Cy3 and of (b) Cy5. The wavelength with maximum absorption or emission is shown above the curves. The vertical line shows the excitation wavelength. (These curves are approximations based on data collected at Amersham Biosciences.)

7.2 Advantages of direct fluorescence

When you use the Typhoon system to scan samples labeled or stained with dyes excitable by one or more of the laser wavelengths, the results appear in a digital image format. These results can be analyzed qualitatively and quantitatively using ImageQuant.

Scanning direct fluorescence on the Typhoon system offers several advantages—

- Nucleic acid and protein gels can be analyzed shortly after electrophoresis. Soak the gels in a dye solution and rinse away the excess dye as you would for the well-known ethidium bromide and Coomassie™ protocols. You can use some of these same gels for follow-on blot experiments.
- You can analyze fluorescently labeled samples that can be detected directly and do not require staining.
- 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 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 Typhoon system provides a linear response to the fluorescent signal intensities.

7.3 Emission filters

The Typhoon instrument contains a standard set of emission filters and can accommodate a total of 14 emission filters. The instrument uses emission filters to reject reflected and scattered excitation light and background fluorescence while allowing the emitted light from the sample to pass through to the PMT. Table 7-1 lists the standard set of emission filters used with fluorescence scanning.

Table 7-1. Standard set of emission filters

Emission filter	Description
520-nm band-pass filter (520 BP 40) (Typhoon 9400 and 9410 only)	Transmits light between 500 nm and 540 nm and has a transmission peak centered at 520 nm. Use this filter with fluorochromes, such as Cy2 and ECL Plus™, or with fluorescein when scanning with the 488-nm wavelength of the blue laser.
555-nm band-pass filter (555 BP 20)	Transmits light between 545 nm and 565 nm and has a transmission peak centered at 555 nm. Use this filter with fluorochromes, such as R6G and HEX.
580-nm band-pass filter (580 BP 30)	Transmits light between 565 nm and 595 nm and has a transmission peak centered at 580 nm. Use this filter with fluorochromes, such as TAMRA and Cy3.
610-nm band-pass filter (610 BP 30)	Transmits light between 595 nm and 625 nm and has a transmission peak centered at 610 nm. Use this filter with fluorochromes, such as ROX, EtBr, SYPRO™ Red, and SYPRO Ruby.
670-nm band-pass filter (670 BP 30)	Transmits light between 655 nm and 685 nm and has a transmission peak centered at 670 nm. Use this filter with fluorochromes, such as Cy5.
526-nm short-pass filter	Transmits light below 526 nm. Use this filter with fluorochromes, such as fluorescein when scanning with the green laser. **Note:** When using the blue laser, the 520 BP 40 might provide better results.
560-nm long-pass filter	Transmits light above 560 nm. Use this filter with fluorochromes, such as TRITC.
390-nm band-pass filter	This filter is used for storage phosphor scanning and is not normally used for fluorescence scanning.

For more information on emission filters, see the Amersham Biosciences publication *Fluorescence Imaging principles and methods*. (See appendix C for the references.)

You use the Fluorescence Setup window in the Scanner Control software to select the appropriate filter (section 9.3). Sections 13.4 and 13.5 describe how to install and remove an emission (optical) filter.

7.4 Beamsplitters

The Typhoon instrument contains three standard beamsplitters and has a slot for one additional custom beamsplitter. You can use the beamsplitters with multilabel fluorescent samples to create multichannel images. Table 7-2 lists the three standard beamsplitters.

Table 7-2. Standard beamsplitters

Beamsplitter	Description
560-nm dichroic	Reflects light of wavelengths shorter than 560 nm and passes light longer than 560 nm.
580-nm dichroic	Reflects light of wavelengths shorter than 580 nm and passes light longer than 580 nm.
630-nm dichroic	Reflects light of wavelengths shorter than 630 nm and passes light longer than 630 nm.

7.5 Linking fluorescent scans

The instrument uses one beamsplitter and two emission filters when you link two scans together in the Fluorescence Setup window in the Scanner Control software (section 9.3.7). You link scans that contain similar scan parameters so that you can acquire signals from two different fluorochromes during one scan. This optimizes scanning of samples containing multiple fluorochromes by reducing the scan time.

Similar to a long-pass emission filter, the beamsplitter separates the emissions from the linked scans so that one PMT collects the longer wavelength emissions while the other PMT collects the shorter wavelength emissions (figure 7-2).

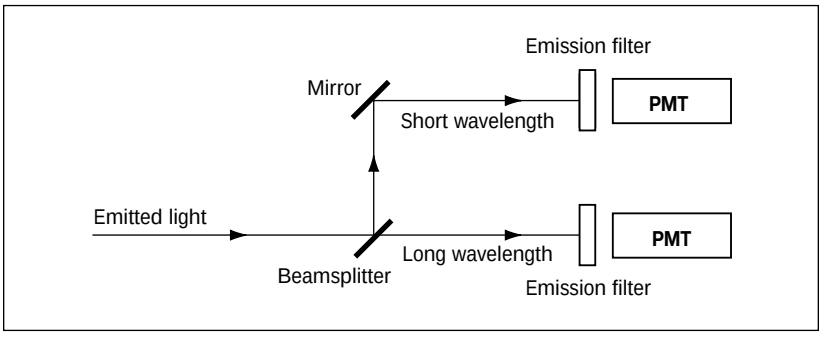


Figure 7-2. How the beamsplitter separates short- and long-wavelength emissions.

Scanner Control determines that two scans can be linked if—

- The scans use the same laser, or they combine the red laser with the green laser.
- The scans use different emission filters or at least one uses no emission filter.
- The scans use the same sensitivity parameter.
- The wavelength of one of the beamsplitters is between the wavelengths of the two emission filters, and the wavelength of the beamsplitter is at least 5 nm from each filter. The filter value is determined as the center wavelength for a band-pass filter, or the cutoff wavelength for a short-pass or a long-pass filter.

Based on the selected emission filters and laser(s), Scanner Control selects the appropriate beamsplitter to use with the linked scans. The possible links appear in the Fluorescence Setup window (figure 7-3). You select whether or not to activate the link.

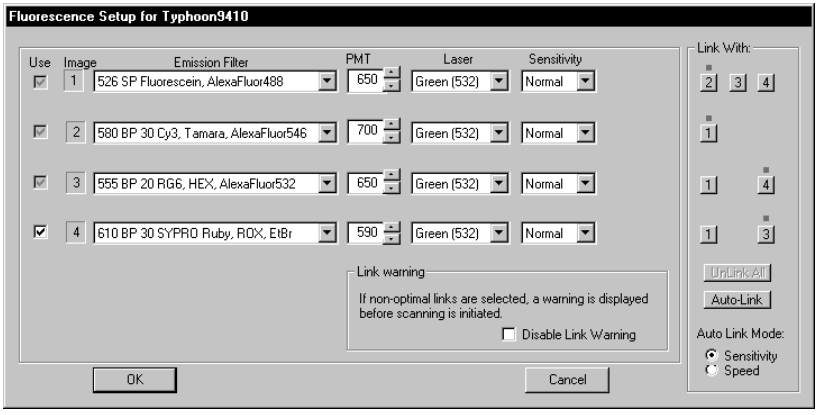


Figure 7-3. Fluorescence Setup window showing possible scans that can be linked.

You can link a red laser scan with the green laser scan. Linking the two scans decreases the scan time because the scans are performed simultaneously. However, the image sensitivity can degrade slightly because the second laser can increase the background of the first sample. This degradation varies based on the sample type and media, and you might see no degradation at all. After you evaluate the needs of the experiment, you might need to make a trade-off between scan time and sensitivity.

(Typhoon 9400 and 9410 only) You cannot link a blue laser scan with a green or red laser scan. You cannot link the 457-nm wavelength and the 488-nm wavelength of the blue laser.

7.6 Multilabel (multichannel) experiments

You can label the sample with multiple fluorescent dyes and then create a multichannel image. A multichannel image is a set of images (channels) that can be viewed individually or as a composite (overlaid). To provide accurate detection and separation of the signals, you label the sample with two, three, or four fluorochromes that can be collected and distinguished from each other using the available filters. You include a spectral separation standard for each fluorochrome in the sample to assist in reducing cross talk (cross-contamination) between the fluorescent emissions.

After the scan, you use the FluorSep™ software to remove the cross talk and create a new image for each fluorochrome in the sample. The multichannel image can be viewed in ImageQuant. In ImageQuant, you can view the set of images (channels) overlaid in different colors, or you can view the channels side-by-side in gray scale. For information on removing cross talk using FluorSep, see the FluorSep Help or user documentation. For information on viewing images in ImageQuant, see the ImageQuant Help or user documentation.

7.7 Common fluorochromes

Table 7-3 lists the absorption and emission maxima of some common fluorochromes. The chemistry of the experiment can alter the absorption and emission spectra slightly.

For more information on selecting fluorochromes and for additional fluorochrome recommendations, see the Amersham Biosciences publication *Fluorescence Imaging principles and methods*. (See appendix C for the references.)

Table 7-3. Fluorochrome absorption and emission maxima*

Fluorochrome	Absorption maximum (nm)	Emission maximum (nm)
Acridine Orange-dsDNA	502	526
BODIPY™ 493/503	493	503
BODIPY FL	503	512
Cy2	489	506
Cy3	550	570

Table 7-3. Fluorochrome absorption and emission maxima* (continued)

Fluorochrome	Absorption maximum (nm)	Emission maximum (nm)
Cy5	649	670
DDAO-Phosphate	646	660
ECF™ Substrate	440	560
ECL Plus	420	460
Ethidium Bromide (EtBr)	526	610
FAM	495	535
Fluorescein (FITC)	490	520
HEX	529	560
NanoOrange™	485	590
Phycoerythrin (R)	480	578
PicoGreen™-dsDNA	480	520
Rhodamine-6-G (R6G)	546	560
RiboGreen™	500	520
ROX	590	605
SYBR™ Green I-dsDNA	497	520
SYBR Green II	497	513
SYBR Gold	495	537
SYPRO Orange	472	570
SYPRO Red	530	625
SYPRO Ruby	450	610
TAMRA	555	580
TET	519	545
Texas Red™	595	615
TOTO™-1-dsDNA	513	532
TRITC	547	572
Vistra Green™	490	520

* 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 Typhoon instrument. The topics in this chapter are—

- Preparing the sample (section 8.1)
- Cleaning the glass platen and sample lid (section 8.2)
- Determining how to position the sample (section 8.3)
- Placing a gel or membrane on the glass platen (section 8.4)
- Placing a sandwich gel on the glass platen (section 8.5)
- Placing the slide holder and microarray slides on the glass platen (Typhoon 8610, 9210, and 9410 only) (section 8.6)
- Placing the gel alignment guide and multiple sandwich gels on the glass platen (section 8.7)

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 (such as bromophenol blue or xylene cyanol). Whenever possible, put the tracking dye in a separate lane. Alternatively, use a dye in the sample loading buffer that is either nonfluorescent or does not enter the gel.
- **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:

- **Green-excited fluorescence—**Detects samples labeled with fluorochromes that are excited at 532 nm.
- **Red-excited fluorescence—**Detects samples labeled with fluorochromes that are excited at 633 nm.
- **(Typhoon 9400 and 9410 only) Blue-excited fluorescence—**Detects samples labeled with fluorochromes that are excited at 457 or 488 nm. **Note:** The laser power at 457 nm is much lower than the laser power at 488 nm.
- **Multilabel fluorescence—**Detects samples labeled with two, three, or four fluorochromes and creates a multichannel image. To minimize the spectral overlap from the fluorochromes, the emission peaks should be greater than 30 nm apart. Use one or more lasers and up to four different emission filters to create the multichannel image. The multichannel image contains one image for each fluorochrome. Using ImageQuant, you can view these images separately or overlaid.

Note: The instrument contains a built-in filter for the red and green lasers. The built-in filter blocks the laser light at 532 nm ±8 nm or 635 nm ±10 nm. For the Typhoon 9400 and 9410 instruments, an additional built-in filter blocks all laser light below 500 nm. The Typhoon system automatically selects the appropriate built-in filter based on the color of the laser you selected. The built-in filters cannot be used simultaneously.

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.

- **Agarose—**FMC SeaKem™ Gold and LE have 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 the 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. If you poured the gel into a plastic tray, remove the gel from the tray before placing the gel on the glass platen of the Typhoon instrument.

  If the agarose gel containing the sample is more than 4-mm thick, scan the sample using the +3 mm focal plane parameter in the Scanner Control window (section 9.2.7). If the agarose gel is less than 4-mm thick, scan the sample using the Platen focal plane parameter. Check the results in ImageQuant to make sure the selected focal plane provides the best limit of detection and spatial resolution.

- **Polyacrylamide**—Polyacrylamide gels are usually clear and thin (less than 1 mm). The background contribution from the gel material is generally very low. Make sure your electrophoresis 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.

Electrophoresis glass plates

When using electrophoresis glass plates with the Typhoon instrument, make sure that—

- The glass has a low-fluorescence background
- The glass is clean before preparing a sandwich gel
- The sandwich gel does not leak

Important **Make sure the electrophoresis glass plate you place on the glass platen is 3 mm thick. The +3 mm focal plane parameter is designed for 3-mm-thick glass. Make sure both the electrophoresis glass plates of a sandwich gel have a low-fluorescence background.**

Gel alignment guides

The gel alignment guides contain spacers that raise the gels 0.2 mm above the glass platen, which prevents optical interference and eliminates the need to use distilled water or buffer on the glass platen. When selecting the Scanner Control parameters, make sure you select the +3 mm parameter for the focal plane, the Press Sample check box, and the correct tray definition.

Membranes

Membranes that generate lower background fluorescence generally offer more optimal imaging results. The background fluorescence of the membranes depends on the experimental conditions. You should scan a test piece of each membrane you plan to use and check that the background is low enough for your purposes. Before scanning the membrane, treat the membrane using the same procedure you will be using in the experiment.

Microarray slide and slide holder (Typhoon 8610, 9210, and 9410 only)

Make sure you use the microarray slide holder when scanning microarray slides. When selecting the Scanner Control parameters—

- Select **MicroArray 2 Slides** in the Tray list.

  Note: If the MicroArray 2 Slides tray definition is not available and you are scanning one slide, select A2 through D2 for the scan area. If you are scanning two slides, select A2 through J2 for the scan area. If you want to create two scan areas, see section 3.6 to create two scan areas—A2 through D2 and F2 through J2.

- Select **Fluorescence** for the acquisition mode.
- Select **+3 mm** for the focal plane.
- Select **10 microns** for the pixel size.
- Select (Я) for the orientation.
- Select the **Press Sample** check box.

Microplates

Because the bottoms of the wells in most microplates raise the sample at least 2 mm above the glass platen, you should use the +3 mm focal plane parameter in the Scanner Control window (section 9.2.7). You might want to scan a test microplate and check the results in ImageQuant to make sure the selected focal plane provides the best limit of detection and spatial resolution. In addition, check the background fluorescence of the microplate to make sure it is within an acceptable range. To prevent the microplate from moving during the scan, make sure you select the Press Sample parameter in the Scanner Control window.

Microplate strips

Scan a test microplate strip using the +3 mm focal plane parameter in the Scanner Control window (section 9.2.7). Check the results in ImageQuant to make sure the selected focal plane provides the best limit of detection and spatial resolution. In addition, check the background fluorescence of the microplate strip to make sure it is within an acceptable range.

Transparency support

To avoid contaminating the glass platen and sample lid, you can place a sample, such as a membrane, in a plastic bag, between two pieces of transparency material, or between sheet protectors (section 8.4). You should check the background generated by the material to make sure it is low enough for your purposes.

Solvents

Spectroscopic-grade solvents have the lowest autofluorescence.

8.2 Cleaning the glass platen and sample lid

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

Important

If you use the Typhoon instrument for storage phosphor scanning, you should periodically check the instrument for contamination from radioactive samples (section 6.1.1).

8.2.1 Cleaning the glass platen

The glass platen of the Typhoon 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.

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 powder-free gloves.**

To clean the glass platen—

1. Push up on the lid release under the center front of the sample lid until the lid opens (figure 8-1).

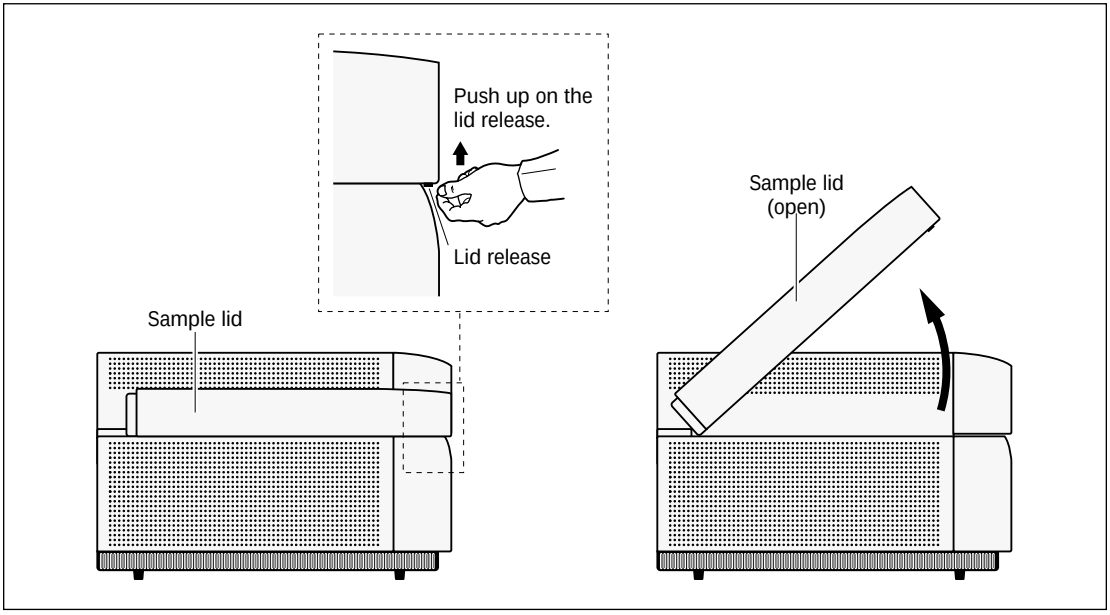


Figure 8-1. Opening the sample lid.

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 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.**

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 Determining how to position the sample

Before you load the sample, you should determine how to position it on the glass platen. Use the following guidelines to determine the sample orientation and the sample placement.

8.3.1 Determining the sample orientation

Determine whether to place the sample face up or face down on the glass platen. The Typhoon 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 sandwich gel, make sure you create the sandwich gel using a 3-mm electrophoresis glass plate. Place the 3-mm electrophoresis glass plate on the glass platen of the instrument.
- As a reference point for the sample orientation, you can cut a corner of the gel or sample.
- If the sample is physically uneven on one side, such as an agarose gel, place the flat side down. This allows the sample to lie flat on the glass platen.
- For a microplate or microplate strip, place the plate or strip on the glass platen so that the edges of the plate or strip align with the grid markings on the glass platen.
- For a microarray slide, place the slide or slides in the microarray slide holder so that the spotted side is face down toward the glass platen.
- For multiple gels, use the gel loading guides to place up to two large gels side-by-side or up to four smaller gels in a 2-by-2 grid.

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 scan time—
  - Place the sample near the A1 corner of the grid. Each lettered or numbered grid segment is marked by two dots.
  - Position a rectangular sample so that the shortest edge is along the numbered side of the glass platen as shown in figure 8-2. Because each pass of the scan head moves the entire length of the glass platen (from front to back), placing the longest edge of the sample along the scan head path minimizes the scan time.
  - (Typhoon 8610, 9210, and 9410 only) If you are scanning only one microarray slide, position the slide in the first well.
- To minimize the image file size—
  - 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.
  - (Typhoon 8610, 9210, and 9410 only) Use the 10-µm pixel size to scan smaller samples and microarray slides. Because the 10-µm pixel size creates a large file, you can scan approximately two-thirds of the total scan area before you exceed the 1-GB file size limit for a single-channel image or for each channel of an unlinked multichannel image. For a linked multichannel image, each channel cannot exceed 400 MB. Scanner Control warns you if you try to select a scan area that exceeds the file size limit. If the scan area exceeds the limit, you can scan the sample twice using two different scan areas.
- To record multiple samples during the same scan without using the gel alignment guides, 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.

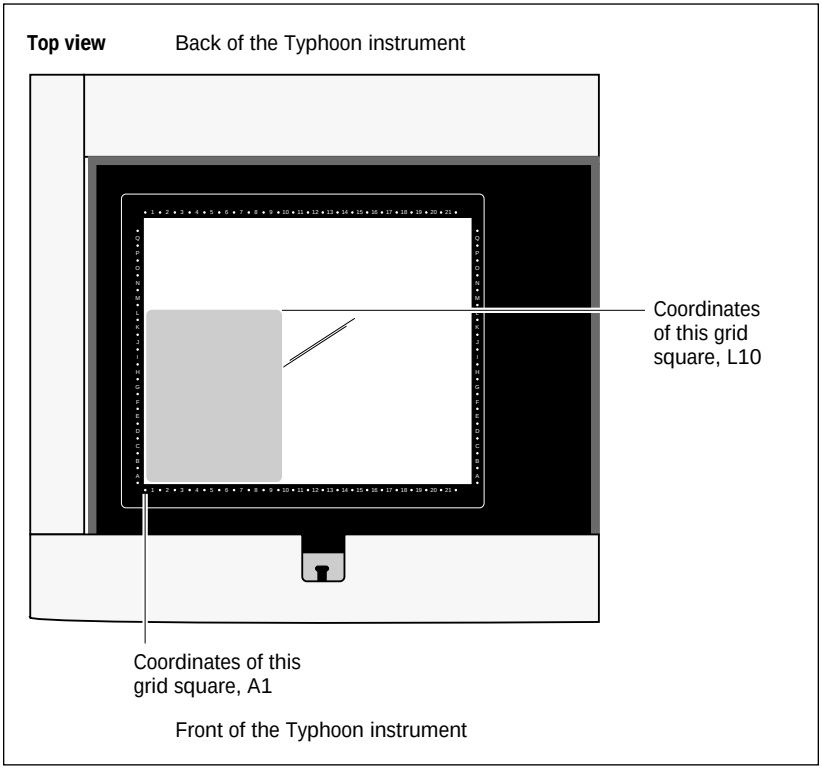


Figure 8-2. Sample placement on the glass platen of the Typhoon instrument.

8.4 Placing a gel or membrane on the glass platen

Caution **If you place the sample directly on the glass platen, make sure you place the sample in the correct position when you first set it down. Although unlikely, any fluorescent material left on the glass after you move a sample can result in a double image.**

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 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 lessons the chance of trapping air bubbles between the sample and the glass platen.

Caution **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.**

To place a gel or membrane on the glass platen of the Typhoon instrument—

1. If the sample lid is not already open, push up on the lid release under the center front of the lid until the lid opens (figure 8-1).
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 platen starting at the A1 corner. Do not trap air bubbles under the sample or scratch the glass. Cover a membrane with a glass plate to keep it flat.

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

4. Make a note of the grid square coordinates of the area you want to scan (figure 8-2). You will use this information later in the Scanner Control window (section 9.2.3).
5. Close the sample lid and press the lid down until the latch clicks. The sample is ready to scan.

8.5 Placing a sandwich gel on the glass platen

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.**

You can use Kapton™ tape to help prevent an optical interaction between the glass platen and the 3-mm electrophoresis glass plate. The Kapton tape prevents the two pieces of glass from coming in contact with each other, which can cause optical interference patterns or rings to appear on the image. Before assembling the sandwich gel, place one strip of Kapton tape on each long side of the 3-mm electrophoresis glass plate. Make sure each piece of Kapton tape covers at least 75% of the long edge of the plate.

To place a sandwich gel on the glass platen of the Typhoon instrument—

1. If the sample lid is not already open, push up on the lid release under the center front of the lid until the lid opens (figure 8-1).
2. Hold the sandwich gel by the edges and make sure the 3-mm electrophoresis glass plate edged with Kapton tape is on the bottom.
3. Place one edge of the sandwich gel on the glass platen of the instrument (figure 8-3).
4. Squirt distilled water on the glass platen. The distilled water increases the sensitivity of the scan by filling the space created by the Kapton tape with water. In addition, the water helps provide a continuous interface for the laser light to pass through on its way to the sample.
5. Lower the sandwich gel onto the glass platen. Make sure you do not trap air bubbles as you lower the sandwich gel onto the glass platen.

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

6. When you can no longer lower the sandwich gel using your fingers, insert the Wonder Wedge™ tool between the glass platen and the 3-mm electrophoresis glass plate.
7. Lower the sandwich gel onto the Wonder Wedge, and then slowly remove the Wonder Wedge. Make sure you do not trap air bubbles between the glass platen and the 3-mm electrophoresis glass plate. Make sure the sandwich gel is aligned on the glass platen.
8. Make a note of the grid square coordinates that are covered by the area of the sample you want to scan (figure 8-2). You will use this information later in the Scanner Control window (section 9.2.3).
9. Close the sample lid and press the lid down until the latch clicks. The sample is ready to scan.

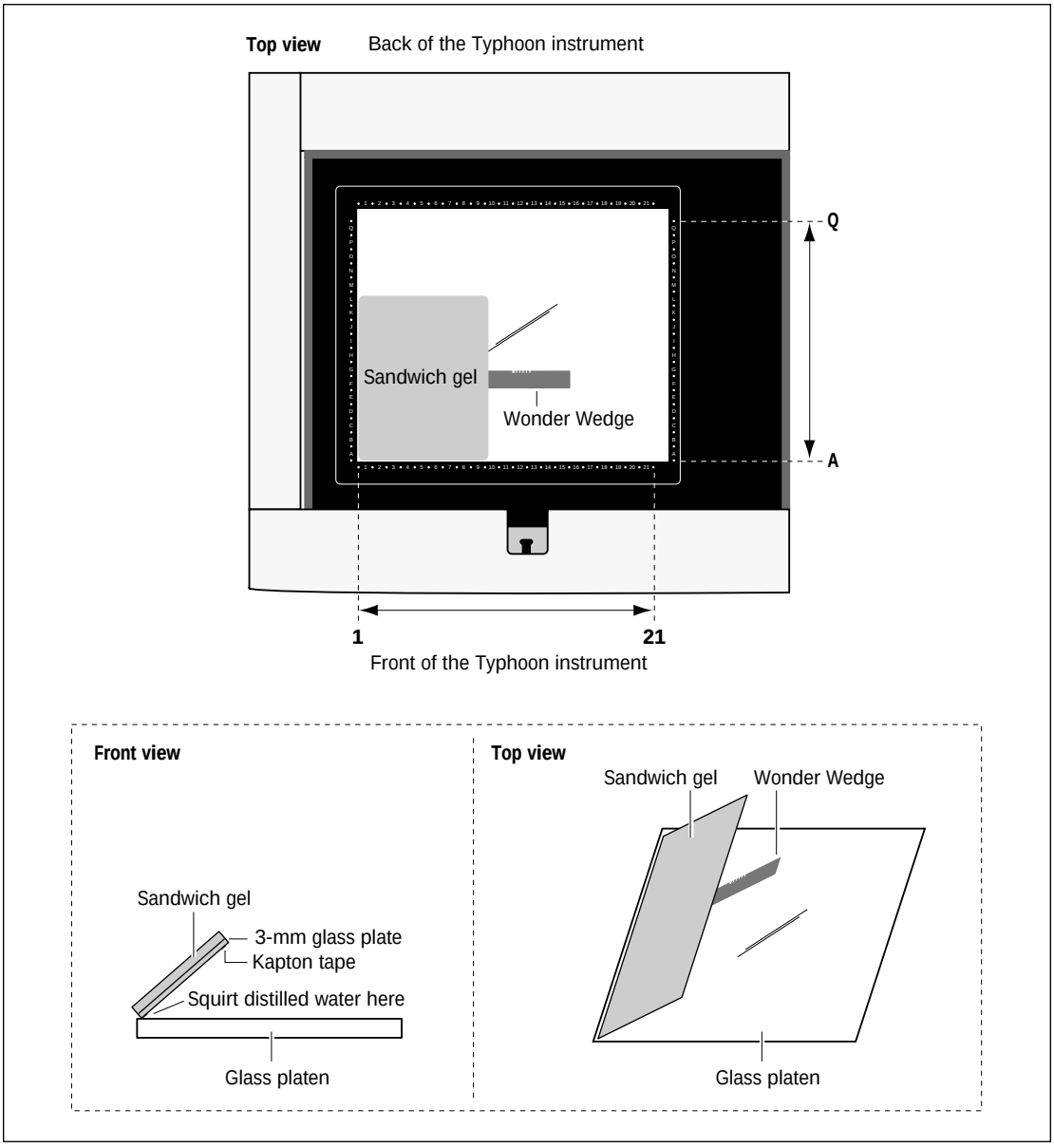


Figure 8-3. Aligning a sandwich gel on the glass platen of the Typhoon instrument.

8.6 Placing the slide holder and microarray slides on the glass platen (Typhoon 8610, 9210, and 9410 only)

To scan microarray slides on the Typhoon instrument, you should use the Microarray Slide Holder Kit (optional accessory). For more information on the kit, contact Technical Support. See Assistance in the preface for contact information.

The slide holder allows you to scan one or two microarray slides. The slide holder positions the slides at the proper height above the glass platen so that the instrument can accurately collect data.

Caution **To prevent anomalies in the image data, make sure the glass platen of the Typhoon instrument, the microarray slide holder, and the slides are clean. Remove any liquid marks, fingerprints, or dust particles from the back of the slides before scanning.**

To position the slide holder and microarray slides on the glass platen—

1. If the sample lid is not already open, push up on the lid release under the center front of the lid until the lid opens (figure 8-1).
2. Moisten a lint-free cloth with distilled water and clean the slide holder.
3. Gently position the slide holder at the front left corner of the glass platen as shown in figure 8-4.

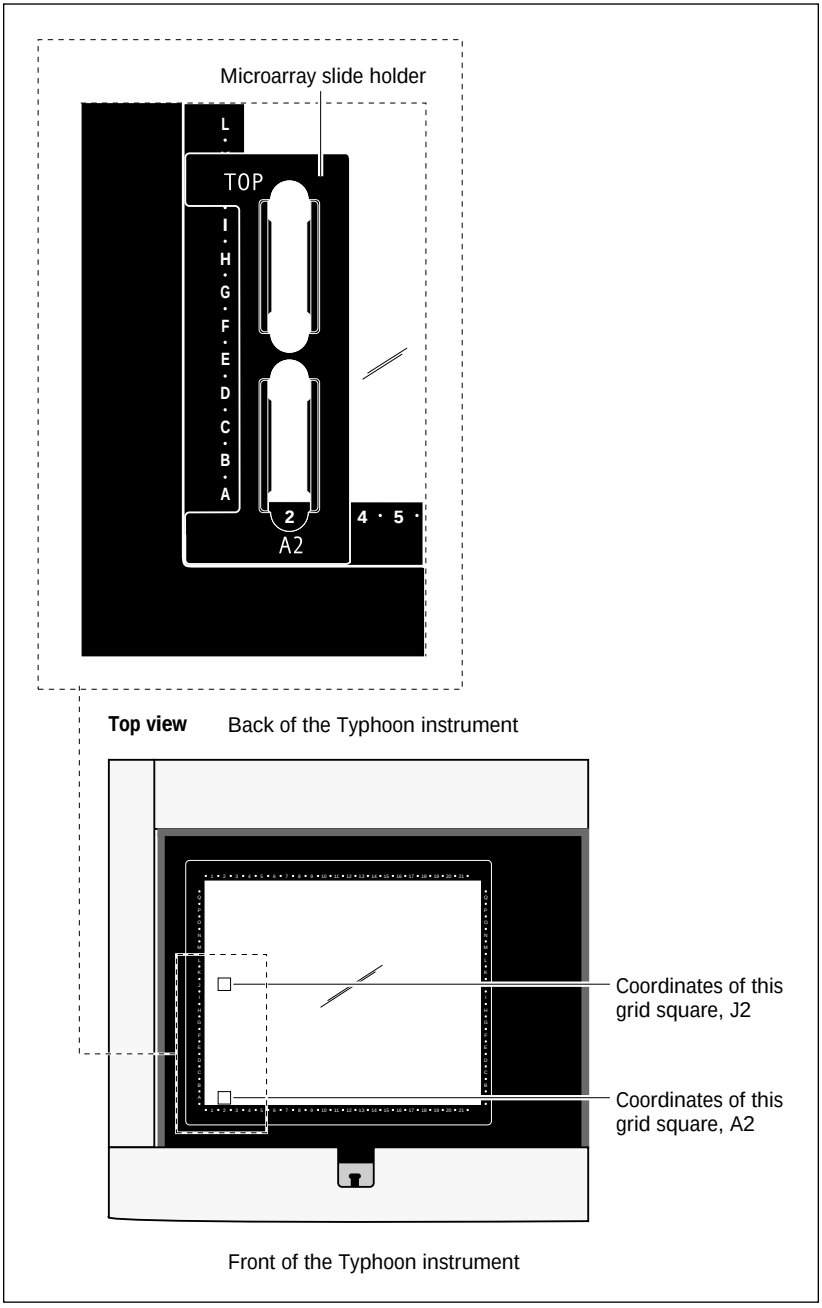


Figure 8-4. Positioning the microarray slide holder on the glass platen.

4. Holding the slide with the spotted side face down, carefully lower the slide into the first well on the slide holder as figure 8-5 shows. (If you are scanning only one slide, positioning the slide in the first well minimizes the scan time.)

   Note: Alternatively, you can place the slides in the slide holder first, and then position the slide holder on the glass platen. However, this method increases the risk of dropping the slides.

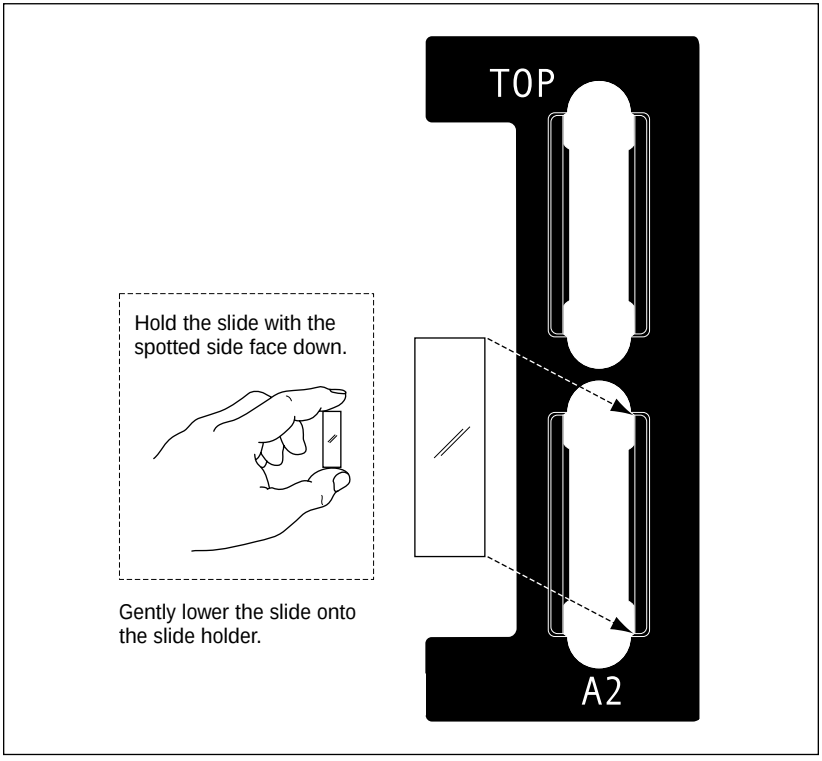


Figure 8-5. Positioning the slides in the slide holder.

5. (Optional) If you are scanning two slides, repeat step 4 to position the slide in the second well on the slide holder.

6. Make sure the slides are seated in the grooves of the slide holder and make sure the slide holder is seated firmly against the front and left metal sides of the platen area as shown in figures 8-4 and 8-5. The positions of the slides and the slide holder keep the slides at the proper angle and location for accurate scanning.

7. Place a slide restraint over each slide as shown in figure 8-6. Make sure each restraint is correctly positioned in the grooves on the slide holder.
8. Close the sample lid and press the lid down until the latch clicks. The microarray slides are ready to scan.

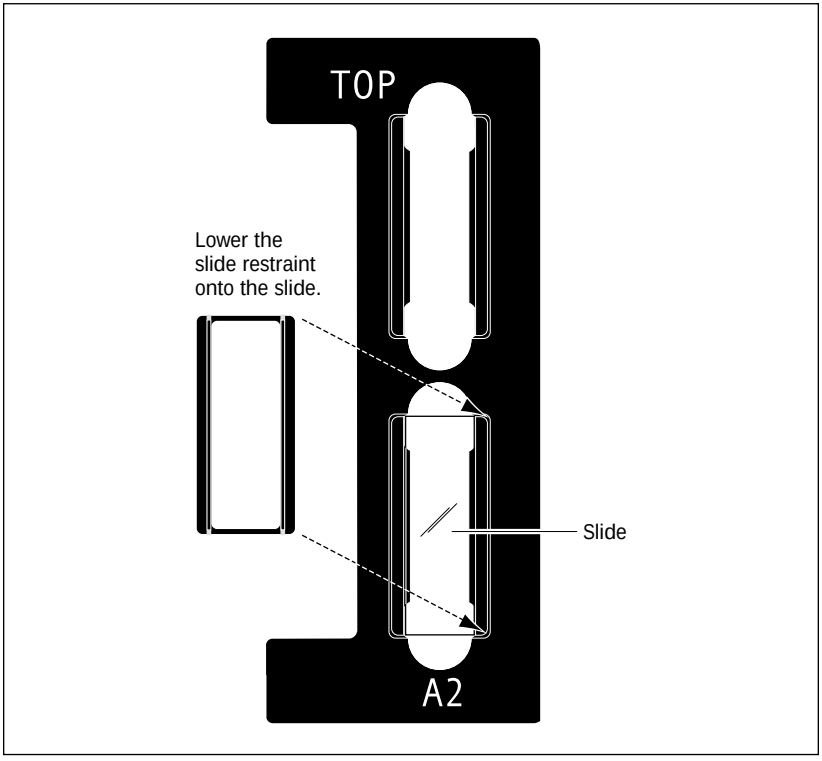


Figure 8-6. Positioning the slide restraint on the slide holder.

8.7 Placing the gel alignment guide and multiple sandwich gels on the glass platen

To scan multiple sandwich gels on the Typhoon instrument, you should use the gel alignment guides (optional accessory). For more information on the guides, contact Technical Support. See Assistance in the preface for contact information.

The gel alignment guides allows you to scan up to two large or four small sandwich gels. The gel alignment guides position the sandwich gels at the proper height above the glass platen so that the instrument can accurately collect data.

Important **Make sure the electrophoresis glass plate you use for the bottom of the sandwich gel is 3 mm thick and has a low-fluorescence background.**

Caution **To prevent anomalies in the image data, wipe any excess liquid from the sandwich gel with a lint-free cloth before scanning.**

Warning **When placing the sandwich gel on the gel alignment guide and glass platen, make sure you hold the sandwich gel with one hand on the gripper and the other hand on the edge of the sandwich gel. Holding the sandwich gel by the gripper only can cause the sandwich gel to fall out of the gripper and onto the glass platen, which could break the electrophoresis glass plates or the glass platen.**

8.7.1 Loading one or two large sandwich gels

To scan one or two large sandwich gels, you use the Ettan DALT gel alignment guide and two grippers (one for each sandwich gel). The large sandwich gels can be up to 21 cm by 27.5 cm (8.2 in by 10.7 in).

Important **You do not need to squirt distilled water or buffer on the glass platen when you use the gel alignment guide. The spacers on the guide and grippers hold the sandwich gel 0.2 mm above the glass platen, which prevents optical interference.**

To position the Ettan DALT gel alignment guide and one or two large sandwich gels on the glass platen—

1. If the Typhoon lid is not already open, push up on the lid release under the center front of the lid until the lid opens (figure 8-1).
2. Moisten a lint-free cloth with distilled water and clean the Ettan DALT gel alignment guide.
3. Place the guide along the front edge of the glass platen as shown in figure 8-7.

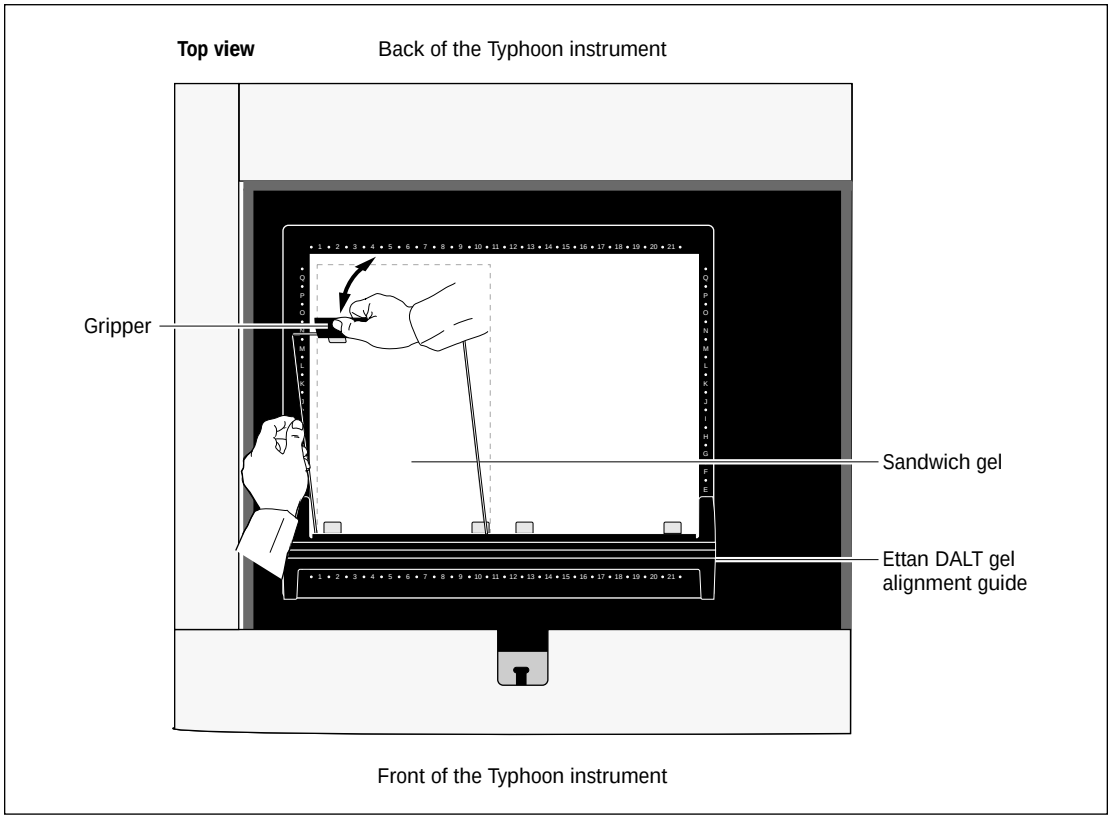


Figure 8-7. Placing the Ettan DALT gel alignment guide and sandwich gel with attached gripper on the glass platen.

4. Attach the gripper to one end of the prepared sandwich gel so that the spacers are on the side of the sandwich gel that you will place on the glass platen.
5. Holding the sandwich gel by the gripper and one edge, place the edge of the sandwich gel without the gripper on the edge of the guide (figure 8-7). Make sure the sandwich gel rests on the two spacers.
6. Lower the sandwich gel and gripper onto the glass platen.
7. (Optional) Repeat steps 4 through 6 for the second sandwich gel.
8. Close the Typhoon lid and press the lid down until the latch clicks. The sandwich gels are ready to scan.

8.7.2 Loading one through four small sandwich gels

To scan one through four small sandwich gels, you use the SE600 gel alignment guide and four grippers (one for each sandwich gel). The small sandwich gels can be up to 16 cm by 18 cm (6 in by 7 in).

Important

You do not need to squirt distilled water or buffer on the glass platen when you use the gel alignment guide. The spacers on the guide and grippers hold the sandwich gel 0.2 mm above the glass platen, which prevents optical interference.

To position the SE600 gel alignment guide and up to four small sandwich gels on the glass platen—

1. If the sample lid is not already open, push up on the lid release under the center front of the lid until the lid opens (figure 8-1).
2. Moisten a lint-free cloth with distilled water and clean the three pieces of the SE600 gel alignment guide.
3. Place the front SE600 guide along the front edge of the glass platen as shown in figure 8-8.
4. Place the back SE600 guide along the back edge of the glass platen as shown in figure 8-8.
5. Place one end of the center SE600 guide in the groove on the back SE600 guide, and place the other end in the groove on the front SE600 guide as shown in figure 8-8.

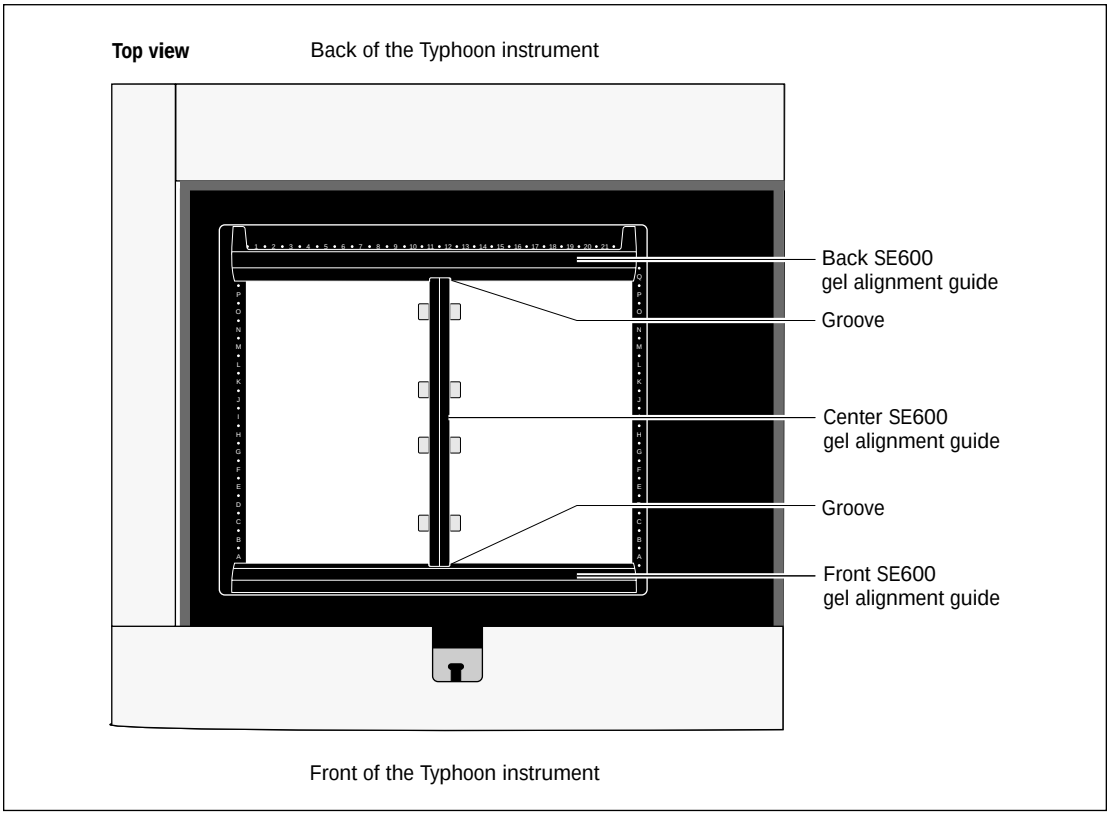


Figure 8-8. Placing the three pieces of the SE600 gel alignment guide on the glass platen.

6. Attach the gripper to one end of the prepared sandwich gel so that the spacers are on the side of the sandwich gel that you will place on the glass platen.
7. Holding the sandwich gel by the gripper and one edge, place the edge of the sandwich gel without the gripper on the edge of the center gel alignment guide (figure 8-9). Make sure the sandwich gel rests on the two spacers.
8. Lower the sandwich gel and gripper onto the glass platen.
9. (Optional) Repeat steps 6 through 8 for the remaining sandwich gels.
10. Close the sample lid and press the lid down until the latch clicks. The sandwich gels are ready to scan.

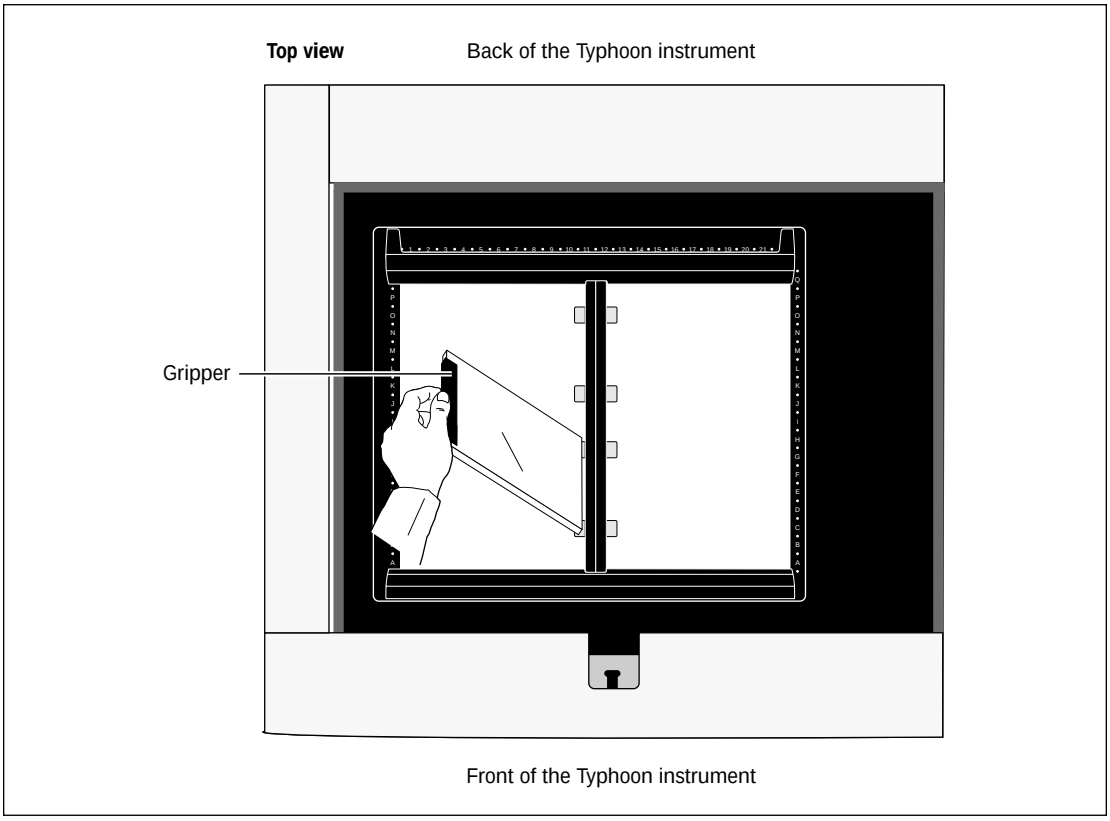


Figure 8-9. Placing a sandwich gel with attached gripper on the SE600 gel alignment guide.

Warning **When placing the sandwich gel on the gel alignment guide and glass platen, make sure you hold the sandwich gel with one hand on the gripper and the other hand on the edge of the sandwich gel. Holding the sandwich gel by the gripper only can cause the sandwich gel to fall out of the gripper and onto the glass platen, which could break the electrophoresis glass plates or the glass platen.**

Chapter 9 Scanning a fluorescent sample

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

- Checking the instrument state (section 9.1)
- Selecting the scan parameters in the Scanner Control window (section 9.2)
- Using the Setup window to select the fluorescence parameters (section 9.3)
- Starting a scan and checking the progress (section 9.4)
- Removing the sample from the Typhoon 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 Checking the instrument state

If time is critical for your experiment, you can make sure the instrument is ready to scan the fluorescent sample by checking the instrument state, which is displayed at the top left of the Scanner Control window (figure 9-1). If the instrument state is Ready, you can select the scan parameters for your scan, place the sample on the glass platen, and start the scan.

If the instrument is in the Sleep state, click the **Initialize Scanner** button to bring the instrument to the Ready state. The Instrument State area displays a **Warming Up** message. The warm-up period takes approximately 5 minutes. During the warm-up period, you can select the scan parameters in the Scanner Control window.

If time is not critical for your experiment, you can select the scan parameters, place the sample on the glass platen, and start the scan while the instrument is in the Sleep state. The instrument takes approximately 4 minutes during initialization to stabilize the lasers and system components before starting the scan. The instrument takes approximately 1 additional minute to position the scan head, select the correct emission filter or filters, and lower the sample lid (if selected).

For more information on instrument states, see section 3.4.

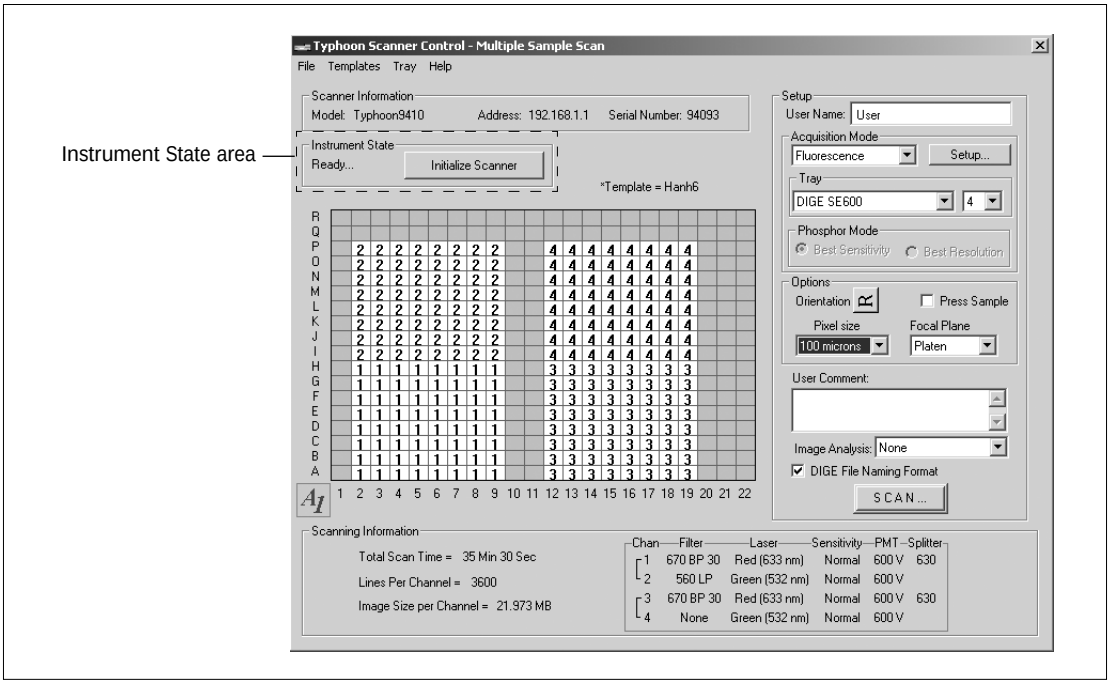


Figure 9-1. The Scanner Control window set for a fluorescence scan.

9.2 Selecting the scan parameters in the Scanner Control window

You use the Scanner Control window (figure 9-1) to select the scan parameters you want to use when you scan the fluorescent sample. If the Scanner Control window is not open, see section 3.3.

9.2.1 Selecting a template

If a template exists with the parameters you want to use, you can select the template, review the parameters, add user comments (section 9.2.9), and then start the scan (section 9.4). For more information on creating and using templates, see section 3.7.

To select a template, choose **Load** from the Templates menu and then select the template name from the list.

If a template does not exist, select the parameters using the instructions in sections 9.2.2 through 9.3.8.

9.2.2 Selecting the fluorescence scan acquisition mode

Make sure Fluorescence is the selected acquisition mode. If it is not, select **Fluorescence** from the Acquisition Mode list. The parameters for the fluorescence scan acquisition mode appear in the Scanner Control window (figure 9-1) and in the additional Fluorescence Setup window.

9.2.3 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 Typhoon instrument. The white rectangle on the grid designates the area you want to scan. You can either use the current scan area, select a predefined scan area from the Tray list, or select a new scan area using the grid.

If you are selecting a predefined scan area from the Tray list, select the tray definition that matches how you are placing the samples on the glass platen. The grid changes to the parameters for the tray definition you selected. If you are scanning fewer samples than defined in the tray definition, select the number in the Tray number list that corresponds to the number of samples you are scanning. The grid changes to reflect the number of scan areas you selected.

Important

In the Tray area, the User Select parameter allows you to select the scan area using the procedure below. The 1 parameter indicates that you have selected only one scan area. However, if you are scanning multiple samples, you can customize the scan area using the Tray Editor to define a separate scan area for each sample (section 3.6).

To select a new scan area using the grid—

1. In the Tray list, choose **User Select.**
2. Place the pointer in the grid square that corresponds to the lowest lettered and numbered grid square of the area you want to scan (figure 9-2). For example, grid square A1 is the lowest lettered and numbered grid square on the glass platen.
3. Drag the pointer to the grid square that corresponds to the highest lettered and numbered grid square of the area you want to scan. For example, R22 is the highest lettered and numbered grid square on the glass platen.
4. Release the mouse button. The scan area you selected appears in white. The Scanning Information area displays the image file size, the approximate scan time, and the number of lines per channel.

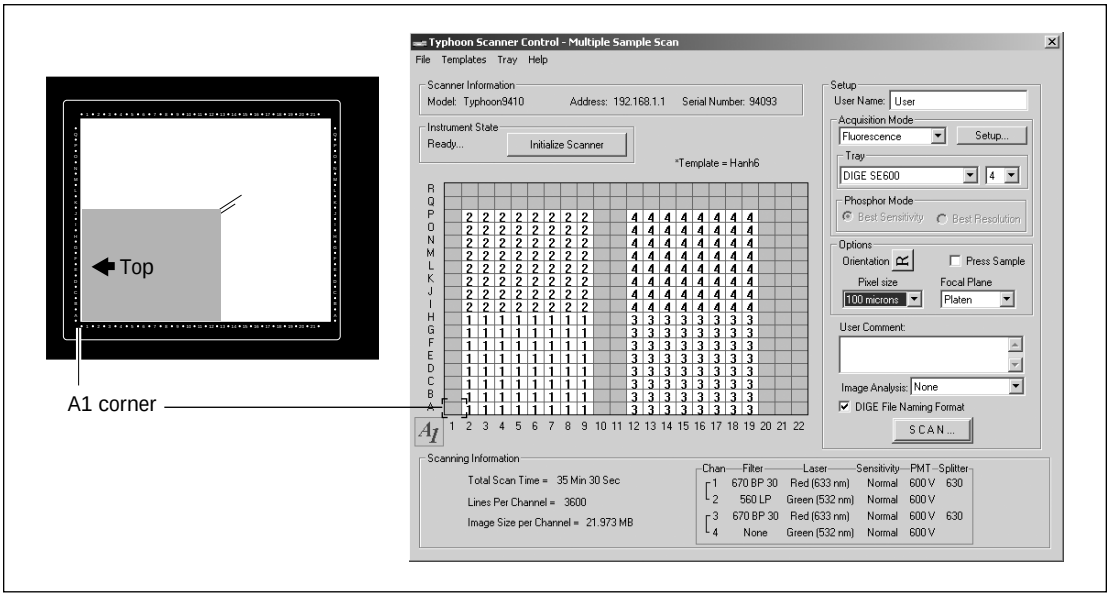


Figure 9-2. Selecting the scan area.

9.2.4 Selecting the sample orientation

You select the sample orientation from the Setup area in the Scanner Control window (figure 9-1). The orientation buttons allow you to change how the scanned image will be displayed in ImageQuant. To do this, select the icon that represents how you aligned the upper left corner of the sample with the grid markings on the glass platen or the gel alignment guide. Figure 9-3 describes each sample orientation parameter.

If you placed the sample on the glass platen so that the top of the sample was aligned with the numbered rows, you can use the orientation buttons to display the top of the sample at the top of the Image window in ImageQuant.

If you placed the sample on a gel alignment guide, use the gel orientation guide (in the Gel Alignment Guide Kit) to help you determine the orientation. Hold the gel orientation guide over the gel making sure that the IEF strip and molecular weight orientation of the guide match that of the gel. The appearance of the letter “R” as seen from the front of the Typhoon instrument shows which letter “R” to select in the Scanner Control software. Selecting the matching letter “R” orients the image correctly for viewing in ImageQuant.

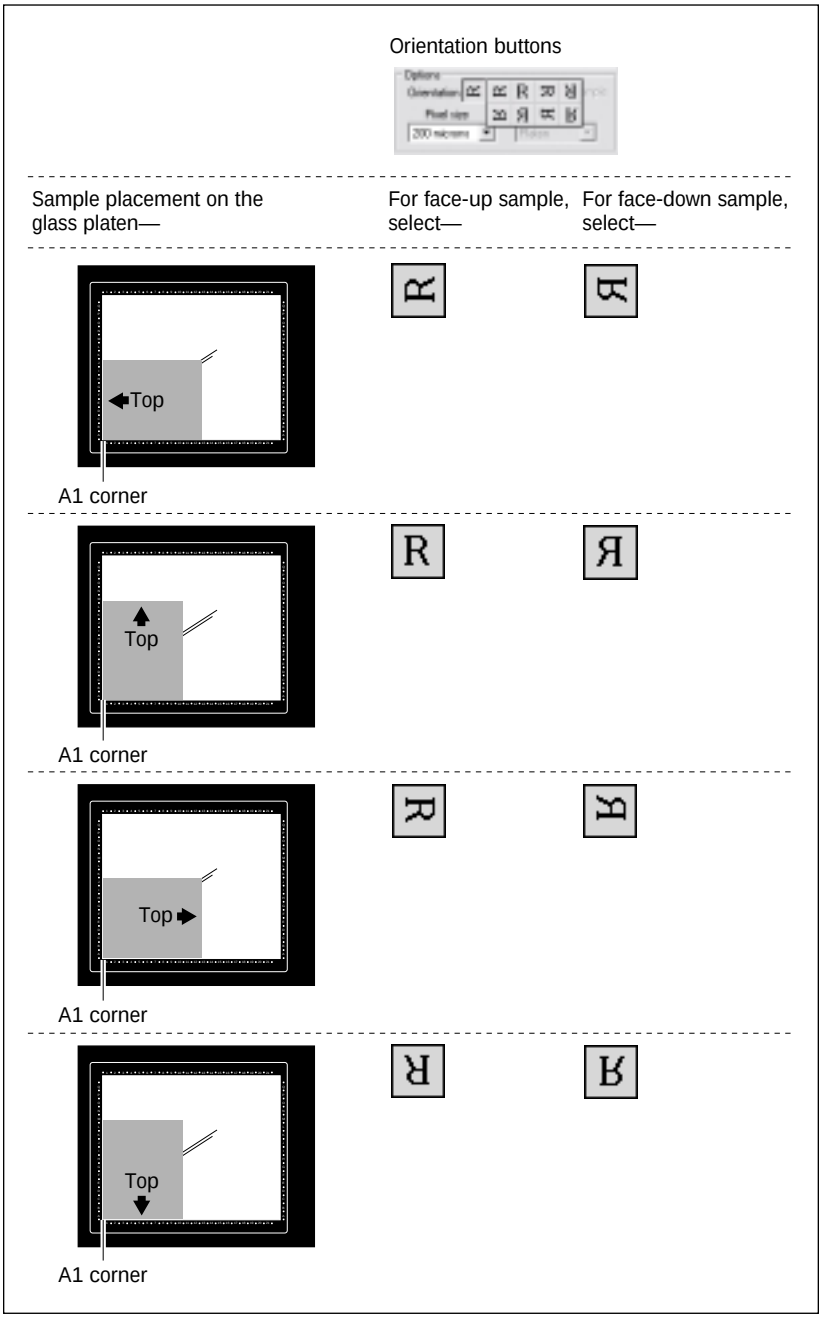


Figure 9-3. Determining which orientation button to use.

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 aligned the sample on the glass platen and release the mouse button. During the scan, the software maps the pixels to permit display of the sample image face up and top-end up in ImageQuant.

9.2.5 Selecting the Press Sample parameter

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 Typhoon 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 sample and the image.**

9.2.6 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 select the pixel size from the Pixel Size list in the Setup area of the Scanner Control window (figure 9-1). After you change the pixel size, Scanner Control updates the image size, the scan time, and the number of data lines per channel in the Scanning Information area.

You should choose the largest pixel size that provides the best resolution for your sample. Using the smaller pixel sizes increases the scan time and the image file size.

You can use the 1 000- and 500-µm pixel sizes to perform a quick prescan of the sample to make sure the experiment was successful or to check the PMT voltage parameters to make sure the data is not saturated.

Table 9-1 lists the pixel sizes.

Table 9-1. Pixel sizes

Pixel Size	Description
1 000 µm	For a very quick scan of the sample, select **1 000 microns.** The 1 000-µm pixel size produces 10 data points per centimeter and 20 data lines per grid square. (**Note:** The 1 000-µm pixel size produces very rough data that is good for a quick prescan, but is not recommended for quantitative analysis.)
500 µm	For a quick scan of the sample, select **500 microns.** The 500-µm pixel size produces 20 data points per centimeter and 40 data lines per grid square.
200 µm	For most samples, select **200 microns.** The 200-micron pixel size produces 50 data points per centimeter and 100 data lines per grid square.
100 µm	For samples that require high resolution, select **100 microns.** The 100-µm pixel size produces 100 data points per centimeter and 200 data lines per grid square.
50 µm	For samples that require a higher resolution, select **50 microns.** The 50-µm pixel size produces 200 data points per centimeter and 400 data lines per grid square.
25 µm	For samples that require an even higher resolution, select **25 microns.** The 25-µm pixel size produces 400 data points per centimeter and 800 data lines per grid square.
10 µm (Typhoon 8610, 9210, and 9410 only)	For samples that require the highest resolution, select **10 microns.** The 10-µm pixel size produces 1 000 data points per centimeter and 2 000 data lines per grid square.

9.2.7 Selecting the focal plane

The Focal Plane area allows you to adjust the focal point of the laser and collection optics. Two parameters are available—

- **Platen—**Use this parameter if you placed a thin (less than 3 mm) or surface sample, such as a gel or membrane, directly on the glass platen of the Typhoon instrument. Scanner Control adjusts the focal point of the laser beam and collection optics to the area just above the glass platen of the Typhoon instrument.
- **+3 mm—**Use this parameter if you placed a microplate or a sandwich gel on the glass platen of the Typhoon instrument. Scanner Control adjusts the focal point of the laser beam and collection optics 3 mm higher than the glass platen of the Typhoon instrument. If you used different thicknesses of electrophoresis glass plates, make sure you place the 3-mm electrophoresis glass plate of the sandwich gel on the glass platen.

9.2.8 Selecting the image analysis software

If you want to analyze the image after you finish scanning, you can select the image analysis software you want to use from the Image Analysis list in the Setup area of the Scanner Control window (figure 9-1). After the instrument finishes scanning, the selected software starts and displays the image.

If you select None from the Image Analysis list, the Scanner Control window remains active so that you can perform another scan.

9.2.9 Entering user comments (optional)

Important **The user comments are not saved as part of a template. If you want to include comments, you must type the comments each time you scan.**

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

9.3 Using the Setup window to select the fluorescence parameters

You use the Fluorescence Setup window to select the fluorescence parameters to use with the sample. You can select from one through four sets of parameters to perform one through four scans. The following sections describe how to use the Fluorescence Setup window (figure 9-4).

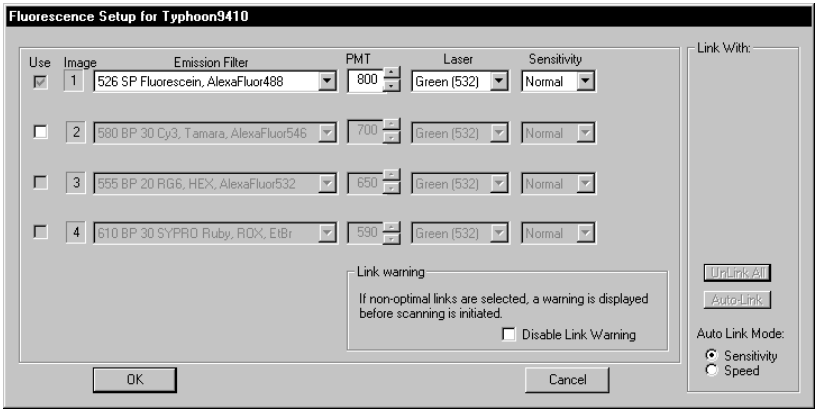


Figure 9-4. The Fluorescence Setup window with Image 1 active.

9.3.1 Displaying the Fluorescence Setup window

To display the Fluorescence Setup window, make sure Fluorescence is the selected acquisition mode, and then click the **Setup** button in the Acquisition Mode area in the Scanner Control window (figure 9-1).

9.3.2 Selecting the number of scans

The Fluorescence Setup window displays the fluorescence parameters for all four scans. As a default, Scanner Control activates the parameters for Image 1 only, as shown in figure 9-4. If your sample contains two or more labels, you can set up scan parameters for all the labels in your sample (up to 4). See section 7.6 for more information on multilabel experiments.

To activate the parameters for Images 2, 3, and then 4, click the **Use** check box for Images 2, 3, and then 4, respectively.

9.3.3 Selecting the emission filter

You select an emission filter from the Emission Filter list for each scan you want to perform. Each list displays the filters that are installed on the Typhoon instrument. If you do not want to use an emission filter, select **None** from the list. For a description of the emission filters, see section 7.3. When you select a filter, Scanner Control automatically suggests the laser you should use with the emission filter you selected. However, if desired, you can select a different laser.

Important

The laser and emission filter combinations listed in the Fluorescence Setup window are based on the excitation and emission maxima of the selected fluorochrome. For most gel microplate and array samples, these combinations provide the best scanning results. However, for membrane samples, selecting a laser and emission filter combination that is close to the excitation and emission maxima might not provide the best results because of the high background interference. For the best results, you should test the membrane samples with different laser and emission filter combinations to determine the optimal combination.

9.3.4 Setting the PMT voltage

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

Guidelines for changing the PMT voltage

For accurate quantitation of high-intensity samples, the pixel intensities in your scanned image should be within a specific range. To determine the correct PMT voltage, scan a test sample or scan the real sample using the 1 000- or 500-µm pixel size, and then display the image in ImageQuant. Use the Gray/Color Adjust feature to display the intensity values of the image. For more information, see the *ImageQuant User's Guide* or Help within the software.

- If the pixel values are more than 1 count and less than 100 000 counts, all spots in your image are within the dynamic range of the instrument.
- If some of the pixel values are equal to or greater than 100 000 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 limit of detection.

- For weak samples, increase the PMT voltage. If you increase the PMT voltage, the image might lose quantitation accuracy at the upper end of the signal range. If the sample background is very low, a PMT voltage above 900 V might increase the PMT noise and cause a loss in sensitivity. Note that the maximum PMT voltage is 1 000 V.
- For high-intensity samples that saturate the system, decrease the PMT voltage to bring high-intensity signals into the linear range of the instrument. Otherwise the image might lose the sensitivity and quantitation accuracy at the lower end of the signal range. If you decrease the voltage below 400 V, the image might lose linearity.

Important **For the best sensitivity, use the High sensitivity parameter with a medium PMT voltage (600–800 V).**

If you change your sample matrix, you should test the PMT values between 400 V and 1 000 V to determine which voltage provides an acceptable background. As a suggested starting point, use 600 V.

Procedure for changing the PMT voltage

To set the voltage in the PMT Voltage box—

- Click the box to select it, and then type the value you want. The maximum value is 1 000 V.
- Use the up or down arrows to change the value in 5-V increments. To approximately double the signal intensity, increase the PMT voltage by 50 V.

Because different labels, lasers, and emission filters affect the signal intensity, you need to select an appropriate PMT voltage for each scan of a multilabel experiment.

9.3.5 Selecting the laser

Based on the emission filter you select, Scanner Control selects the laser you should use for the scan. You can change the laser displayed in the Laser list to the other nonoptimal parameter. The parameters are the **Green (532)** or **Red (633)** laser.

(Typhoon 9400 and 9410 only) Two additional laser parameters appear in the list: **Blue (488)** and **Blue (457)**. **Note:** The laser power of the 457 wavelength is much lower than the laser power of the 488 wavelength. You might need to adjust the PMT voltage accordingly.

9.3.6 Selecting the sensitivity (limit of detection)

You use the sensitivity parameters to help improve the limit of detection of the Typhoon instrument. The sensitivity parameter controls how long the Typhoon instrument collects data from each pixel. The Sensitivity parameters are—

- **Normal (default)—**Collects data from each pixel once. In general, you should use the Normal parameter.
- **Medium—**Collects data from each pixel four times and averages the results. Using the Medium parameter to detect weak signals improves the sensitivity by reducing the background noise. Selecting Medium will increase 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.

  (Typhoon 8610, 9210, and 9410 only) For the 10-µm pixel size, the system collects data from each pixel twice and then averages the values.)
- **High—**Collects data from each pixel eight times and averages the results. Using the High parameter to detect weak signals improves the sensitivity by reducing the background noise. Selecting High will increase 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. **Note:** This parameter is not available for the 10- and 25-µm pixel sizes.

If you are performing multiple scans, you set sensitivity parameters for each scan.

9.3.7 Linking scans

You link scans that contain similar parameters so that you can acquire signals from two different fluorochromes during one scan. This optimizes the scanning of samples containing multiple fluorochromes by reducing the scan time. The beamsplitter separates the emissions from the linked scans so that one PMT collects the longer wavelength emissions while the other PMT collects the shorter wavelength emissions. For a description of the beamsplitters in the Typhoon instrument, see section 7.4. For information on how scans are linked, see section 7.5.

Determining if scans can be linked

Scans can be linked if they meet the following criteria:

- The scans use the same laser, or they combine the red laser with the green laser. Make sure you select the Speed mode if you combine the red and green laser.

  (Typhoon 9400 and 9410 only) The 457 and 488 wavelengths of the blue laser are not considered to be the same laser and cannot be linked.

- The scans use different emission filters or at least one uses no emission filter.
- The scans use the same sensitivity parameter.
- The wavelength of one of the beamsplitters is between the wavelengths of the two emission filters, and the wavelength of the beamsplitter is at least 5 nm from each filter.

If Scanner Control determines that scans can be linked, the Link With area becomes active. Scan number buttons appear in the Link With area for the scans that can be linked (figure 9-5), and the Auto-Link button becomes active. After you link two scans, the Unlink All button becomes active so that you can remove the links if you do not want to use them.

Scanner Control selects the optimal link by determining which scans provide the best limit of detection and the best spectral separation. The Link With area displays the scans that can be linked and uses colored dots above the scan numbers to show the optimal link for each scan. The color of the dot represents the selected laser, and the number represents the scan that will be linked if you click the Auto-Link button.

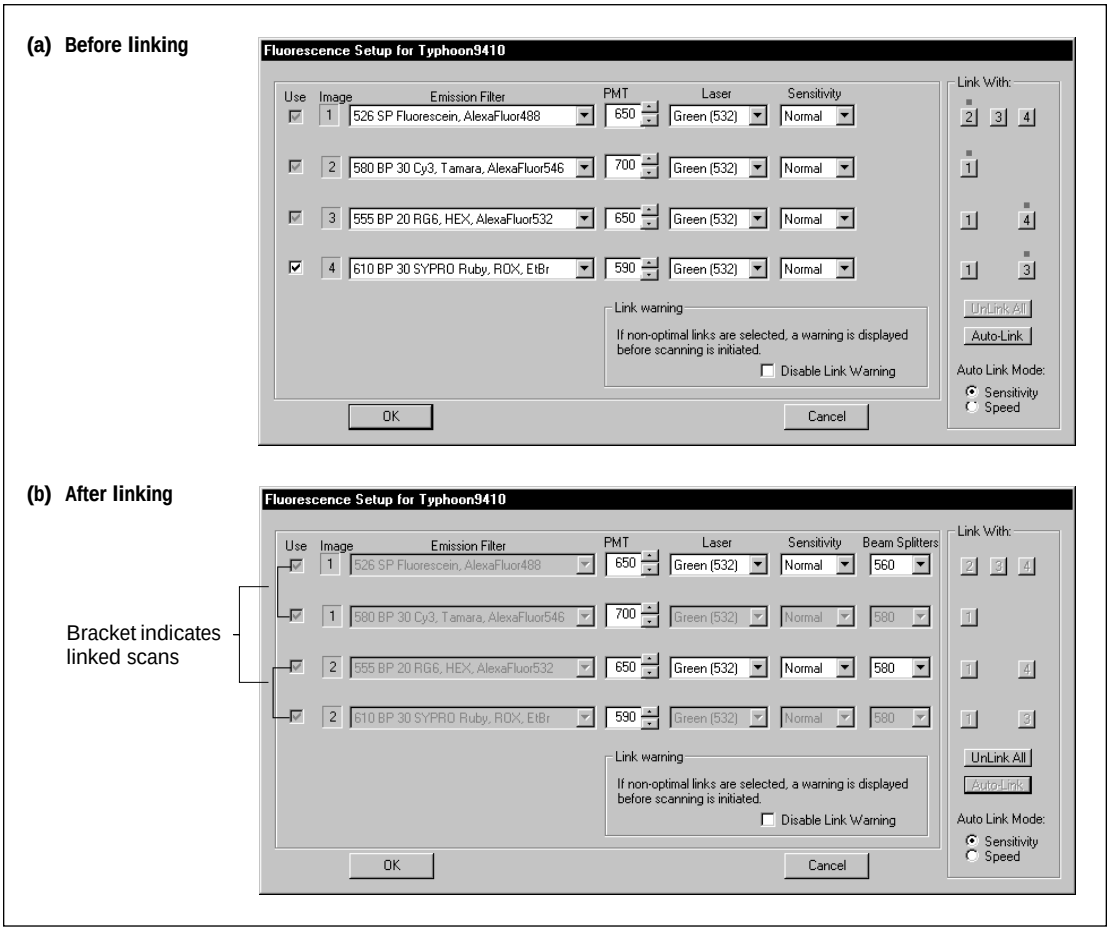


Figure 9-5. The Fluorescence Setup window. (a) The example shows the scans that can be linked. (b) The example shows that the first and second scans and the third and fourth scans have been linked.

Automatically linking scans

The Fluorescence Setup window allows you to link scans automatically. After you select the scans you want to perform and select the parameters for each scan, Scanner Control determines which scans can be linked, activates the Link With area, and displays colored dots above the scan numbers to show the optimal links.

For optimal linking, Scanner Control requires more than 5 nm between the peak of a band-pass filter and the edge of the beamsplitter, or more than 5 nm between the edge of a short-pass or long-pass filter and the edge of the beamsplitter.

If you select emission filters that are separated by less than 15 nm, Scanner Control disables the Auto-Link button. If you select None for any of the filters, Scanner Control disables the Auto-Link feature. To activate the Auto-Link button, change the emission filters to more optimal pairs.

If you link scans, you need to select an auto-link mode. The parameters are—

- **Sensitivity**—Select Sensitivity if you want to avoid cross talk between the fluorochromes that are excited by the different lasers. The Sensitivity parameter only allows auto-linking of fluorochromes that use the same laser. The scans requiring different lasers are not linked and are performed sequentially. Selecting Sensitivity improves the signal-to-noise ratio by as much as 20% for the red laser.
- **Speed**—Select Speed if you want to decrease the total scan time by linking scans that use different lasers. For example, you can link the Cy3 dye using the green laser with the Cy5 dye using the red laser. Both the red and green lasers excite the sample simultaneously. Scanning with two lasers simultaneously can cause the emission light from one dye to appear in the other channel for some fluorochromes. The resulting cross talk can reduce the sensitivity for some fluorochromes. However, for some samples, the Speed mode does not reduce the sensitivity.

Depending on the sample, you should experiment using both modes so that you can make a reasonable trade-off between scan time and sensitivity. If you are unsure and need the best results for your multilabel experiment, you should use the Sensitivity parameter.

After you click the Auto-Link button, Scanner Control links the scans that display the colored dots. Brackets appear connecting the Use check boxes, and the Image numbers change to indicate which scans Scanner Control combined into one scan (figure 9-5).

Manually linking scans

If you do not want to use the Auto-Link feature, you can manually link two scans using the link buttons in the Link With area. For each scan that can be linked, link buttons (1 2 3 4) appear in the Link With area. An optimal link displays a colored dot above the link button. Nonoptimal links do not display a colored dot. You can create both optimal and nonoptimal links.

In the Link With area for one of the scans you want to combine, click the **link** button that corresponds to the scan you want to combine with this scan. After you click the link button, brackets appear connecting the Use check boxes for the linked scans.

For example, figure 9-5(a) shows Image 1 can be linked to either Image 2, 3, or 4. The colored dot above the link button for Image 2 shows that Image 2 is the optimal link for Image 1. To manually link Image 1 and Image 2, you click one of the link buttons that has a colored dot. Figure 9-5(b) shows the Fluorescence Setup window after the scans have been linked. Note that both scans now display a 1 as the image number.

Scanner Control selects the beamsplitter you should use for the linked scan. You can change to any beamsplitter displayed in the Beamsplitter list. Scanner Control warns you that you selected a nonoptimal beamsplitter when you close the Fluorescence Setup window.

Figure 9-5(a) also shows that you could link Image 1 with Image 3 or Image 4, but these links are not the optimal links.

To link Image 1 with Image 3, click the **Image 1 link** button in the Link With area for Image 3. After you click the link button, brackets appear connecting the Use check boxes for the original Image 1 and Image 3. In addition, the Image numbers change to indicate which scans you combined into one scan. After you link nonoptimal scans and click **OK,** Scanner Control warns you that your selection is not optimal, saves the parameters, and closes the Fluorescence Setup window.

If you do not want Scanner Control to warn you that you selected nonoptimal links, click the **Disable link warning** check box. Otherwise, after you click **OK** to save the parameters and close the Fluorescence Setup window, Scanner Control will display a message warning you of the nonoptimal links.

Unlinking scans

If you want to make changes to the scan parameters, you must unlink the scan first. To unlink the linked scans, click the **Unlink All** button. Scanner Control removes all the links. You no longer see the brackets connecting the Use check boxes, and the Image numbers return to their original numbers.

9.3.8 Closing the Fluorescence Setup window

After you finish selecting the fluorescence parameters, click **OK** to save the parameters and close the Fluorescence Setup window. Scanner Control warns you if you have selected a nonoptimal link, laser, or beamsplitter.

If you do not want to save the parameters, click **Cancel** to return to the Scanner Control window.

The Scanning Information area in the Scanner Control window displays the parameters you selected in the Fluorescence Setup window, including the linking information.

Important **In the Scanner Control window, the scans are labeled by channel number, not scan number. If you linked scans, the channel numbers might not match the order of the Image number in the Fluorescence Setup window. Scanner Control reorders the scans so that linked scans appear as contiguous channel numbers. For example, if you linked Images 1 and 3, they become channels 1 and 2 in the Scanning Information area. In addition, the scans are arranged so that the scan with the longest wavelength is listed first in the channel order. ImageQuant uses the channel number to display multichannel images. For more information, see the *ImageQuant User's Guide* or Help within the software.**

9.4 Starting a 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.**

Caution **Never turn off the Typhoon instrument or disconnect the Ethernet cable while scanning. You can damage the internal mechanism of the instrument.**

Important **Keep the sample lid closed during the scan. Opening the lid causes the instrument to close the laser shutter and block the laser light. Then Scanner Control aborts the scan and saves the data already collected.**

During the scanning process, light illuminates each position on the sample only while the corresponding pixel is being recorded. The position is minimally exposed during the rest of the scan.

Scanner Control creates a single image file or a set of files and automatically adds the correct file extensions. The image file extensions are—

- **Data File (.gel)—**A single image file. If you are scanning the sample once using one laser or you selected the DIGE File Naming Format check box, Scanner Control adds this extension.

- **Dataset File (.ds)—**The image file required for viewing a multichannel image. If you linked scans or are scanning the sample more than once by selecting more than one scan in the Fluorescence Setup window, Scanner Control adds this extension.

  If you select Dataset File (.ds), Scanner Control creates a Directory folder (.dir) in the Data folder (or the location you selected) and names the .dir folder using the same name as the .ds file. The .dir folder contains a backup .ds file and one .gel file for each channel of the multichannel image (up to four channels).

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 a single-channel scan of a single sample or single scan area

If you are scanning a single sample or included multiple samples in a single scan area, you create one image file.

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 Fluorescence Setup windows.
2. In the Scanner Control window, click the **Scan** button. The Save as window appears (figure 9-6).

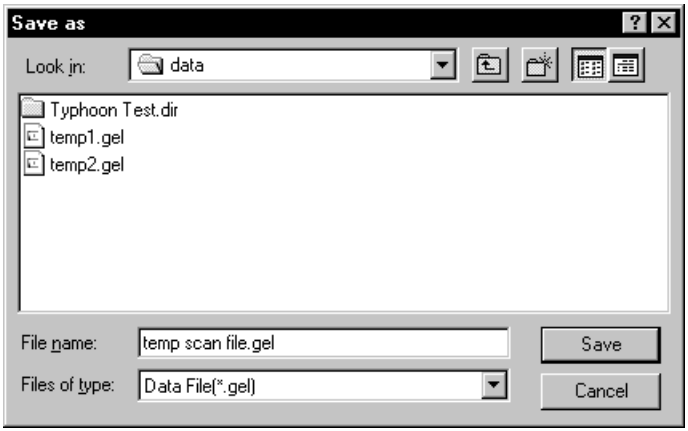


Figure 9-6. The Save as window.

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

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

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

Caution **Using a network to save the scanned data to a folder located on a remote computer can slow or delay the scan. For the best results, select a folder on the local hard drive. After you scan the sample, move the image files to the remote computer.**

5. To start the scan, click **Save.**

If you selected **Press Sample** in section 9.2.5, the inner lid of the Typhoon instrument lowers to hold the sample flat against the glass platen of the instrument.

9.4.2 Starting a multichannel (non-DIGE) scan of multiple samples and multiple scan areas

If you are scanning multiple (non-DIGE) samples and you created separate scan areas for each sample, you can create a separate multichannel dataset file for each scan area.

To start the scan—

1. Make sure that the sample or samples are in place, the sample lid is shut, and all the parameters are correct in the Scanner Control and Fluorescence Setup windows.

Important **Make sure the tray definition and number of samples in the Tray area match the positions of the samples on the glass platen.**

2. In the Scanner Control window, click the **Scan** button. The Multiple Sample Naming window appears (figure 9-7).

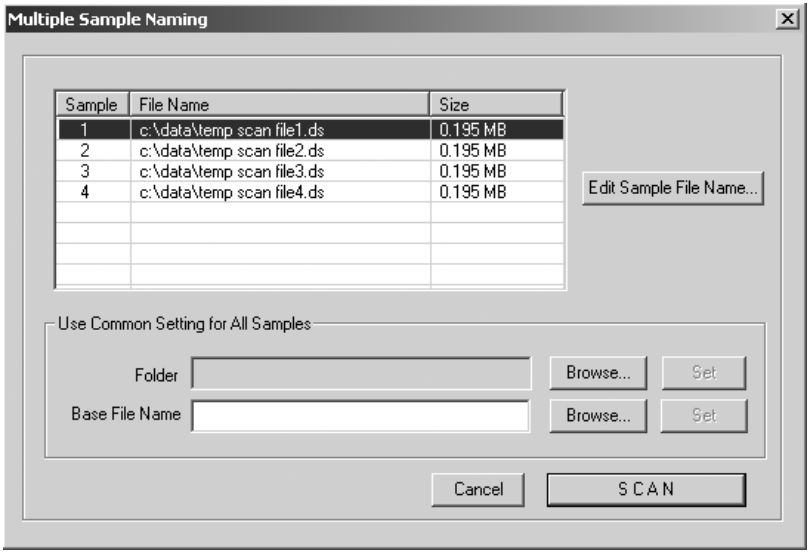


Figure 9-7. The Multiple Sample Naming window.

3. In the Use Common Setting for All Samples area, click **Browse** and locate an existing folder from the Browse for Folder window. Click **OK** to close the window and return to the Multiple Sample Naming window. Click **Set.**

Caution **Using a network to save the scanned data to a folder located on a remote computer can slow or delay the scan. For the best results, select a folder on the local hard drive. After you scan the sample, move the image files to the remote computer. For dataset image files, make sure you move both the .ds file and the .dir folder.**

4. To name—

   - **Each dataset file individually**—In the File Name list, click the file name that you want to change and then click **Edit Sample File Name.** The Save As window appears. Type the name you want to use in the File Name box, and click **Save** to close the window and return to the Multiple Sample Naming window. Click the next file name that you want to change and repeat the steps in this bullet.

   - **Each dataset file using the same base file name**—In the Use Common Setting for All Samples area, type a name in the Base File Name box, and click **Set.** The software displays the base file name in the File Name list.

- **Each dataset file using an existing base file name**—In the Use Common Setting for All Samples Area, click **Browse,** and locate the file name you want to use in the Name File window. Click **OK** to close the window, and then click **Set** in the Multiple Sample Naming window.

5. Click **Scan** to start the scan.

After the scan, the software saves each dataset in the folder you selected.

- If you used unique names, the folder contains a dataset file (.ds) and dataset folder (.dir) for each scan area.
- If you used a base file name, the software appends a number to each dataset file (.ds) and dataset folder (.dir) to make the names unique. For example, the file name for sample one will be basefilename1, where basefilename is the name you entered in the Base File Name box and 1 is the number appended to the name to indicate the first sample.

9.4.3 Starting a multichannel (DIGE) scan of multiple samples and multiple scan areas

If you are scanning samples that you want to save in the DIGE file naming format, you can create a separate image file for each channel in each scan area.

To start the scan—

1. Make sure that the samples are in place, the sample lid is shut, and all the parameters are correct in the Scanner Control and Fluorescence Setup windows.

Important **Make sure the tray definition and number of samples in the Tray area match the positions of the samples on the glass platen.**

2. Make sure the **DIGE File Naming Format** check box is selected.
3. In the Scanner Control window, click the **Scan** button. The Multiple Sample Naming window appears (figure 9-7). Default file names appear for each sample.
4. In the Use Common Setting for All Samples area, click **Browse** and locate an existing folder from the Browse for Folder window. Click **OK** to close the window and return to the Multiple Sample Naming window. Click **Set.**

Caution **Using a network to save the scanned data to a folder located on a remote computer can slow or delay the scan. For the best results, select a folder on the local hard drive. After you scan the sample, move the image files to the remote computer.**

5. To name the files from the Multiple Sample window, either—
   - In the Use Common Setting for All Samples area, type a name in the Base File Name box, and click **Set.** The software displays the base file name in the File Name list.
   - In the Use Common Setting for All Samples Area, click **Browse,** and locate an existing file name you want to use in the Name File window. Click **OK** to close the window, and then click **Set** in the Multiple Sample Naming window.

   To uniquely identify each dataset, the software appends a number to each base file name.

6. (Optional) If you want to change the dataset name or the individual file names for a sample, click the file name that you want to change and then click the **Edit Sample File Names** button. The DIGE File Naming Format window appears and displays the default names for each image of the multichannel image (figure 9-8).

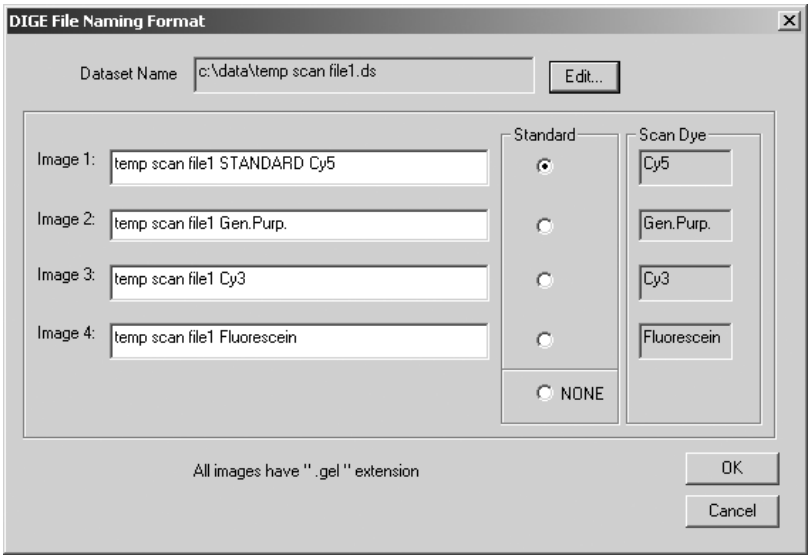


Figure 9-8. The DIGE File Naming Format window.

You can—

- **Change the dataset name**—Click the **Edit** button and type a new name in the dataset name box. After you click OK, the name appears in the Dataset Name box and in each image box.
- **Change each image name**—Delete the default image name and then type a new image name.
- **Identify the image to use as the standard**—Click the **Standard** button to the right of the image that you want to use as a standard. If you do not want to identify an image as the standard, click **None.**

After you make all the changes, click **OK** to close the DIGE File Naming Format window and return to the Multiple Sample Naming window.

Important **After you type unique file names in the DIGE File Naming Format window and close the window, do not type a name in the Base File Name box in the Multiple Sample Naming window. Doing so causes the unique file names to be overwritten.**

7. (Optional) If you want to change the file name for another sample, repeat step 6.
8. Click **Scan** to start the scan.

After the scan, the software saves each dataset in the folder you selected.

- If you used unique names, the folder contains a datset file (.ds) and dataset folder (.dir) for each scan area. The dataset folder contains the uniquely named image files (.gel).
- If you used a base file name, the software appends a number to each dataset file (.ds) and dataset folder (.dir) to make each multichannel dataset name unique. The dataset folder contains the image files (.gel). To make each image file name unique the software appends the fluorescent label associated with the emission filter used to collect the image to the file name. For example, the file name for the first scan of sample one will be basefilename1dye, where basefilename is the name you entered in the Base File Name box, 1 is the number appended to the name to indicate the first sample, and dye is the fluorescent label.

9.4.4 The initialization process

Before the Typhoon instrument begins the scan, an initialization process occurs. Depending on the state of the instrument and the scan parameters you selected in Scanner Control, the initialization can take as long as 5 minutes. A timer displays the time estimated for initialization. The timer counts down as the initialization proceeds.

The initialization procedures include—

- Moving the scan head into position
- Changing the filters to match the scan parameters
- Stabilizing the laser if the laser has been idle, shut down, or recently turned on
- Lowering the inner lid of the Typhoon instrument to hold the sample flat against the glass platen (if selected)

Note: To avoid the extra 5 minutes of initialization time used to stabilize the lasers when the instrument has been in the Sleep state, click the **Initialize Scanner** button in the Scanner Control window several minutes before you plan to start the scan.

9.4.5 Canceling the scan

If you decide you need to cancel the scan, click **Cancel Scan** in the ImageQuant Preview window. Scanner Control saves the data collected before you canceled the scan.

9.4.6 Monitoring the scan progress

After you start the scan, the ImageQuant Preview window appears (figure 9-9), and the green Scan indicator light on the top of the instrument blinks. As the Typhoon instrument scans the sample, Scanner Control displays the image in the ImageQuant Preview 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.

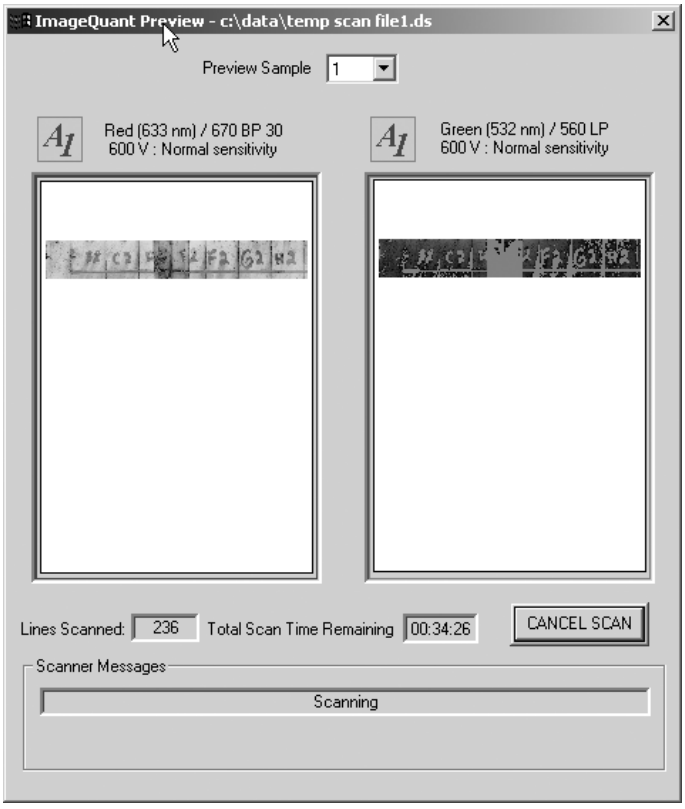


Figure 9-9. The ImageQuant Preview window during a scan set up for multiple scan areas.

A More Info button appears in the ImageQuant Preview window when the Typhoon instrument reports errors. Click the **More Info** button to view the error message.

- If the error is a nonfatal error, the instrument continues to scan, and Scanner Control saves the file when the scan is finished. You should check the image in ImageQuant to make sure the data are not corrupted.
- If the error is a fatal error, the instrument aborts the scan, and Scanner Control saves the data collected before the fatal error occurred. You should check the image in ImageQuant. Depending on where the fatal error occurred in the scan, you might be able to use the data. If you cannot use the image for quantitation, you must scan again.

If you are performing a multichannel scan, the ImageQuant Preview window displays the current scan. For example, if you selected parameters for four unlinked scans in the Fluorescence Setup window, the instrument scans the sample four times. You see an image in the ImageQuant Preview window for each scan, one at a time.

If you used the link feature to combine two scans, you see one channel image that was created using one emission filter, and you see one channel image that was created using the second emission filter (figure 9-9).

If you create a multichannel image from both linked and unlinked scans, you see two channel images for the linked scan, and you see one channel image for the unlinked scans. Only the active scan image is shown in the ImageQuant Preview window.

If the instrument is scanning multiple scan areas, you see the Preview Sample box at the top of the window, which lists the number of scan areas. To view the progress of a different scan area, select the scan area from the list.

9.4.7 Viewing the preview image for saturated data

While the instrument is scanning, an image appears in the ImageQuant Preview window that shows the part of the sample that has been scanned. Saturated data appear in red in the image. If the data portions of the image appear saturated, you will not be able to perform quantitative analysis on the image. Because saturation causes a total loss of the ability to distinguish the signal intensity fluctuations, a saturated image can be used for viewing only the shapes and positions of the spots on the sample.

To avoid saturation of the data, lower the PMT voltage you selected in the Fluorescence Setup window (section 9.3.4). **Note:** Do not select a PMT voltage below 500 V.

Important **Before repeating the scan, verify in ImageQuant that the image is saturated (100 000 rfu) and that you cannot perform accurate quantitation.**

9.4.8 Completing the scan

Scanner Control saves the image or images using the file name you typed in the Save As or Multiple Sample Naming window. The ImageQuant Preview window displays a **Complete** message, and then closes. The Scan indicator light on the Typhoon 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 Scanner Control window remains active for more scanning.

9.5 Removing the sample from the Typhoon instrument

After you finish scanning, you should remove the sample from the Typhoon 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 an image analysis software in the Scanner Control window, the software starts and displays the image. For detailed information, see the user documentation or Help for the selected software.

If you created a multichannel image, the channel numbers displayed in the Scanning Information area of the Scanner Control window are the same as the channel numbers displayed in ImageQuant.

Part four

Scanning in the chemiluminescence mode

Chapter 10 About chemiluminescence scanning

This chapter describes the theory behind chemiluminescence. The topics in this chapter are—

- How chemiluminescence works (section 10.1)
- Advantages of chemiluminescence (section 10.2)
- How to optimize the chemiluminescent experiment (section 10.3)
- Beamsplitters (section 10.4)

10.1 How chemiluminescence works

Chemiluminescence is the release of light energy from a chemical reaction. Chemiluminescent reactions used in biological assays typically emit visible light from the enzymatic conversion of a synthetic substrate.

Most common chemiluminescent substrates react with enzymes, such as peroxidase, alkaline phosphatase, or galactosidase. Each enzyme can initiate multiple chemiluminescent reactions.

Depending on the system you use, the reaction kinetics and the wavelength of the emitted light will vary. To assure saturation kinetics, you use an excessive amount of the substrate. In addition, environmental influences, such as temperature, affect the efficiencies of some chemiluminescent reactions. See the manufacturer's documentation for information about how the environment affects the chemistry you are using.

You can detect the light emission from a chemiluminescent reaction using film autoradiography or using a light-sensitive instrument, such as the Typhoon system.

10.2 Advantages of chemiluminescence

Chemiluminescence is a popular and convenient alternative to radioactive, fluorescent, and colorimetric detection methods. Unlike colorimetric or fluorescent measurements, chemiluminescent samples contribute very little native background because an exogenous light source (illumination or excitation) is not required for detection. The lack of inherent background

and an extended output of the light signal (caused by enzyme catalysis) contribute to sensitive detection over a wide dynamic range.

A variety of chemiluminescent substrates are commercially available for different enzymes. The substrates are optimized for either solution or solid-phase assays.

You can use the Typhoon system for membrane-based chemiluminescent detection of protein and nucleic acid blots. In addition, you can use the Typhoon system to scan microplate assays.

10.3 How to optimize the chemiluminescent experiment

When you design the chemiluminescent experiment, you need to evaluate how to prepare the sample so that the image you create using the Typhoon instrument can be used for quantitation. Several factors affect the quality of the experiment.

For accurate quantitation and limit of detection, time is a critical factor in the chemiluminescent experiment. You should make sure you understand the effects the following have on the quality of the scan:

- **Substrate—**You should test several substrates and evaluate which substrate emits light for the length of time required for the Typhoon instrument to scan the sample. If the signal intensity of the substrate diminishes significantly before the instrument completes the scan, you might not be able to perform accurate quantitation on the last part of the sample scanned by the instrument (figure 10-1).

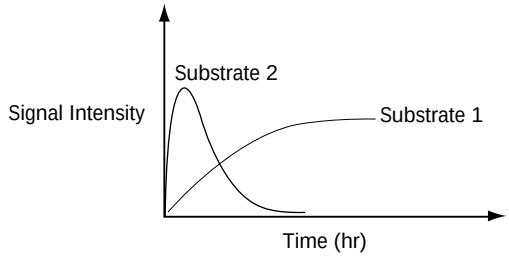


Figure 10-1. How signal intensity of a substrate and time affect the scan.

- **Scanner Control parameters—**The parameters you select in Scanner Control affect how long the instrument needs to scan the sample. The parameters that can increase the scan time are sensitivity (section 12.3.2), which sets the limit of detection, and pixel size (section 12.2.6).
- **Sample size and position—**The size of the scan area and the position of the sample on the glass platen also affect the amount of time the Typhoon instrument needs to scan the sample (section 11.3).
- **Instrument state—**An instrument in the Sleep state requires approximately 4 minutes to warm up before entering the Ready state. You can reduce the time required to scan a sample by 4 minutes if you bring the instrument to the Ready state before starting the scan (section 3.4).

When selecting the parameters in the Scanner Control window and the Chemiluminescence Setup window, note the time change in the Scanning Information area at the bottom of the Scanner Control window. If the signal intensity of the sample diminishes significantly before the instrument finishes the scan, the accuracy of the collected data can be affected.

10.4 Beamsplitters

The Typhoon instrument contains three standard beamsplitters and has a slot for one additional custom beamsplitter. You can use the beamsplitters with chemiluminescent detection to collect light emitted at different wavelengths. Table 10-1 lists the three standard beamsplitters.

Table 10-1. Standard beamsplitters

Beamsplitter	Description
560-nm dichroic	Reflects light of wavelengths shorter than 560 nm and passes light longer than 560 nm.
580-nm dichroic	Reflects light of wavelengths shorter than 580 nm and passes light longer than 580 nm.
630-nm dichroic	Reflects light of wavelengths shorter than 630 nm and passes light longer than 630 nm.

The instrument uses the beamsplitter you select in the Chemiluminescence Setup window of the Scanner Control software (section 12.3.3) to create two images from a single scan. This optimizes scanning of samples containing two chemiluminescent substrates by reducing the scan time. The beamsplitter separates the emissions from the substrates so that one PMT collects the longer wavelength emissions while the other PMT collects the shorter wavelength emissions (figure 10-2).

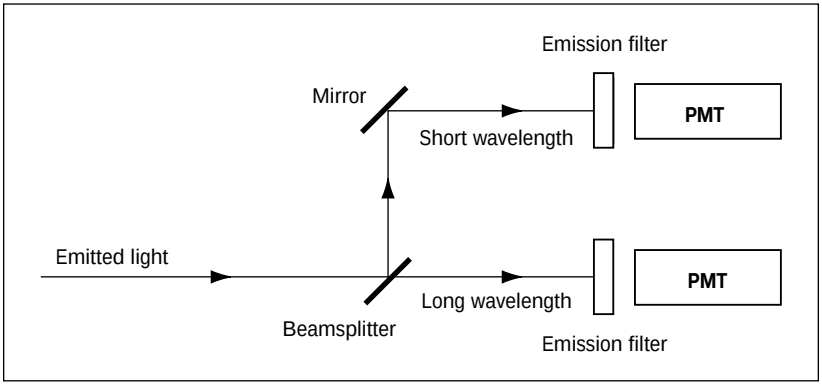


Figure 10-2. How the beamsplitter separates short- and long-wavelength emissions.

Chapter 11 Setting up for chemiluminescence scanning

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

- Preparing the sample (section 11.1)
- Cleaning the glass platen and sample lid (section 11.2)
- Loading the sample (section 11.3)

11.1 Preparing the sample

You should follow the manufacturer's guidelines for preparing membranes or microplates for chemiluminescence scanning.

To avoid compromising the chemistry on the membrane, place the membrane between two pieces of transparency material or between sheet protectors. Alternatively, you can place the membrane in a plastic bag to keep the sample wet and surrounded by substrate.

11.2 Cleaning the glass platen and sample lid

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

Important **If you use the Typhoon instrument for storage phosphor scanning, you should periodically check the instrument for contamination from radioactive samples (section 6.1.1).**

11.2.1 Cleaning the glass platen

The glass platen of the Typhoon instrument should be kept free of sources of contamination (such as substrate). It is good practice to 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 use the Typhoon instrument for fluorescence scanning, make sure the gloves are powder free.**

To clean the glass—

1. Push up on the lid release under the center front of the sample lid until the lid opens (figure 11-1).

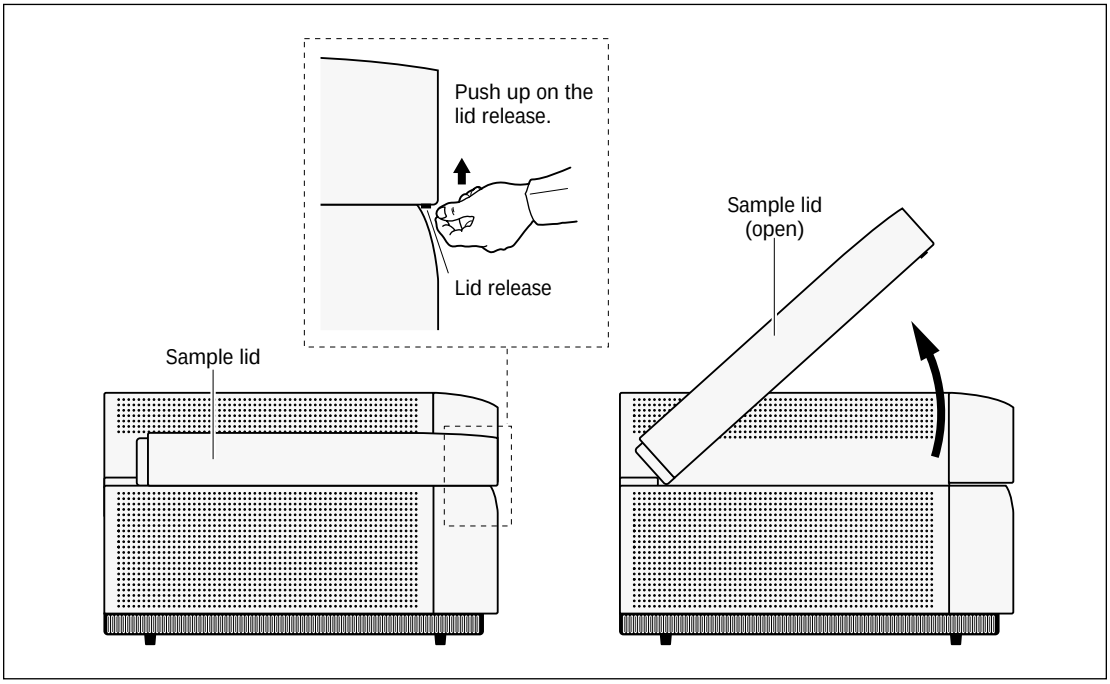


Figure 11-1. Releasing and opening the sample lid.

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.**

11.2.2 Cleaning the sample lid

The surface of the inner lid should be clean and free of chemiluminescent 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 getting inside the lid or rolling down onto the glass, do not spray liquid on the lid.

11.3 Loading the sample

Important **If time is a critical factor for scanning the sample, you should set up the scan parameters using the procedures in chapter 12 before you place the sample on the glass platen.**

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

11.3.1 Determining the sample orientation

The Typhoon instrument collects data from underneath the sample. Use the following guidelines when determining the sample orientation:

- **Membranes—**Place the sample face down on the glass platen.
- **Microplate—**Place the microplate on the glass platen so that the edges of the plate are aligned with the grid markings on the glass platen.

Note: Make a note of the grid square coordinates covered by the sample. You will use this information in the Scanner Control window (section 12.2.4).

11.3.2 Determining the sample placement

Use the following guidelines and figure 11-2 to determine where on the glass platen to place the sample:

- To minimize the scan time—
  - Place the sample near the A1 corner of the grid. Each lettered or numbered grid segment is marked by two dots.
  - Position a rectangular sample so that the shortest edge is along the numbered side of the glass platen as shown in figure 11-2. Because each pass of the scan head moves the entire length of the glass platen (from front to back), placing the longest edge of the sample along the scan head path minimizes the scan time.
- 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.
  - (Typhoon 8610, 9210, and 9410 only) Use the 10-µm pixel size to scan smaller samples only. Because the 10-µm pixel size creates a large file, you can scan approximately two-thirds of the total scan area before you exceed the 1-GB file size limit for a single-channel image. For a linked multichannel image, each channel cannot exceed 400 MB. Scanner Control warns you if you try to select a scan area that exceeds the two-thirds limit.
- To record multiple samples 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.

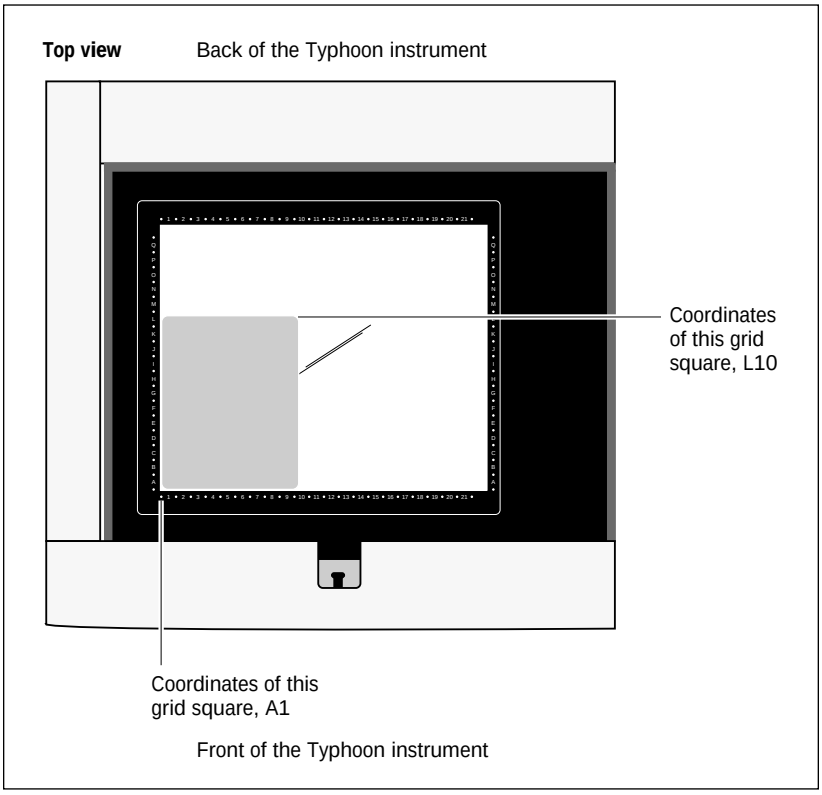


Figure 11-2. Sample placement on the glass platen of the Typhoon instrument.

11.3.3 Placing a sample on the glass platen

You can place the sample directly on the glass platen. If you are scanning a membrane, place the membrane inside transparency material, sheet protectors, or a plastic bag, and then place this on the glass platen.

You place the sample on the glass platen so that the sample creates a smooth surface on the glass. 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 platen. The buffer or distilled water lowers the chance of trapping air bubbles between the sample and the glass platen.

Important **Make sure you wipe up 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.**

To place a sample on the glass platen of the Typhoon instrument—

1. If the sample lid is not already open, push up on the lid release under the center front of the lid until the lid opens (figure 11-1).
2. (Optional) For a wet sample, squirt a little buffer or distilled water on the glass platen.
3. Hold the sample by the edges or use forceps to handle the sample. Gently lower the sample onto the glass platen 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 Typhoon instrument. Oil from fingerprints and powder from gloves, even thoroughly washed gloves, can leave a mark that affects the quality of the image.**

4. Make a note of the grid square coordinates of the area you want to scan (figure 11-2). You will use this information later in the Scanner Control window (section 12.2.3).
5. Close the sample lid and press the lid down until the latch clicks. The sample is ready to scan.

Chapter 12 Scanning a chemiluminescent sample

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

- Checking the instrument state (section 12.1)
- Selecting the scan parameters in the Scanner Control window (section 12.2)
- Using the Setup window to select the chemiluminescence parameters (section 12.3)
- Starting a scan and checking the progress (section 12.4)
- Removing the sample from the Typhoon instrument (section 12.5)
- Cleaning the glass platen and sample lid (section 12.6)
- Analyzing or preprocessing the scanned image (section 12.7)

12.1 Checking the instrument state

If time is critical for your experiment, you can make sure the instrument is ready to scan the chemiluminescent sample by checking the instrument state, which is displayed at the top left of the Scanner Control window (figure 12-1). If the instrument state is Ready, you can select the scan parameters for your scan, place the sample on the glass platen, and start the scan.

If the instrument is in the Sleep state, click the **Initialize Scanner** button to bring the instrument to the Ready state. The Instrument State area displays a **Warming Up** message. The warm-up period takes approximately 5 minutes. During the warm-up period, you can prepare the sample and select the scan parameters in the Scanner Control window.

If time is not critical for your experiment, you can select the scan parameters, place the sample on the glass platen, and start the scan while the instrument is in the Sleep state. The instrument takes 4 minutes during initialization to stabilize the lasers and system components before starting the scan. The instrument takes approximately 1 additional minute to position the scan head, select the correct beamsplitter, and lower the sample lid (if selected).

For more information on instrument states, see section 3.4.

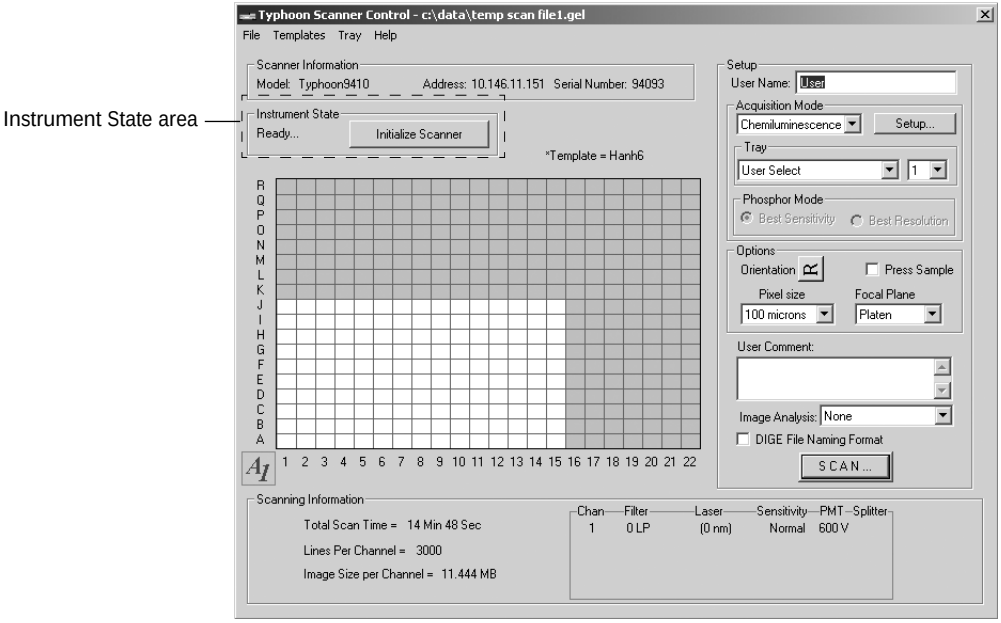


Figure 12-1. The Scanner Control window set for a chemiluminescence scan.

12.2 Selecting the scan parameters in the Scanner Control window

You use the Scanner Control window (figure 12-1) to select the parameters you want to use when you scan the chemiluminescent sample. If the Scanner Control window is not open, see section 3.3.

12.2.1 Selecting a template

If a template exists with the parameters you want to use, you can select the template, review the parameters, add user comments (section 12.2.9), and then start the scan (section 12.4). For more information on creating and using templates, see section 3.7.

To select a template, choose **Load** from the Templates menu and then select the template name from the list.

If a template does not exist, select the parameters using the instructions in sections 12.2.2 through 12.3.4.

12.2.2 Selecting the chemiluminescence scan acquisition mode

Make sure Chemiluminescence is the selected scan acquisition mode. If it is not, select **Chemiluminescence** from the Acquisition Mode list. The selections for the chemiluminescence scan acquisition mode appear in the Scanner Control window (figure 12-1) and in the additional Chemiluminescence Setup window.

12.2.3 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 Typhoon instrument. The white rectangle on the grid designates the area you want to scan. You can either use the current scan area, select a predefined scan area from the Tray list, or select a new scan area using the grid.

If you are selecting a predefined scan area from the Tray list, select the tray definition that matches how you are placing the samples on the glass platen. The grid changes to the parameters for the tray definition you selected. If you are scanning fewer samples than defined in the tray definition, select the number in the Tray number list that corresponds to the number of samples you are scanning. The grid changes to reflect the number of scan areas you selected.

Important

In the Tray area, the User Select parameter allows you to select the scan area using the procedure below. The 1 parameter indicates that you have selected only one scan area. However, if you are scanning multiple samples, you can customize the scan area using the Tray Editor to define a separate scan area for each sample (section 3.6).

To select a new scan area using the grid—

1. Place the pointer in the grid square that corresponds to the lowest lettered and numbered grid square of the area you want to scan (figure 12-2). For example, grid square A1 is the lowest lettered and numbered grid square on the glass platen.
2. Drag the pointer to the grid square that corresponds to the highest lettered and numbered grid square of the area you want to scan. For example, R22 is the highest lettered and numbered grid square on the glass platen.
3. Release the mouse button. The scan area you selected appears in white. The Scanning Information area displays the image file size, the approximate scan time, and the number of lines per channel.

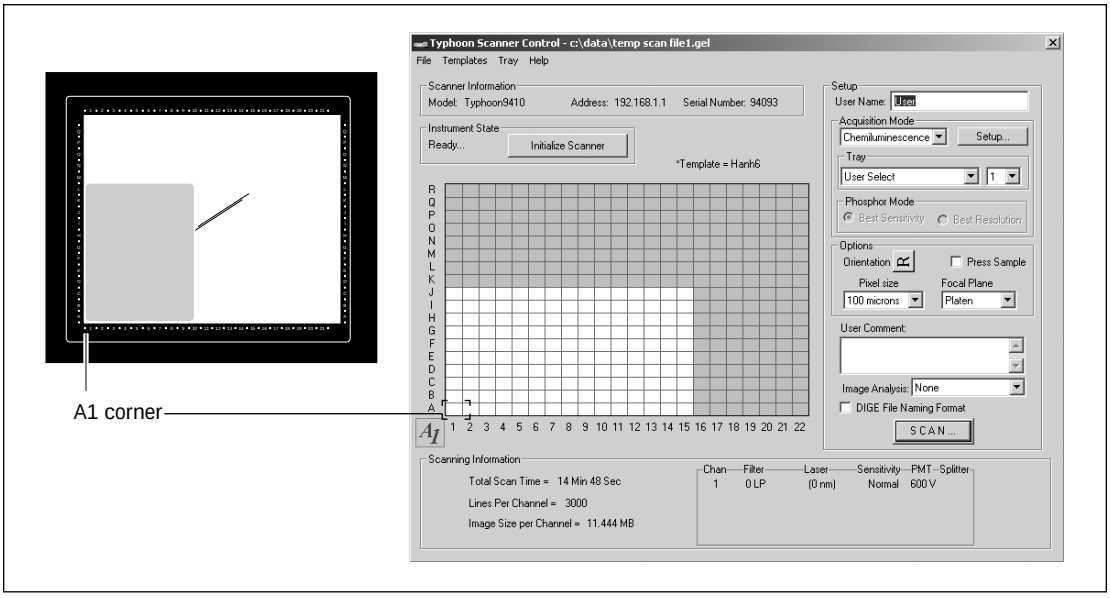


Figure 12-2. Selecting the scan area.

12.2.4 Selecting the sample orientation

You select the sample orientation from the Setup area in the Scanner Control window (figure 12-1). The orientation buttons allow you to change how the scanned image will be displayed in ImageQuant. To select an orientation, select the icon that represents how you aligned the upper left corner of the sample with the grid markings on the glass platen. Figure 12-3 describes each orientation parameter.

Note: If you placed the sample on the glass platen so that the top of the sample was aligned with the numbered rows, you can use the orientation buttons to display the top of the sample at the top of the Image window in ImageQuant.

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 aligned 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.

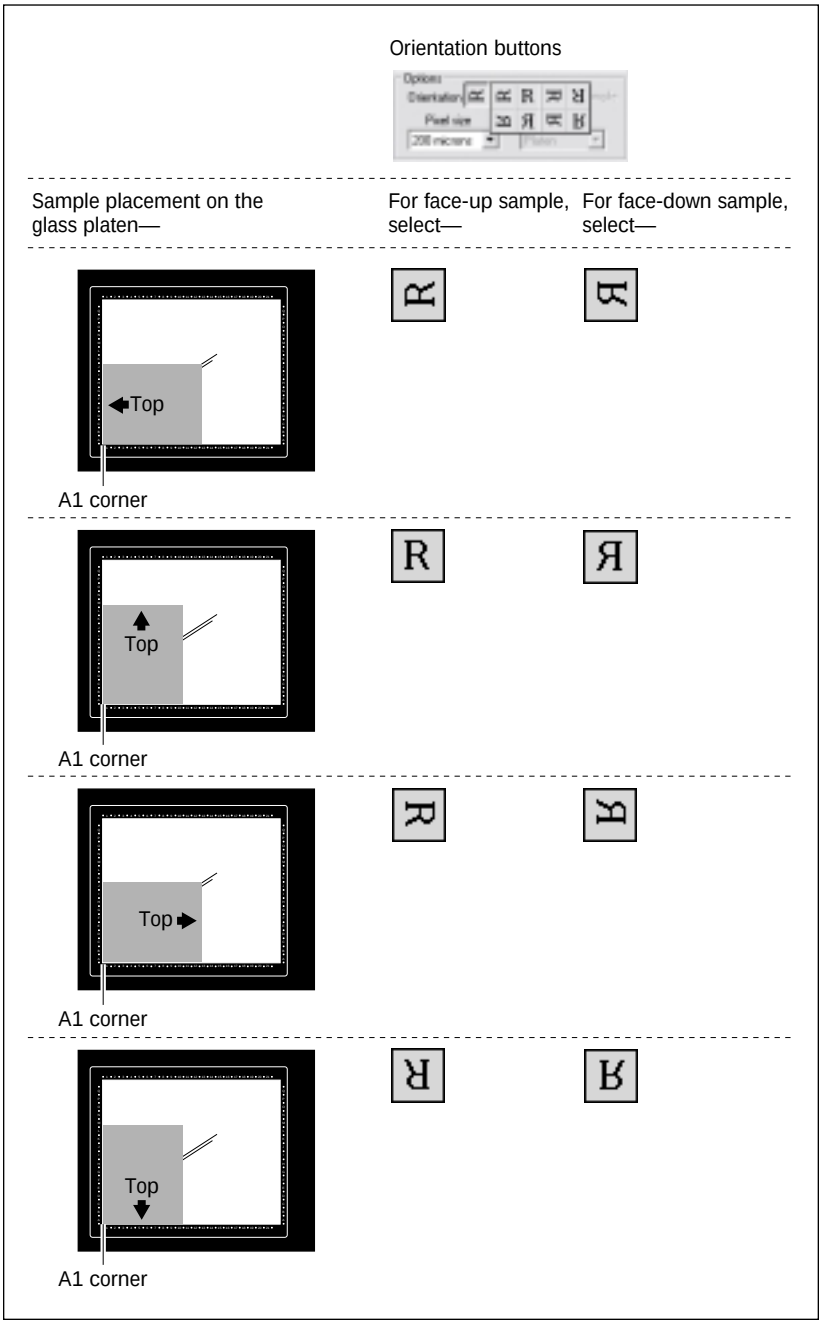


Figure 12-3. Determining which orientation button to use.

12.2.5 Selecting the Press Sample parameter

If you are scanning a dry membrane that does not lie flat against the glass platen of the instrument, you can select the Press Sample check box. When you scan the sample, the inner lid of the Typhoon instrument lowers to press the sample flat. Alternatively, lay an electrophoresis glass plate over the sample after you place the sample on the glass platen of the instrument.

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. Pressing wet membranes that are not in a plastic bag can damage the instrument.**

12.2.6 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 select the pixel size from the Pixel Size list in the Setup area of the Scanner Control window (figure 12-1). After you change the pixel size, Scanner Control updates the image size, the scan time, and the number of data lines per channel in the Scanning Information area.

The pixel size you select affects the amount of time required to scan the sample. Because chemiluminescent samples decay over time, the signal intensity collected at the beginning of the scan is stronger than at the end of the scan. Make sure you select a pixel size that causes the least amount of signal intensity degradation for your scan; for example, use the 500-µm pixel size. For more information on optimizing your chemiluminescent experiment, see section 10.3.

Table 12-1 lists the pixel sizes.

Table 12-1. Pixel sizes

Pixel Size	Description
1 000 µm	For a very quick scan of the chemiluminescent sample, select **1 000 microns.** The 1 000-µm pixel size produces 10 data points per centimeter and 20 data lines per grid square. **Note:** If you use the 1 000-µm pixel size, place your sample toward the center of the scan area along the J row. The 1 000-µm pixel size provides very rough data and is not recommended for quantitative analysis.
500 µm	For a quick scan of the chemiluminescent sample, select **500 microns.** The 500-µm pixel size produces 20 data points per centimeter and 40 data lines per grid square.

Table 12-1. Pixel sizes (continued)

Pixel Size	Description
200 µm	For most samples, select **200 microns.** The 200-µm pixel size produces 50 data points per centimeter and 100 data lines per grid square.
100 µm	For small samples that require high resolution, select **100 microns.** The 100-µm pixel size produces 100 data points per centimeter and 200 data lines per grid square.
50 µm	The 50-µm pixel size produces 200 data points per centimeter and 400 data lines per grid square. **Note:** The amount of time required to scan using this pixel size could exceed the signal intensity of the chemiluminescent sample.
25 µm	The 25-µm pixel size produces 400 data points per centimeter and 800 data lines per grid square. **Note:** The amount of time required to scan using this pixel size could exceed the signal intensity of the chemiluminescent sample.
10 µm (Typhoon 8610, 9210, and 9410 only)	The 10-µm pixel size produces 1 000 data points per centimeter and 2 000 data lines per grid square. **Note:** The amount of time required to scan using this pixel size could exceed the signal intensity of the chemiluminescent sample.

12.2.7 Selecting the focal plane

The Focal Plane area allows you to adjust the focal point of the collection optics. Two parameters are available—

- **Platen—**Use this parameter if you placed a thin (less than 3 mm) membrane on the glass platen of the Typhoon instrument. Scanner Control adjusts the focal point of the collection optics to the area just above the glass platen of the Typhoon instrument.
- **+3 mm—**Use this parameter if you are scanning a microplate or you want to place the membrane on a piece of 3-mm electrophoresis glass plate before setting the membrane on the glass platen of the Typhoon instrument. Scanner Control adjusts the focal point of the collection optics 3 mm higher than the glass platen to accommodate the height of the electrophoresis glass plate used to support the membrane.

12.2.8 Selecting the image analysis software

If you want to analyze the image after you finish scanning, you can select the image analysis software you want to use from the Image Analysis list in the Setup area of the Scanner Control window (figure 12-1). After the instrument finishes scanning, the selected software starts and displays the image.

If you select None from the Image Analysis list, the Scanner Control window remains active so that you can perform another scan.

12.2.9 Entering user comments (optional)

Important **The user comments are not saved as part of a template. If you want to include comments, you must type the comments each time you scan.**

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

12.3 Using the Setup window to select the chemiluminescence parameters

You use the Chemiluminescence Setup window to select the chemiluminescence parameters to use with the sample. The following sections describe how to use the Chemiluminescence Setup window (figure 12-4).

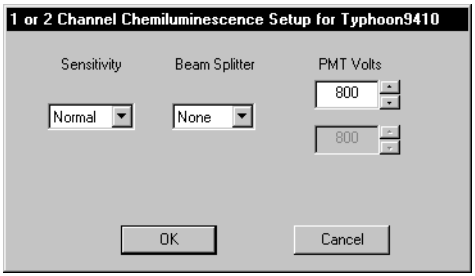


Figure 12-4. The Chemiluminescence Setup window contains parameters for scanning a chemiluminescent sample.

12.3.1 Displaying the Chemiluminescence Setup window

To display the Chemiluminescence Setup window, make sure Chemiluminescence is the selected acquisition mode, and then click the **Setup** button in the Acquisition Mode area in the Scanner Control window (figure 12-1).

12.3.2 Selecting the sensitivity (limit of detection)

You use the sensitivity parameters to help improve the limit of detection of the Typhoon instrument. The sensitivity parameter controls how long the Typhoon instrument collects data from each pixel. The Sensitivity parameters are—

- **Normal (default)—**Collects data from each pixel once. In general, you should use the Normal parameter.
- **Medium—**Collects data from each pixel four times and averages the results. Using the Medium parameter to detect weak signals improves the sensitivity by reducing the background noise. Selecting Medium 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.

  (Typhoon 8610, 9210, and 9410 only) For the 10-µm pixel size, the system collects data from each pixel twice and then averages the values.)
- **High—**Collects data from each pixel eight times and averages the results. Using the High parameter to detect weak signals improves the sensitivity by reducing the background noise. Selecting High will increase 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. **Note:** This parameter is not available for the 10- and 25-µm pixel sizes.

12.3.3 Selecting a beamsplitter for a multichannel scan

The Typhoon instrument contains three standard beamsplitters and has a slot for one additional custom beamsplitter. Table 10-1 lists the three standard beamsplitters.

If you want to scan the chemiluminescent sample and create a multichannel image, you must select a beamsplitter from the Beamsplitter list. The default selection is None, which is the correct parameter for a single-channel chemiluminescent scan.

12.3.4 Setting the PMT voltage

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

Guidelines for changing the PMT voltage

For accurate quantitation of high-intensity samples, the pixel intensities in your scanned image should be within a specific range. To determine the correct PMT voltage, scan a test sample and display the image in ImageQuant. Use the Gray/Color Adjust feature to display the intensity values of the image. For more information, see the *ImageQuant User's Guide* or Help within the software.

- If the pixel values are more than 1 count and less than 100 000 counts, all spots in your image are within the dynamic range of the instrument.
- If some of the pixel values are equal to or greater than 100 000 counts, part of your image is at or near saturation. Lower the PMT voltage, rescan, and confirm that the image is not saturated.
- For high-intensity samples that saturate the system, decrease the PMT voltage. This brings high-intensity signals into the linear range of the instrument. The image might lose the sensitivity and quantitation accuracy at the lower end of the signal range. If you decrease the PMT voltage below 400 V, the image 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 should test the PMT values between 400 V and 1 000 V to determine which voltage provides an acceptable background. As a suggested starting point, use 600 V.

Procedure for changing the PMT voltage

To set the voltage in the PMT Voltage box—

- Click the box to select it, and then type the value you want. The maximum value is 1 000 V.
- Use the up or down arrows to change the value in 5-V increments. To approximately double the signal intensity, increase the PMT voltage by 50 V.

If you are using a beamsplitter to perform a multichannel scan, you can select a PMT voltage for each scan.

12.3.5 Closing the Chemiluminescence Setup window

After you finish selecting the chemiluminescence parameters, click **OK** to save the parameters and close the Chemiluminescence Setup window. If you do not want to save the parameters, click **Cancel** to return to the Scanner Control window.

The Scanning Information area in the Scanner Control window displays the parameters you selected in the Chemiluminescence Setup window.

12.4 Starting a 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.

Caution

Never turn off the Typhoon instrument or disconnect the Ethernet cable while scanning. You can damage the internal mechanism of the instrument.

Important

Keep the sample lid closed during the scan. Opening the lid causes Scanner Control to abort the scan and save the data already collected.

During the scanning process, the chemiluminescent sample emits light, which is collected by either one or two PMTs.

Scanner Control creates a single image file or a set of files and automatically adds the correct file extensions. The image file extensions are—

- **Data File (.gel)—**A single image file.
- **Dataset File (.ds)—**The image file required for viewing a multichannel image. If you are scanning the sample using a beamsplitter, Scanner Control adds this extension.

  If you select Dataset File (.ds), Scanner Control creates a Directory folder (.dir) in the Data folder (or the location you selected) and names the .dir folder using the same name as the .ds file. The .dir folder contains a backup .ds file and one .gel file for each channel of the multichannel image.

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

12.4.1 Starting a single-channel scan of a single sample or scan area

If you are scanning a single sample or included multiple samples in a single scan area, you create one image file.

To start a 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 Chemiluminescence Setup windows.
2. In the Scanner Control window, click the **Scan** button. The Save as window appears (figure 12-5).

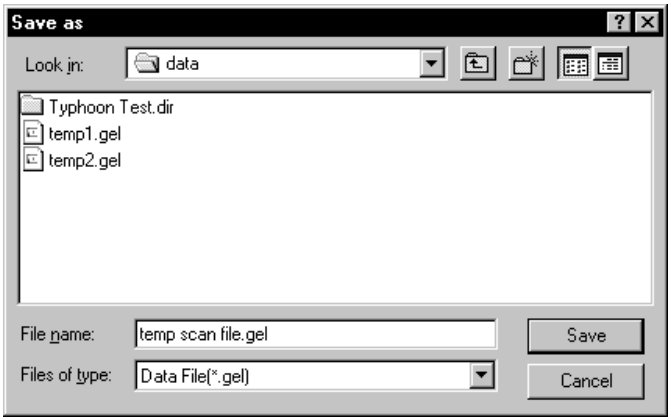


Figure 12-5. The Save as window.

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

Caution **If you type a name that is already used, a message appears and asks you if you want to replace the existing file. Before you click Yes, make sure you want to replace the existing image file. Clicking Yes deletes the existing image and all the associated auxiliary files. If you have analyzed the image using ImageQuant, all the data will be deleted.**

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

Caution **Using a network to save the scanned data to a folder located on a remote computer can slow or delay the scan. For the best results, select a folder on the local hard drive. After you scan the sample, move the image files to the remote computer. For dataset image files, make sure you move both the .ds file and the .dir folder.**

5. To start the scan, click **Save.**

If you selected Press Sample in section 12.2.5, the inner lid of the Typhoon instrument lowers to hold the sample flat against the glass platen of the instrument.

12.4.2 Starting a multichannel (non-DIGE) scan of multiple samples and scan areas

If you are scanning multiple samples and you created separate scan areas for each sample, you can create a separate multichannel dataset file for each scan area.

To start the scan—

1. Make sure that the sample or samples are in place, the sample lid is shut, and all the parameters are correct in the Scanner Control and Chemiluminescence Setup windows.

Important **Make sure the tray definition and number of samples in the Tray area match the positions of the samples on the glass platen.**

2. In the Scanner Control window, click the **Scan** button. The Multiple Sample Naming window appears (figure 12-6). Default file names appear for each sample.

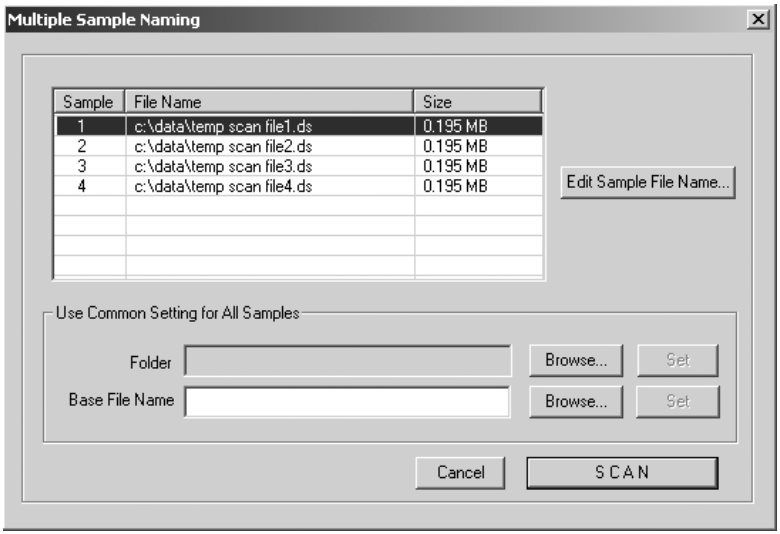


Figure 12-6. The Multiple Sample Naming window.

3. In the Use Common Setting for All Samples area, click **Browse** and locate an existing folder from the Browse for Folder window. Click **OK** to close the window and return to the Multiple Sample Naming window. Click **Set.**

Caution **Using a network to save the scanned data to a folder on a remote computer can slow or delay the scan. For the best results, select a folder on the local hard drive. After you scan the screen, move the image file to the remote computer.**

4. To name—

   - **Each dataset file individually**—In the File Name list, click the file name that you want to change and then click **Edit Sample File Name.** The Save As window appears. Type the name you want to use in the File Name box, and click **Save** to close the window and return to the Multiple Sample Naming window. Click the next file name that you want to change and repeat the steps in this bullet.
   - **Each dataset file using the same base file name**—In the Use Common Setting for All Samples area, type a name in the Base File Name box, and click **Set.** The software displays the base file name in the File Name list.
   - **Each dataset file using an existing base file name**—In the Use Common Setting for All Samples Area, click **Browse,** and locate the file name you want to use in the Name File window. Click **OK** to close the window, and then click **Set** in the Multiple Sample Naming window. The software displays the base file name in the File Name list.

5. Click **Scan** to start the scan.

After the scan, the software saves each dataset in the folder you selected.

- If you used unique names, the folder contains a dataset file (.ds) and dataset folder (.dir) for each scan area.
- If you used a base file name, the software appends a number to each dataset file (.ds) and dataset folder (.dir) to make the names unique. For example, the file name for sample one will be basefilename1, where basefilename is the name you entered in the Base File Name box and 1 is the number appended to the name to indicate the first sample.

12.4.3 Starting a multichannel (DIGE) scan of multiple samples and multiple scan areas

If you are scanning samples that you want to save in the DIGE file naming format, and you are creating a dataset, you can specify the file name for each gel file within the dataset.

To start the scan—

1. Make sure that the samples are in place, the sample lid is shut, and all the parameters are correct in the Scanner Control and Fluorescence Setup windows.

Important **Make sure the tray definition and number of samples in the Tray area match the positions of the samples on the glass platen.**

2. Make sure the **DIGE File Naming Format** check box is selected.
3. In the Scanner Control window, click the **Scan** button. The Multiple Sample Naming window appears (figure 12-6). Default file names appear for each sample.
4. In the Use Common Setting for All Samples area, click **Browse** and locate an existing folder from the Browse for Folder window. Click **OK** to close the window and return to the Multiple Sample Naming window. Click **Set.**

Caution **Using a network to save the scanned data to a folder located on a remote computer can slow or delay the scan. For the best results, select a folder on the local hard drive. After you scan the sample, move the image files to the remote computer.**

5. To name the files from the Multiple Sample window, either—
   - In the Use Common Setting for All Samples area, type a name in the Base File Name box, and click **Set.** The software displays the base file name in the File Name list.
   - In the Use Common Setting for All Samples Area, click **Browse,** and locate an existing file name you want to use in the Name File window. Click **OK** to close the window, and then click **Set** in the Multiple Sample Naming window.

   To uniquely identify each dataset file, the software appends a number to the file name.
6. (Optional) If you want to change the dataset name or the individual file names, click the file name that you want to change and then click the **Edit Sample File Names** button. The DIGE File Naming Format window appears and displays the default names for each image of the multichannel image (figure 12-7).

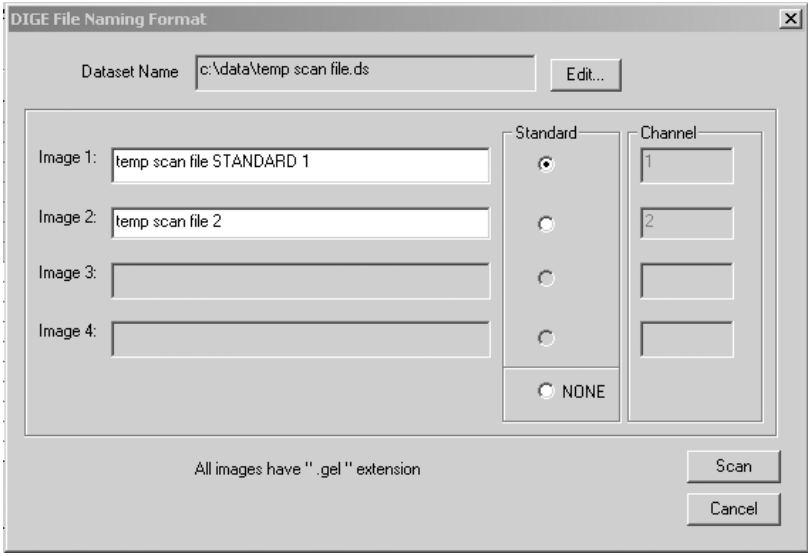


Figure 12-7. The DIGE File Naming Format window.

You can—

- **Change the dataset name**—Click the **Edit** button and type a new name in the dataset name box. After you click OK, the name appears in the Dataset Name box and in each image box.
- **Change each image name**—Double-click to highlight the default image name and then type a new image name.
- **Identify the image to use as the standard**—Click the **Standard** button to the right of the image that you want to use as a standard. If you do not want to identify an image as the standard, click **None.**

After you make all the changes, click **OK** to close the DIGE File Naming Format window and return to the Multiple Sample Naming window.

Important **After you type unique file names in the DIGE File Naming Format window and close the window, do not type a name in the Base File Name box in the Multiple Sample Naming window. Doing so causes the unique file names to be overwritten.**

7. (Optional) If you want to change the file name for another sample, repeat step 6.
8. Click **Scan** to start the scan.

After the scan, the software saves each dataset in the folder you selected.

- If you used unique names, the folder contains a dataset file (.ds) and dataset folder (.dir) for each scan area. The dataset folder contains the uniquely named image files (.gel).
- If you used a base file name, the software appends a number to each dataset file (.ds) and dataset folder (.dir) to make the names unique. For example, the file name for the first scan of sample one will be basefilename 1, where basefilename is the name you entered in the Base File Name box and 1 is the number appended to indicate the first sample.

12.4.4 The initialization process

Before the Typhoon instrument begins the scan, an initialization process occurs. Depending on the state of the instrument and the scan parameters you selected in Scanner Control, the initialization can take as long as 5 minutes. A timer displays the amount of time estimated to be used during initialization. The timer counts down as the initialization proceeds.

The initialization procedures include—

- Moving the scan head into position
- Changing the filters to match the scan parameters
- Stabilizing the PMT cooler if it has been idle, shut down, or recently turned on
- Lowering the inner lid of the Typhoon instrument to hold the sample flat against the glass platen (if selected)

Note: To avoid delays in initialization caused by the instrument warm-up time, click the **Initialize Scanner** button several minutes before you plan to start the scan.

12.4.5 Canceling the scan

If you decide you need to cancel the scan, click **Cancel Scan** in the ImageQuant Preview window. Scanner Control saves the data collected before you canceled the scan.

12.4.6 Monitoring the scan progress

After you start the scan, the ImageQuant Preview window appears (figure 12-8), and the green Scan indicator light on the top of the instrument blinks. As the Typhoon instrument scans the sample, Scanner Control displays the image in the ImageQuant Preview 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.

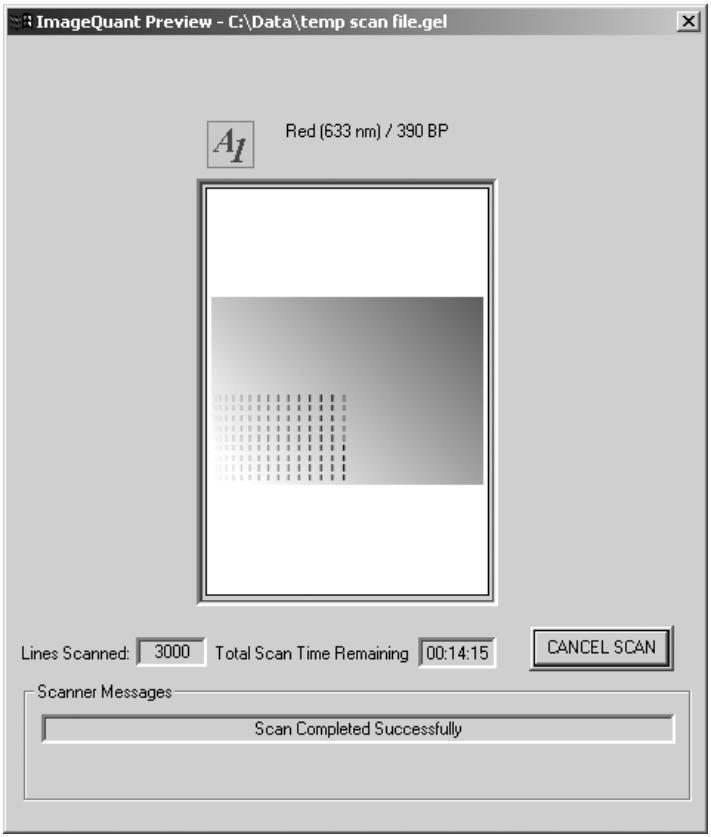


Figure 12-8. The ImageQuant Preview window, showing a single-channel scan of a chemiluminescent sample.

A More Info button appears in the ImageQuant Preview window when the Typhoon instrument reports errors. Click the **More Info** button to view the error message.

- If the error is a nonfatal error, the instrument continues to scan, and Scanner Control saves the file when the scan is finished. You should check the image in ImageQuant to make sure the data are not corrupted.
- If the error is a fatal error, the instrument aborts the scan, and Scanner Control saves the data collected before the fatal error occurred. You should check the image in ImageQuant. Depending on where the fatal error occurred in the scan, you might be able to use the data. If you cannot use the image for quantitation, you must scan again.

If you are scanning a sample using a beamsplitter, the ImageQuant Preview window displays two images.

If the instrument is scanning multiple scan areas, you see the Preview Sample box at the top of the window, which lists the number of scan areas. To view the progress of a different scan area, select the scan area from the list.

12.4.7 Viewing the preview image for saturated data

While the instrument is scanning, an image appears in the ImageQuant Preview window that shows the part of the sample that has been scanned. Saturated data appear in red in the image. If the data portions of the image appear saturated, you will not be able to perform quantitative analysis on the image. Because saturation causes a total loss of the ability to distinguish the signal intensity fluctuations, a saturated image can be used for viewing only the shapes and positions of the spots on the sample.

To avoid saturation of the data, lower the PMT voltage you selected in the Chemiluminescence Setup window (section 12.3.4), prepare a new sample, and scan again.

Important **Before repeating the experiment, verify in ImageQuant that the image is saturated (100 000 rfu) and that you cannot perform accurate quantitation.**

12.4.8 Completing the scan

Scanner Control saves the image or images using the file name you typed in the Save as or Multiple Sample Naming window. The ImageQuant Preview window displays a **Complete** message and then closes. The Scan indicator light on the Typhoon 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 Scanner Control window remains active for more scanning.

12.5 Removing the sample from the Typhoon instrument

After you finish scanning, you should remove the sample from the Typhoon 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.

12.6 Cleaning the glass platen and sample lid

To remove chemiluminescent contamination, you should clean the glass platen and sample lid after you finish each scan. See section 11.2 for details.

12.7 Analyzing or preprocessing the scanned image

You should display the image of the sample to determine the quality of the scan. If you selected an image analysis software in the Scanner Control window, the software starts and displays the image. For detailed information, see the user documentation or Help for the selected software.

Part five

Maintaining the Typhoon system hardware

Chapter 13 Maintaining the Typhoon instrument

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

- Starting the Typhoon Direct Instrument Access software (section 13.1)
- Selecting a laser shut-off time (section 13.2)
- Changing the TCP/IP address (section 13.3)
- Installing an optical filter (section 13.4)
- Removing an optical filter (section 13.5)
- Changing the definition of an optical filter (section 13.6)
- Changing the fuses (section 13.7)
- Moving the Typhoon instrument (section 13.8)

13.1 Starting the Typhoon Direct Instrument Access software

Several maintenance procedures use the Typhoon Direct Instrument Access software to control the instrument or change parameters that affect the instrument.

To use the Typhoon Direct Instrument Access software, you must have the Internet Explorer browser software installed on the computer controlling the Typhoon instrument.

Important **If you use a proxy server to access the Internet, you must modify the Internet options to exclude the address 192.168.1.1 (or the IP number assigned to the Typhoon instrument) from the proxy server. For more information on proxy servers, see your browser software documentation, or the following Web site: www.amershambiosciences.com.**

To start the Typhoon Direct Instrument Access software—

1. Close the Scanner Control software.

Caution **Before using the Typhoon Direct Instrument Access software, make sure you close the Scanner Control software. Leaving Scanner Control running while you use the window might cause problems in Scanner Control.**

2. Start the Internet Explorer browser software.

3. Type the following in the Address box:

   http://192.168.1.1/

   Alternatively, type the IP address of the Typhoon instrument if the address has been changed (section 13.3).

4. Click **Go.** The Typhoon Direct Instrument Access window appears in the browser window (figure 13-1).

The Typhoon Direct Instrument Access window lists the maintenance procedures. If you want to—

- Select a new laser shut off time, see section 13.2.
- Change the Typhoon IP address and subnet mask, see section 13.3.
- Install a new optical filter, see section 13.4.
- Remove an optical filter, see section 13.5.
- Prepare the instrument for moving or shipping, see section 13.8.

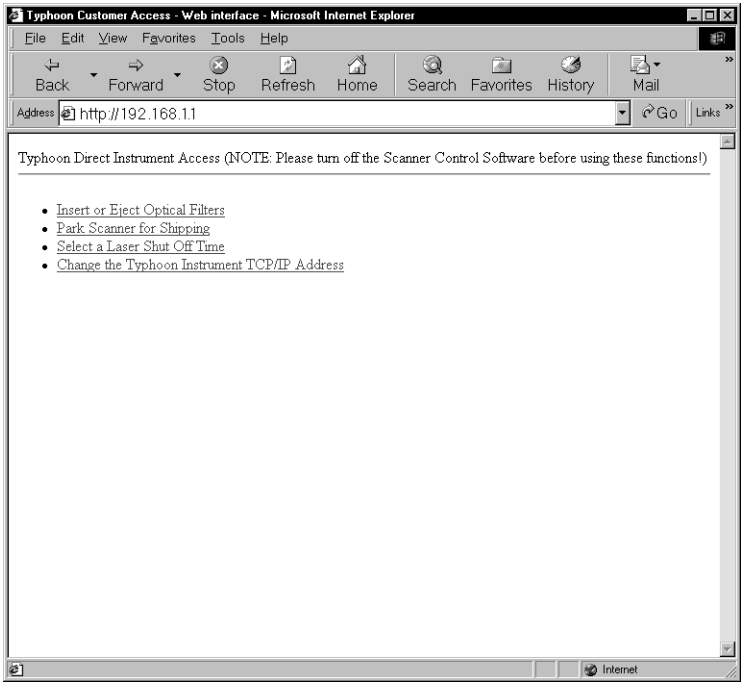


Figure 13-1. The Typhoon Direct Instrument Access window.

13.2 Selecting a laser shut-off time

Shutting down the lasers after the instrument has been idle helps to prolong the life of the lasers. You can use the Typhoon Direct Instrument Access software to select the amount of time you want the instrument to remain idle before the software shuts off the lasers. The lasers turn on automatically when you turn on the instrument, when you start a scan, or when you click the **Initialize Scanner** button in the Scanner Control window.

Caution **Before using the Typhoon Direct Instrument Access software, make sure you close the Scanner Control software. Leaving Scanner Control running while you use the window might cause problems in Scanner Control.**

To select a shut-off time for the lasers—

1. In the Typhoon Direct Instrument Access window, click **Select A Laser Shut Off Time.** The Select A Laser Shut Off Time window appears (figure 13-2).

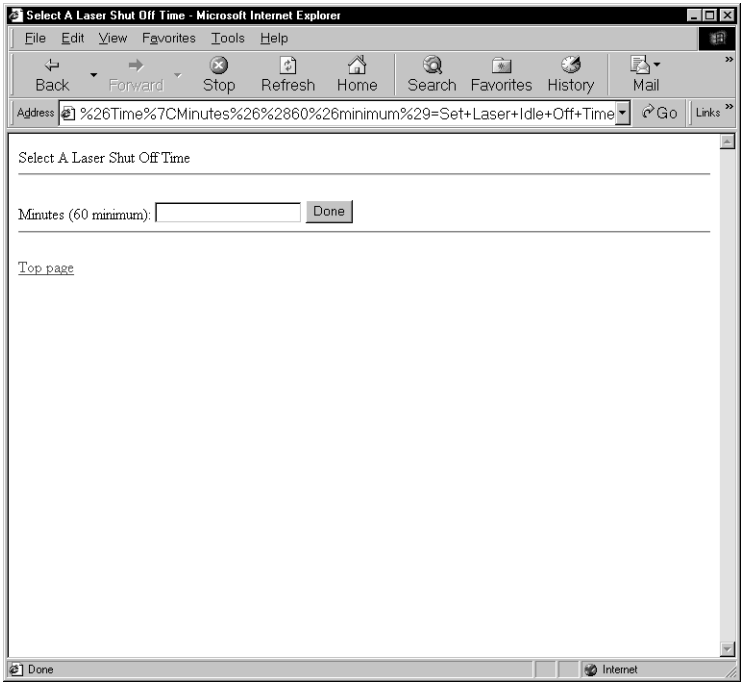


Figure 13-2. The Select A Laser Shut Off Time window.

2. In the **Minutes** box, type the number of minutes you want the instrument to remain idle before the lasers shut off. The minimum value is 60 minutes.

3. Click **Done** to set the shut-off time and return to the Typhoon Direct Instrument Access window.
4. Close the Typhoon Direct Instrument Access software.

Whenever the Typhoon instrument is idle for the time period you specified, the lasers shut off. To reactivate the lasers, you can click the **Initialize Scanner** button in the Scanner Control window, start a scan, or turn the instrument off and then turn the instrument back on.

13.3 Changing the TCP/IP address

Caution **Before using the Typhoon Direct Instrument Access software, make sure you close the Scanner Control software. Leaving Scanner Control running while you use the window might cause problems in Scanner Control.**

When you received the Typhoon instrument, the default IP address was 192.168.1.1 and the default subnet mask was 255.255.0.0.

To change the default TCP/IP address of the Typhoon instrument—

1. Consult the administrator for your laboratory network to determine an appropriate new TCP/IP address and subnet mask for the instrument.

Important **The IP address must be dedicated to the Typhoon instrument only.**

2. In the Typhoon Direct Instrument Access window, click **Change the Typhoon TCP/IP Address.** The Change Instrument IP Address window appears (figure 13-3a).
3. Click **Change IP Address.** The Enter New IP Address window appears (figure 13-3b).
4. In the IP address box, type the IP address that you received from the administrator of the laboratory network.
5. Click **Done.** A message appears listing the old IP address and the new IP address. Make sure the new address is correct. Do not restart the instrument at this time.

Important **Write down the new address here.**

New IP address _____

6. Click the **Back** button once to return to the Change Typhoon Instrument IP Address window (figure 13-3c).

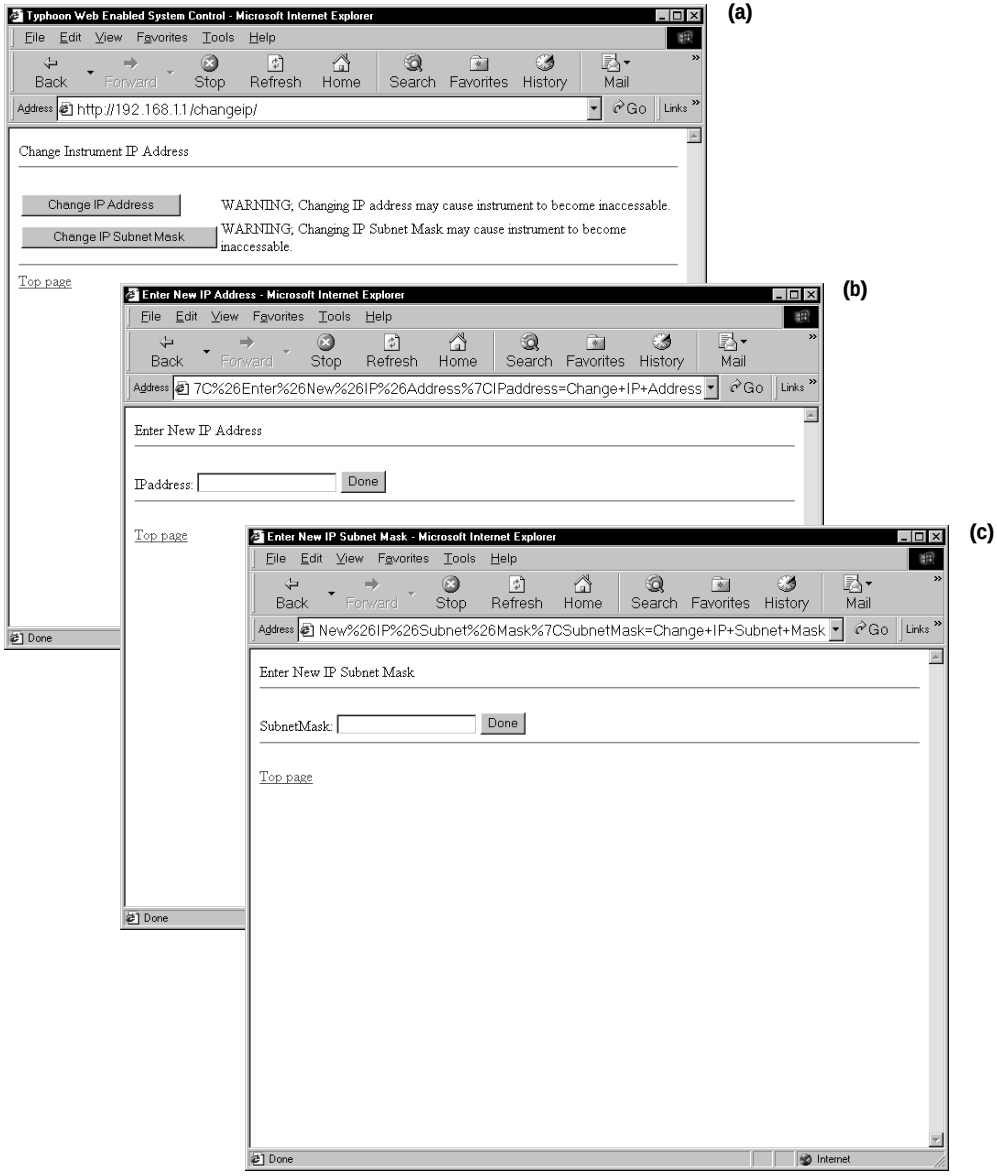


Figure 13-3. Changing the IP address and subnet mask using (a) the Change Instrument IP Address window, (b) the Enter New IP Address window, and (c) the Enter New IP Subnet Mask window.

7. Click **Change IP Subnet Mask.** The Enter New IP Subnet Mask window appears.
8. In the Subnet Mask box, type the subnet mask that you received from the administrator of the laboratory network.
9. Click **Done.** A message appears and lists the old subnet mask and the new subnet mask. Make sure the new subnet mask is correct.

Important

Write down the new subnet mask here.

New subnet mask _____

10. Close the Typhoon Direct Instrument Access software.
11. Restart the Typhoon instrument to reestablish the communication between the instrument and the computer.

13.4 Installing an optical filter

Warning

You must leave the Typhoon instrument turned on when you change the optical filter selection. After you remove the front cover of the Typhoon instrument and open the access and filter doors, make sure you follow the procedures in this section to avoid injury.

When you receive the Typhoon instrument, the instrument contains a standard set of optical (emission) filters that you use for fluorescence scanning (section 7.3), one additional filter for storage phosphor scanning, and one blank filter for multichannel experiments. The filter changer in the Typhoon instrument can contain a maximum of 14 filters. To purchase additional filter holders and for a list of possible filter vendors, contact Technical Support. See Assistance in the preface for contact information.

The Typhoon instrument uses a bar code to identify each optical filter installed in the filter changer. Bar codes 1 through 20 are reserved for optical filters defined by Amersham Biosciences. Bar codes 21 through 62 can be used for optical filters that you define. If you purchase custom optical filters from another source and you do not have a sheet of bar code labels, contact Technical Support.

You use the Typhoon Direct Instrument Access software to install an optical filter. To start the software, see section 13.1.

13.4.1 Moving the filter changer to the access door

The filter changer, which is attached to the slow stage of the Typhoon instrument, is stored at the left side of the Typhoon instrument. The access door for changing the filters is at the right side of the instrument. Before you can install an optical filter, you must move the filter changer to the access door.

The following procedure moves the slow stage and positions the filter changer at the access door so that you can add an optical filter.

Caution **Before using the Typhoon Direct Instrument Access software, make sure you close the Scanner Control software. Leaving Scanner Control running while you use the window might cause problems in Scanner Control.**

1. Start the Typhoon Direct Instrument Access software (section 13.1).
2. In the Typhoon Direct Instrument Access window (figure 13-1), click the **Insert or Eject Optical Filters** option. The Filter Changer Maintenance Page window appears (figure 13-4).

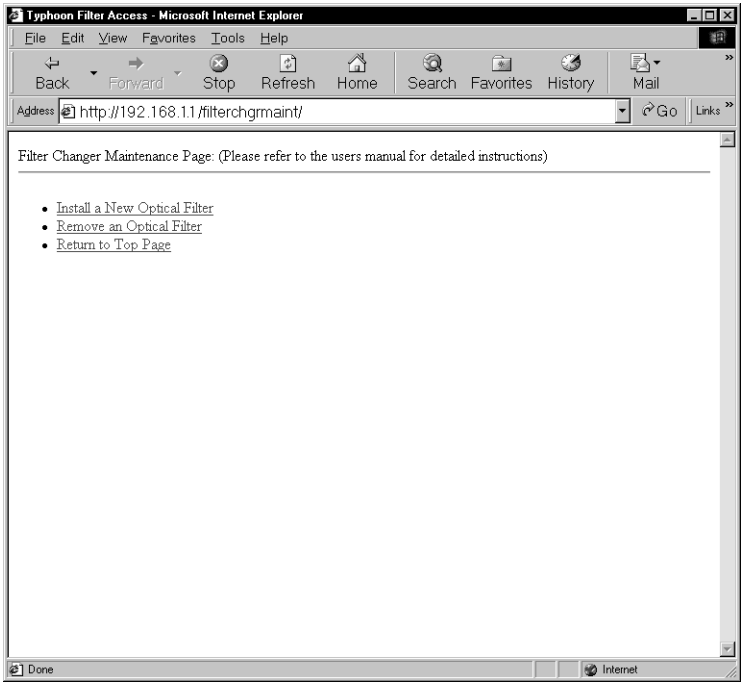


Figure 13-4. The Filter Changer Maintenance Page window.

3. Make sure the sample lid is closed, and then click **Install a New Optical Filter.** The Insert a New Optical Filter into the Filter Changer window appears (figure 13-5).

4. Click **Access Filter Rod.**

The slow stage begins to move from the left to the right side of the instrument, and the Scan indicator light blinks red. When the Scan indicator light stops blinking, the slow stage is in position to access the filter changer.

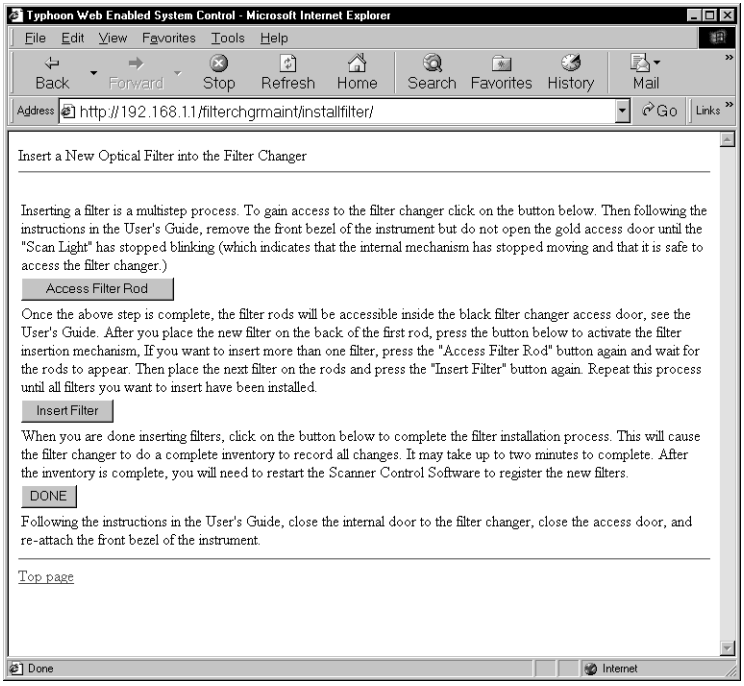


Figure 13-5. The Insert a New Optical Filter into the Filter Changer window.

13.4.2 Accessing the filter changer

Warning

You must leave the Typhoon instrument turned on when you change the optical filter selection. After you remove the front cover of the Typhoon instrument and open the access and filter doors, make sure you follow the procedures in this section to avoid injury.

To access the filter changer, you remove the front cover of the Typhoon instrument and open the access and filter doors.

1. Make sure the slow stage has stopped moving, and the Scan indicator light is no longer blinking.

2. Use a 9/64-inch hex wrench to loosen the two socket-head screws on the bottom of the front panel (figure 13-6). Turn the screws several times, but do not remove them. Pull off the front cover.

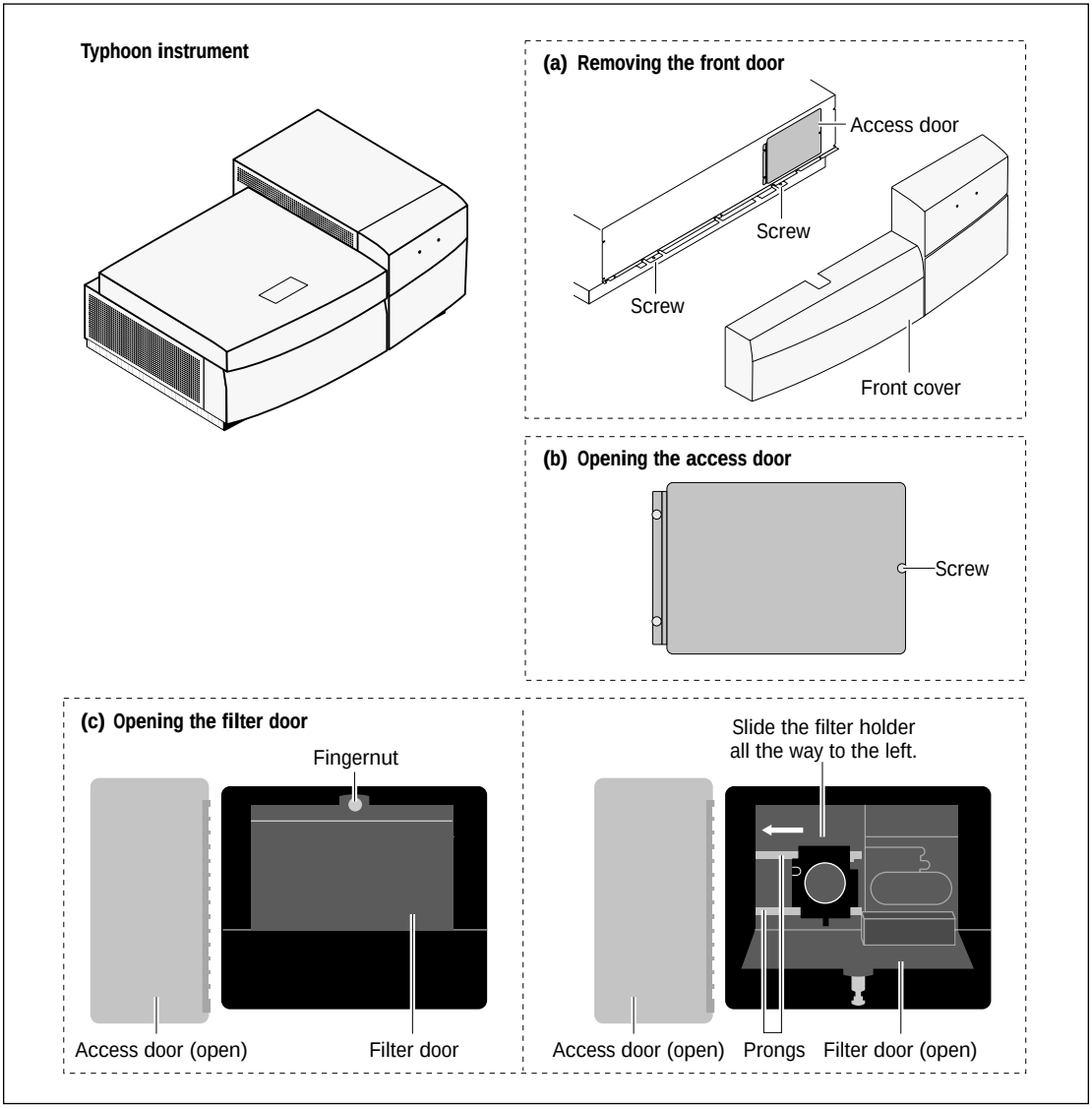


Figure 13-6. Removing the front cover and opening the access and filter doors.

3. Use a 9/64-inch hex wrench to loosen the screw on the access door and open the access door.
4. Loosen the fingernut on the filter door and open the filter door.

If the slow stage has not moved the filter changer into position, call Technical Support. See Assistance in the preface for contact information.

13.4.3 Inserting a new optical filter

Warning

You must leave the Typhoon instrument turned on when you change the optical filter selection. After you remove the front cover of the Typhoon instrument and open the access and filter doors, make sure you follow the procedures in this section to avoid injury.

Before you insert the optical filter into the filter changer make sure the filter is clean. If the filter is not clean—

- Remove dust using puffs from a dust-free blower.
- Remove water spots using a soft cloth.
- Remove fingerprints using a lens tissue and a few drops of spectroscopic grade isopropyl alcohol.

Caution

Before using the Typhoon Direct Instrument Access software, make sure you close the Scanner Control software. Leaving Scanner Control running while you use the window might cause problems in Scanner Control.

You use the Insert a New Optical Filter into the Filter Changer window to insert a new filter.

1. Make sure a bar-code label is attached squarely to the top of the filter holder as shown in figure 13-7.
2. Write down the bar code and the wavelength of the filter you are installing. After you install the filter, you will need this information to register the filter in the Scanner Control software (section 13.4.5).
3. Make sure the filter changer is in position.
4. Hold the optical filter so that the bar code is facing up and the metal notch on the holder is on the left (figure 13-7).
5. Align the holes in the filter holder with the prongs on the filter changer (figure 13-6).

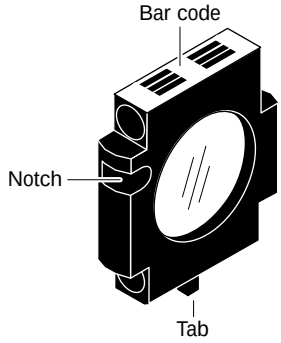


Figure 13-7. The optical filter and holder with the bar-code label attached to the top of the holder and the metal notch to the left.

6. Slide the filter holder all the way to the left. Make sure the bar code is on the top and the metal notch is on the left.

Important **Insert one filter at a time.**

7. In the Insert a New Optical Filter into the Filter Changer window (figure 13-5), click **Insert Filter.** The filter changer moves back inside the Typhoon instrument. The instrument scans the bar code and moves the filter holder to a slot farther back in the changer.

   Messages appear for the following:

   - If the instrument cannot insert the filter
   - If you inserted the filter holder backwards
   - If you did not push the filter holder all the way to the left
   - If the filter holder does not have a bar-code label
   - If you clicked Insert Filter before you inserted the filter
   - If the instrument locates another filter that uses the same bar code as the one you are installing

8. (Optional) To install additional filters, click **Access Filter Rod,** and then repeat steps 1 through 7.

9. Click **Done.** The Typhoon instrument scans the bar codes of all the installed filters and records the changes. After a minute, you see a list of all the filters installed in the Typhoon instrument. Make sure the ID code for the filter(s) you installed appears in the list.

10. Click **OK** to close the window and return to the Typhoon Direct Instrument Access window.

11. Close the Typhoon Direct Instrument Access software.

13.4.4 Closing the doors and replacing the front cover

Warning

You must leave the Typhoon instrument turned on when you change the optical filter selection. After you remove the front cover of the Typhoon instrument and open the access and filter doors, make sure you follow the procedures in this section to avoid injury.

Use the following procedure and figure 13-6 to close the access doors and replace the front cover.

1. Close the filter door and tighten the fingernut.

Important

Make sure you tighten the fingernut. The Typhoon instrument will not scan if the fingernut is loose.

2. Close the access door and use a 9/64-inch hex wrench to tighten the screw.

3. Align the pins on the instrument frame with the holes on the front cover and gently push the cover back into place.

4. Use a 9/64-inch hex wrench to tighten the two socket-head screws at the bottom of the front cover.

13.4.5 Registering a new optical filter in Scanner Control

Every time you start the Scanner Control software, Scanner Control checks the current optical (emission) filters installed in the instrument. If Scanner Control detects a filter with a new bar code, Scanner Control displays the Define the Filter window so that you can define the filter.

1. Start the Scanner Control software. The Define the Filter window appears (figure 13-8).

Important

If you installed more than one filter, make sure you enter the correct filter information for the bar code number displayed in the window.

2. In the Type list, select the **filter type** that you installed. The filter types are Long Pass, Short Pass, and Band Pass.

3. In the Name box, type a **name for the filter**. For example, use the name of a fluorescent dye that you will scan using this filter.

Important

If you will be using the DIGE file naming format, you should use the fluorescent dye as the filter name. The software appends the filter name to the individual image files created using the DIGE file naming format. For more information on the DIGE file naming format, see section 9.4.3.

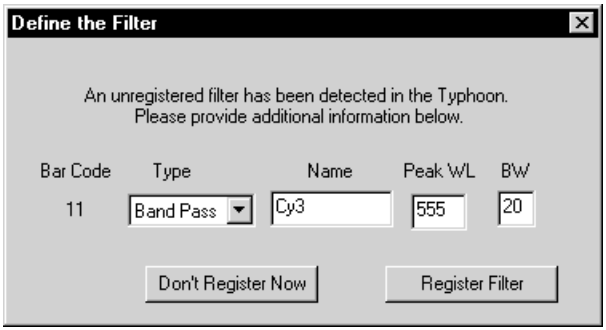


Figure 13-8. The Define the Filter window.

4. In the Peak WL box, type the **wavelength** of the filter. For example, type 555 for a 555-nm filter.
5. If the filter type you selected in step 2 is band pass, the BW box is active. Type the **bandwidth** of the band-pass filter. For example, type 20 if the bandwidth is 20 nm.
6. Click **Register Filter.** (If you click Don't Register Now, you can use the Typhoon instrument for scanning, but you will not be able to use the new filter.)
7. (Optional) If you installed more than one filter, the Define the Filter window appears again and displays the bar code number for the next filter you inserted. Repeat steps 1 through 6 to register the additional filter(s).

You can now use the new optical filter(s) for scanning.

13.5 Removing an optical filter

Warning

You must leave the Typhoon instrument turned on when you change the optical filter selection. After you remove the front cover of the Typhoon instrument and open the access and filter doors, make sure you follow the procedures in this section to avoid injury.

You can remove an optical filter you no longer want to use. Make sure that this filter is not used in any active templates created using the Scanner Control software (section 3.7). If the filter is used in a template, you should modify or delete the template. If you do not, Scanner Control removes the template from the template list, and you cannot use it.

The following procedure moves the slow stage and positions the filter changer at the access door so that you can remove an optical filter.

Caution **Before using the Typhoon Direct Instrument Access window, make sure you close the Scanner Control software. Leaving Scanner Control running while you use the window might cause problems in Scanner Control.**

1. In the Typhoon Direct Instrument Access window (figure 13-1), click the **Insert or Eject Optical Filters** option. The Filter Changer Maintenance Page window appears (figure 13-4).
2. Make sure the sample lid is closed, and then click **Remove an Optical Filter.** The Remove an Optical Filter from the Filter Changer window appears (figure 13-9).

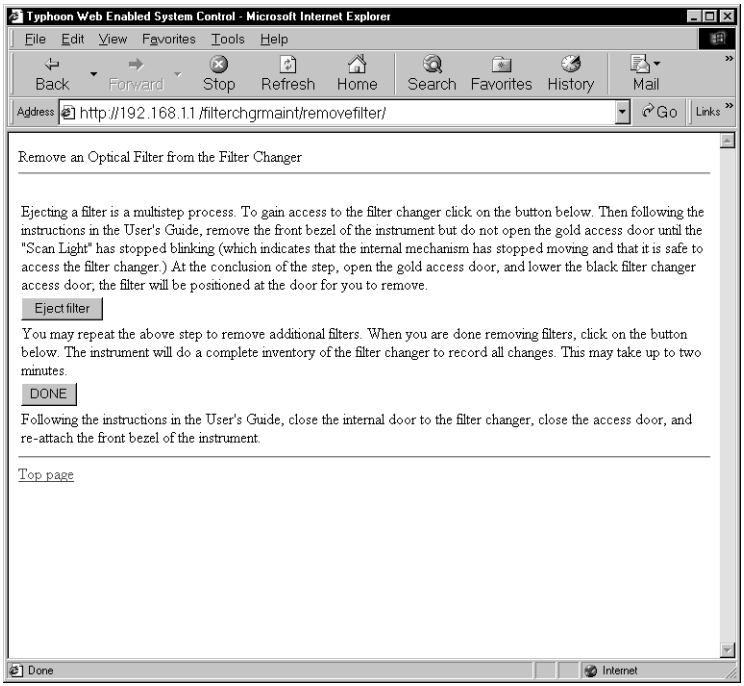


Figure 13-9. The Remove an Optical Filter from the Filter Changer window.

3. Click **Eject Filter.** The Select Filter ID to Eject window appears and displays a list of the bar codes for the installed filters (figure 13-10).

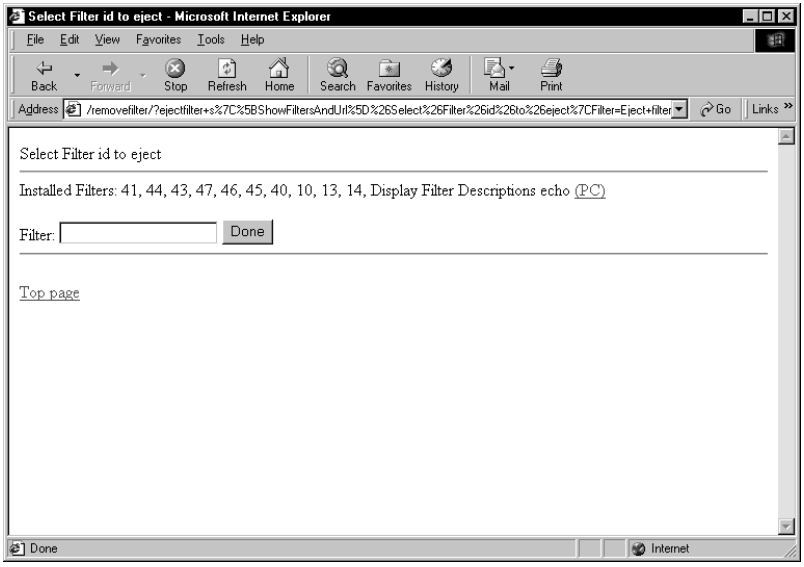


Figure 13-10. The Select Filter ID to Eject window.

4. In the Filter box, type the bar code for the filter you want to remove. (If you do not know the bar code, click **Display Filter Descriptions** to view a list of the installed filters. Click **Back** to return to the Select Filter ID to Eject window and type the bar code.)

5. In the Select Filter ID to Eject window, click **Done** to return to the Remove an Optical Filter from the Filter Changer window. The slow stage begins to move from the left to the right side of the instrument, and the Scan indicator light blinks red. You can lift the sample lid and watch the slow stage move into position.

6. After the slow stage stops moving and the Scan indicator light stops blinking, use the procedure in section 13.4.2 to remove the front cover and open the access and filter doors.

7. Slide the filter holder all the way to the right and remove the filter. Make sure you do not touch the glass filter.

8. Store the filter in a lint-free box.

9. (Optional) To remove additional filters, click **Eject Filter,** type the bar code for the next filter you want to remove, and then repeat steps 7 and 8.

10. In the Remove an Optical Filter from the Filter Changer window, click **Done.** The instrument scans all the bar codes and displays a list of the installed filters.
11. Click **OK** to close the window and return to the Typhoon Direct Instrument Access window.
12. Close the Typhoon Direct Instrument Access software.
13. Use the procedure in section 13.4.4 to replace the front cover.

13.6 Changing the definition of an optical filter

After you install an optical (emission) filter in the Typhoon instrument and define the filter in Scanner Control, you can change the definitions for the filter. To do this—

1. In the Scanner Control window, select **Fluorescence** as the scan acquisition mode and then click the **Setup** button. The Fluorescence Setup window appears.
2. Select the emission filter you want to redefine from the Emission Filter list.
3. To display the Define the Filter window, right-click the selected emission filter in the Emission Filter list. The Define the Filter window appears and displays the current definition of the filter you selected in step 2 (figure 13-11).
4. Modify any of the following:
   - Select a new **filter type** from the Type list.
   - Type a new **filter name** in the Name box.

Important **If you will be using the DIGE file naming format, you should use the fluorescent dye as the filter name. The software appends the filter name to the individual image files created using the DIGE file naming format. For more information on the DIGE file naming format, see section 9.4.3.**

   - Type a new **peak wavelength** in the Peak WL box.
   - If the filter is a band-pass filter, type a new **bandwidth** in the BW box.
5. Click **Register Filter** to redefine the filter and close the window. (If you do not want to redefine the filter, click **Don't Register Now** to close the window.)

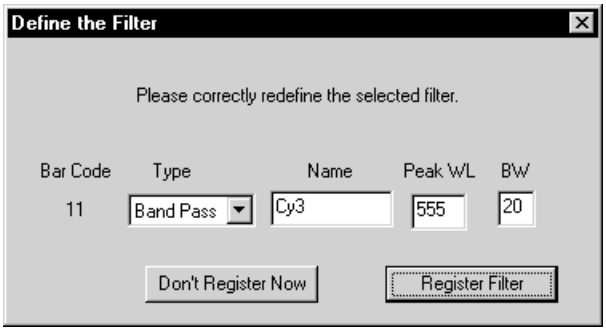


Figure 13-11. The Define the Filter window.

13.7 Changing the fuses

Warning

If fuses must be replaced repeatedly, the Typhoon instrument could have an electrical problem. Do not use the instrument. Contact Technical Support. See Assistance in the preface for contact information.

You can use the Typhoon instrument with either a 220–240 V (230V) power source or a 100–120 V (115V) 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 13-12).

The Typhoon instrument takes two 5A, 250V (⊟, T5A, 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.

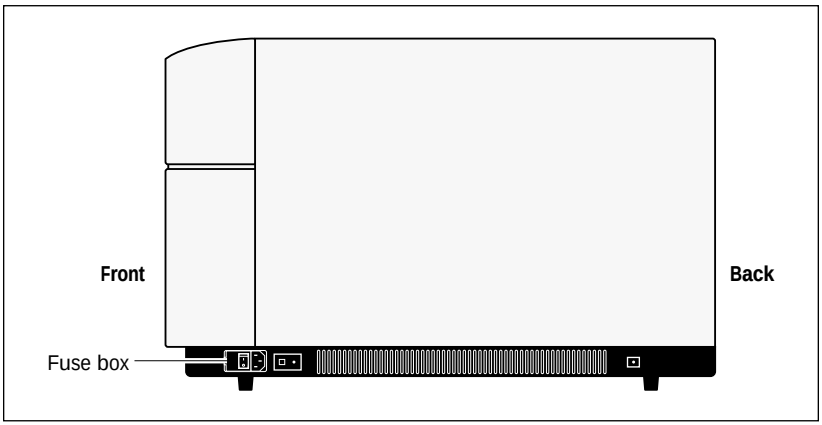


Figure 13-12. Right side view of the Typhoon instrument.

(Typhoon 9400 and 9410 only) **Note:** You cannot change the fuses in the blue laser module. If the blue laser module does not power on, contact Technical Support. See Assistance in the preface for contact information.

To change a fuse—

1. Turn off the Typhoon instrument (section 3.9) and disconnect the power cord.
2. Place the end of a small flathead screwdriver into the groove to the right of the fuse box and pry open the fuse box (figure 13-13).
3. 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.
4. One or both fuses may be blown. Replace each blown fuse with a new fuse of the same type and rating. Fuse specifications are listed at the beginning of this section and on the serial number and fuse labels located on either side of the fuse box.
5. Insert the fuse holder into the fuse box.
6. Snap the cover of the fuse box back into place.
7. Reconnect the power cord and turn on the Typhoon instrument (section 3.1).

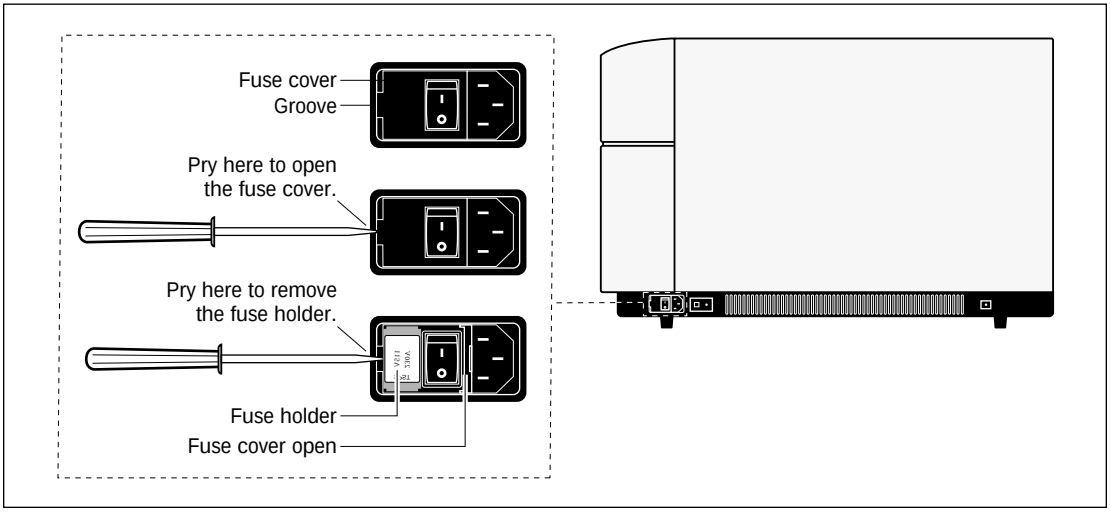


Figure 13-13. Opening the fuse box.

13.8 Moving the Typhoon instrument

If you need to move the instrument, contact Technical Support. See Assistance in the preface for contact information. Use this section to determine a new location for the instrument and to prepare for the move.

Warnings

The Typhoon instrument weighs approximately 160 kg (350 lb). Only authorized Technical Support representatives should move the instrument using the proper lifting equipment. Injury to personnel and damage to the instrument can result from attempting to lift the instrument without authorized personnel and proper equipment.

If you use unauthorized personnel to move the Typhoon instrument, Amersham Biosciences is not responsible for damage to the instrument or injury to personnel. Damaging the instrument can void the warranty and can affect the accuracy of the collected data.

(Typhoon 9400 and 9410 only) The blue laser module weighs approximately 29.5 kg (65 lb). Only authorized Service Support engineers should move the module using the proper lifting equipment. Injury to personnel and damage to the blue laser module can result from attempting to lift the module without authorized personnel and proper equipment.

(Typhoon 9400 and 9410 only) To protect you from laser light exposure, only authorized Service Support engineers can connect and disconnect the fiber-optic cable from the blue laser power unit to the Typhoon instrument.

13.8.1 Determining a new location for the instrument

If you need to move the Typhoon instrument, make sure the new location for the instrument is away from strong air currents, such as near an air vent. The airflow can carry dust or dirt particles into sensitive parts of the instrument. In addition, you should place the instrument away from direct sunlight or other very bright light. Bright light can cause excessive heat or compromise the light-tight operation of the instrument.

Make sure the instrument has at least 5 cm (2 in) of clearance on both sides and behind it. Make sure you have 1.2 m (4 ft) of clearance above the instrument. If the temperature of the room containing the instrument exceeds 30 °C (86 °F), increase the clearance on all sides by 2.5 cm (1 in).

Caution

Blocking the airflow to the instrument can cause the instrument to overheat, which can impact the instrument's performance.

(Typhoon 9400 and 9410 only) Make sure the blue laser module has at least 7.5 cm (3 in) of clearance behind it and 20 cm (8 in) of clearance on both sides. If the blue laser module is located under a laboratory bench, you must provide 30 cm (12 in) of clearance on both sides of the module. If the temperature in the room exceeds 30 °C (86 °F), you should use an external fan to vent the exhaust out of the room.

Caution **(Typhoon 9400 and 9410 only) If you do not use an external exhaust fan, the top of the blue laser module can get very warm. Do not place any temperature-sensitive materials on top of the blue laser module.**

13.8.2 Preparing to move the Typhoon instrument

To prepare the Typhoon instrument for moving, you use the Typhoon Direct Instrument Access software to park the scanner.

Caution **If you do not park the scanner, the optical system of the Typhoon instrument can be damaged during the move.**

To park the scanner—

1. Start the Typhoon Direct Instrument Access software (section 13.1).
2. In the Typhoon Direct Instrument Access window, click **Park Scanner for Shipping.** The Park Scanner for Shipping window appears (figure 13-14).
3. Click **Park Scanner Now.** The scan head moves to a secure position in the Typhoon instrument.
4. Close the Typhoon Direct Instrument Access software.
5. Use the procedures in sections 3.9 and 3.10 to turn off the instrument and computer.

Warning **Do not disconnect the cables with the power on.**

6. Disconnect the power cords and the Ethernet connections.
7. Wait for authorized Technical Support representatives to move the instrument to the new location.

Warning **(Typhoon 9400 and 9410 only) Do not disconnect the fiber-optic cable connecting the blue laser module to the Typhoon instrument. Wait for the authorized Support Service engineer.**

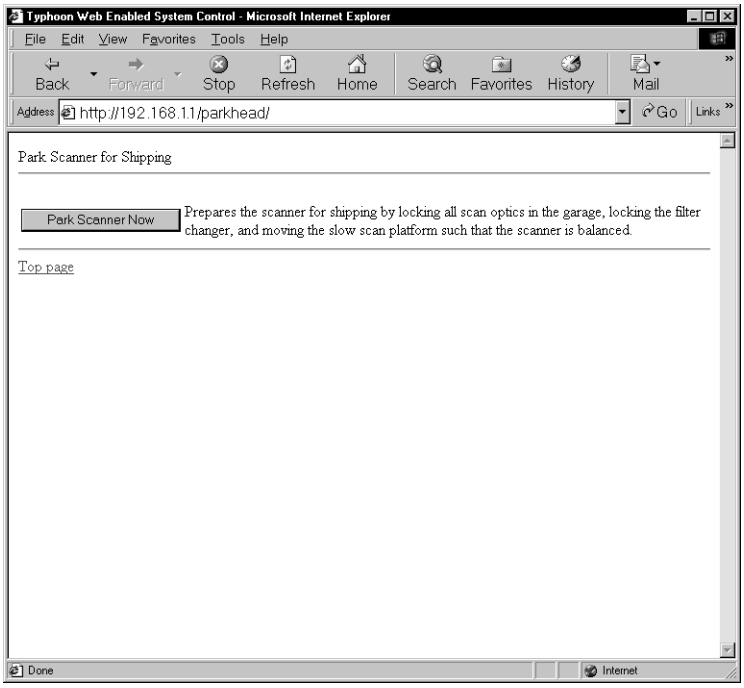


Figure 13-14. The Park Scanner for Shipping window.

13.8.3 Completing the installation after the move

If you connect the Typhoon instrument to a different computer than the one used previously, you must make sure that the computer has a dedicated Ethernet card to communicate with the Typhoon instrument. If you use an Ethernet connection for your local area network (LAN), you will need two Ethernet cards: one for communicating with the Typhoon instrument and one for communicating with your building network. See the following Web site for the Approved Computer list: www.amershambiosciences.com. For the Ethernet configuration procedure, see the same Web site and the instructions provided with the Ethernet cards. In addition, you must make sure the computer is running the Microsoft Windows operating system and that the Internet Explorer browser software is installed on the computer.

After the instrument and computer have been moved to the new location, you should—

1. Reconnect the instrument and computer.
2. Make sure you plug the instrument and computer into a surge protector, which is plugged into a properly grounded outlet.

3. Use the procedures in sections 3.1 and 3.2 to turn on the instrument and computer.
4. Use the procedure in section 3.3 to start the Scanner Control software.

   Scan a test sample to make sure the instrument is working properly. If the instrument is not working properly, contact Technical Support. See Assistance in the preface for contact information.

Chapter 14 Maintaining the Typhoon system accessories

This chapter provides information on the care and maintenance of the Typhoon system accessories. The topics in this chapter are—

- Cleaning the Image Eraser (section 14.1)
- Changing the bulbs in the Image Eraser (section 14.2)
- Changing the fuses in the Image Eraser (section 14.3)
- Cleaning and protecting the exposure cassettes (section 14.4)
- Cleaning and protecting the microarray slide holder (Typhoon 8610, 9210, and 9410 only) (section 14.5)
- Cleaning and protecting the gel alignment guides (section 14.6)

14.1 Cleaning the Image Eraser

Important **To protect your hands from the ethanol used in this procedure, you should wear gloves.**

The surface of the Image Eraser must be clean and free of contamination from radioactive samples 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. Do not allow liquid to seep into the Image Eraser. Use a damp cloth only. Do not pour or spray liquid over the surface. Make sure the eraser is totally dry before you reconnect the power cord.**

1. Turn off the Image Eraser and disconnect the power cord (figure 14-1).
2. Clean the surface of the eraser with a damp 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.

Caution **Do not use powdered detergents. Any undissolved particles can scratch the surface of the eraser.**

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

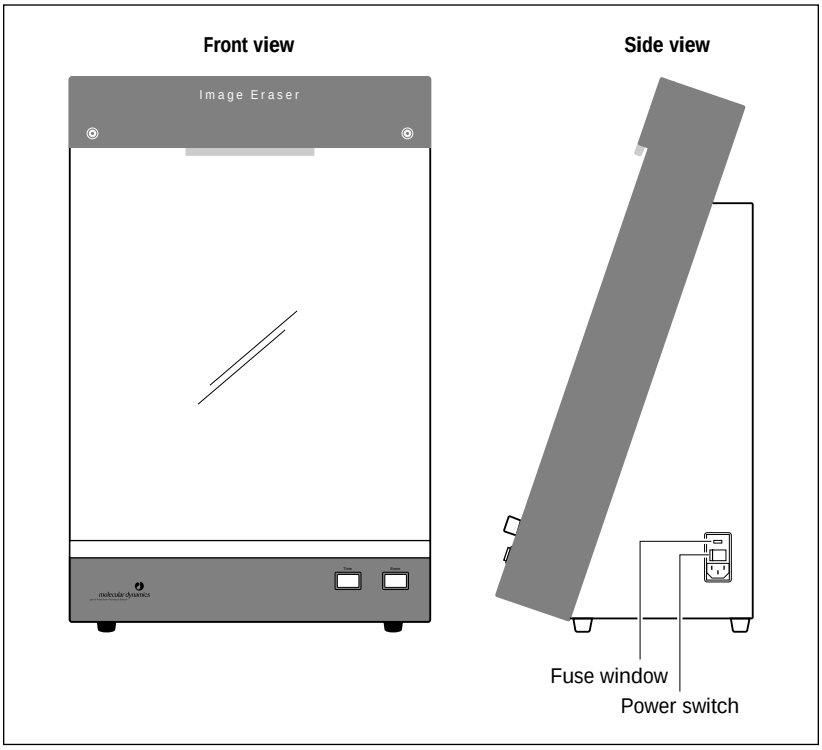


Figure 14-1. The Image Eraser.

14.2 Changing the bulbs in the Image Eraser

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

To replace the bulbs—

1. Turn off the Image Eraser and disconnect the power cord (figure 14-1).
2. Remove the two screws that hold the clip support and diffuser screen in place.
3. Lift off the clip support and diffuser screen.
4. Unscrew the bulb and fixture from the receptacle and replace the bulb and fixture with a new bulb and fixture (figure 14-2).

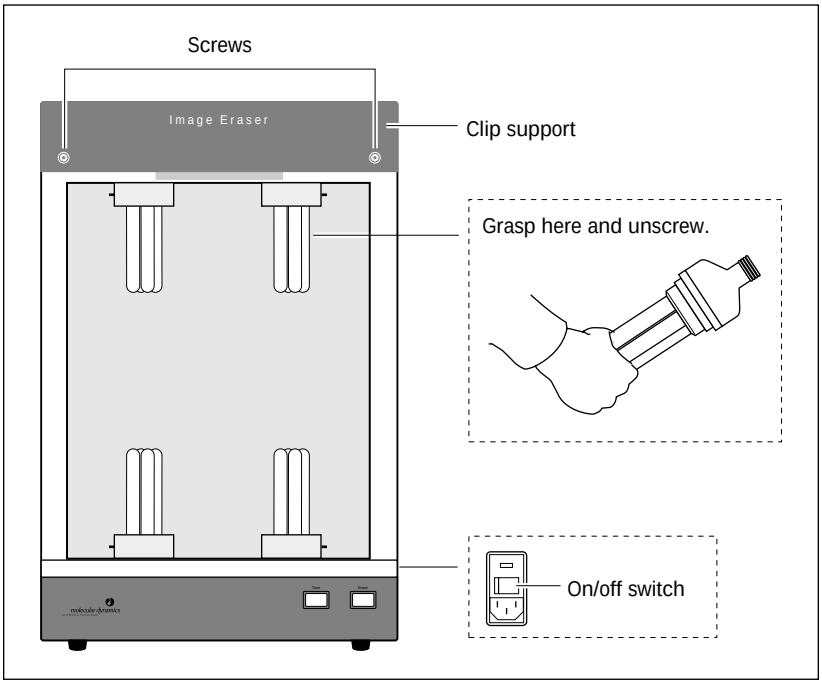


Figure 14-2. Removing a bulb from the Image Eraser.

5. Repeat step 4 to replace the three remaining bulbs and fixtures.
6. Replace the front diffuser screen and clip support, and reinsert the two screws.
7. Reconnect the power cord and turn on the Image Eraser (figure 14-1).

14.3 Changing the fuses in the Image Eraser

Warning

If the fuses must be replaced repeatedly, the Image Eraser could have an electrical problem. Do not use the Image Eraser. Contact 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 100–120 V (115 V) power source. The selected operating voltage of the Image Eraser appears in the fuse window on the lower right side of the eraser, next to the power switch (figure 14-1).

The fuse holder of the Image Eraser takes two 1.5A, 250V (⊟, T1.5A, 250V) slow-acting fuses. The fuse holder accepts both 0.25- × 1.25-in English fuses (designated 3AG fuses) and 5- × 20-mm metric fuses.

To change a fuse—

1. Turn off the Image Eraser and disconnect the power cord (figure 14-1).
2. Note the voltage (115V or 230V) showing in the fuse window (figure 14-3).
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 14-3).
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 may be blown. Replace each blown fuse with a new fuse of the same type and rating. Fuse specifications are listed on the serial number label located to the left of the fuse box.
7. Insert the fuse holder into the fuse box, making sure the correct voltage rating for your power supply is right side up.

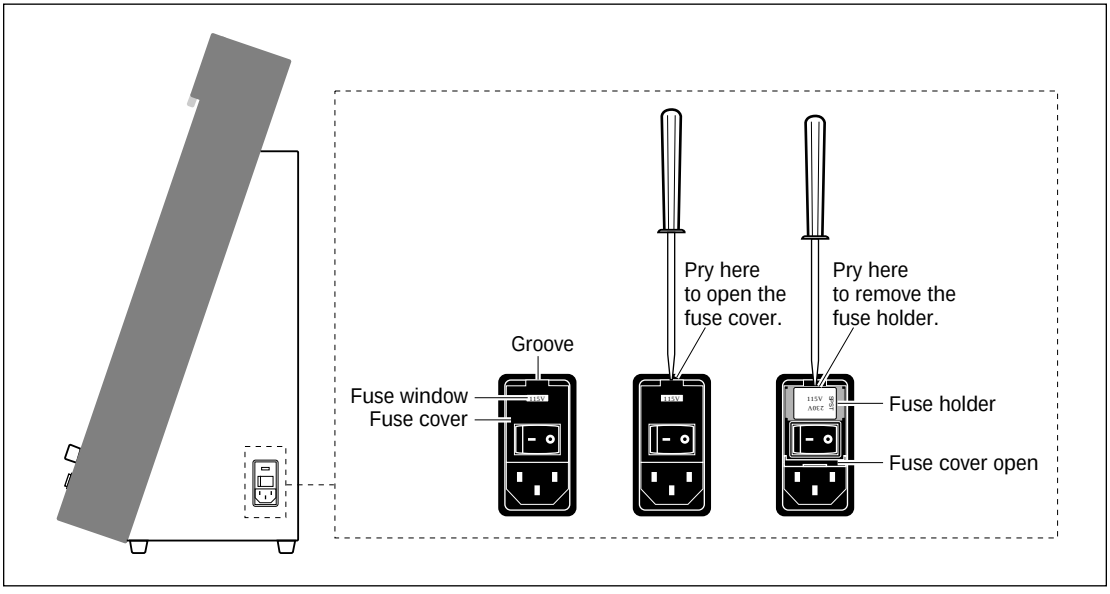


Figure 14-3. Opening the fuse box.

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.

Caution **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 14-1).

14.4 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.

Important **To protect your hands from the ethanol used to clean the exposure cassette, you should wear gloves.**

- Immediately before placing the sample in the cassette, clean the grid surface of the cassette with a damp cloth moistened with a small amount of distilled water. If visible spots still remain, clean the surface first with 75% ethanol and then with distilled water.

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.**

14.5 Cleaning and protecting the microarray slide holder (Typhoon 8610, 9210, and 9410 only)

To protect the microarray slide holder from contamination and damage, observe the following precautions and cleaning procedure:

- After each use, clean the microarray slide holder and slide restraints with a lint-free cloth moistened with distilled water.
- Store the microarray slide holder and slide restraints in a box or drawer lined with a lint-free cloth.

14.6 Cleaning and protecting the gel alignment guides

Caution **Exposing the gel alignment guides to high heat can damage the guides. For example, do not use an autoclave to clean the guides.**

To protect the gel alignment guides from contamination and damage, observe the following precautions and cleaning procedure:

- After each use, clean the gel alignment guides with a lint-free cloth moistened with distilled water.
- Store the gel alignment guides in the gel alignment guides storage box.

Part six

Appendixes

Appendix A Troubleshooting

If you are having problems with the Typhoon instrument or the Scanner Control software, use the troubleshooting sections below to locate the description that matches your problem. If you cannot find a solution, call 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 Typhoon instrument will not turn on.

- The instrument could be unplugged, or the surge protector switch could be turned off. Plug in the instrument or turn on the surge protector.
- The wall outlet could be faulty. Test the outlet or try another one.
- The fuse(s) could have blown. To change the fuse(s), see section 13.7.

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

- You could be using the wrong type of Ethernet cable, or the cable could be bad. The Typhoon instrument requires a crossover Ethernet cable. If you are using the cable, make sure you are using the red Ethernet cable supplied with the instrument.
- The Ethernet cable might not be properly plugged into the computer or the instrument. Check the connections to make sure they are plugged in and are securely fastened (figure A-1).
- If the New TCP IP Address window appears when you open Scanner Control, the IP address has changed. Type the correct address in the IP Address box and click **OK.** If you do not know the IP address, check with your network administrator.
- If the problem still occurs, contact Technical Support. See Assistance in the preface for contact information.

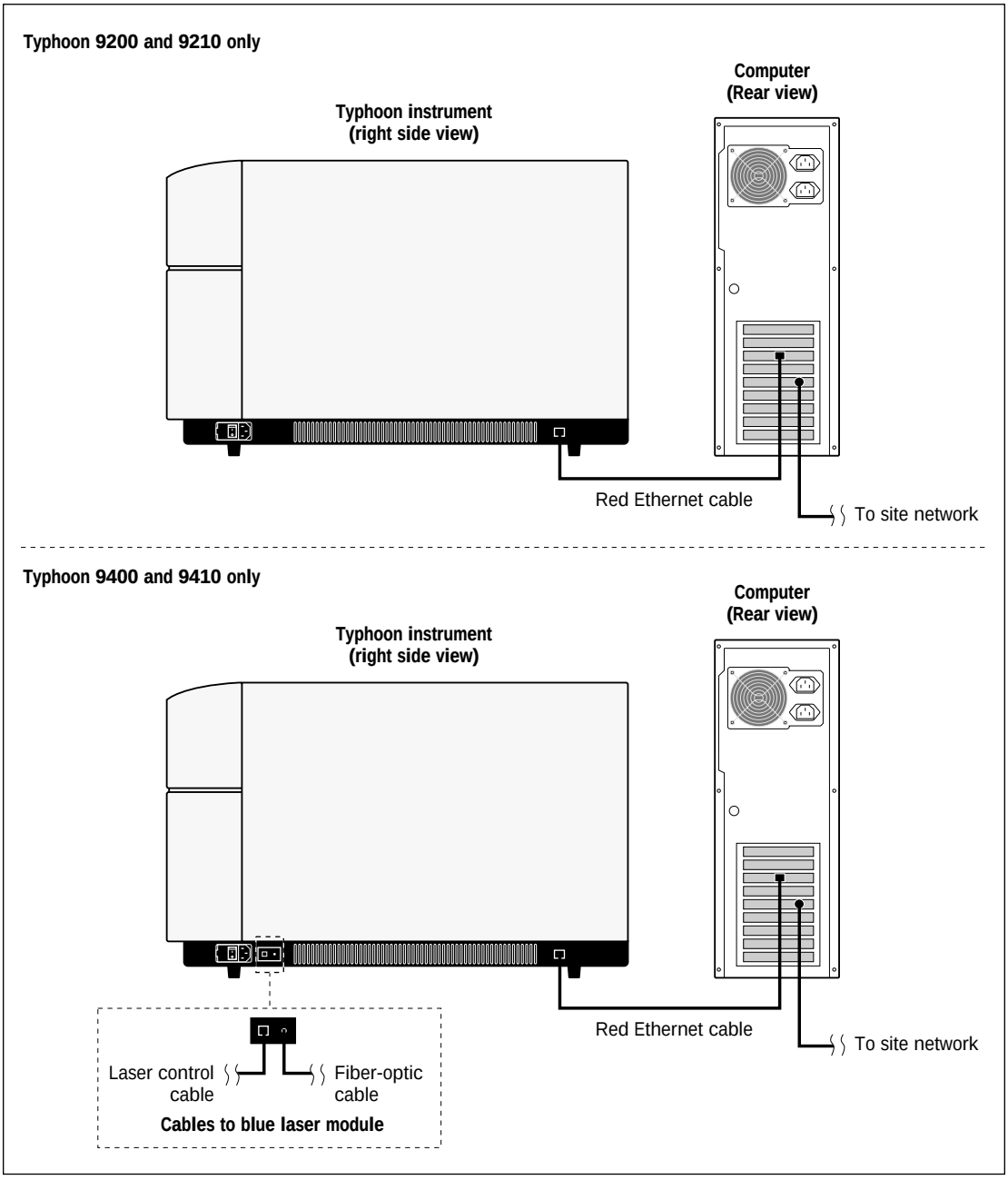


Figure A-1. Ethernet connections on the Typhoon instrument and computer.

(Typhoon 9400 and 9410 only) The Typhoon instrument is on, but the external blue laser module does not come on or the Connection light on the instrument is not lit.

- The laser communication cable is not connected between the instrument and the blue laser module. Alternatively, the cable might be the wrong type. You must use the blue Ethernet cable that was included with the instrument.
- The blue laser module is not plugged in or the wall outlet is faulty.
- If the problem still occurs, contact Technical Support. See Assistance in the preface for contact information.

(Typhoon 9400 and 9410 only) The Laser On light on the blue laser module is not lit.

- After you turn on the instrument or click the **Initialize Scanner** button, approximately 30 seconds elapse before the light turns on. If the Laser On light is not on after 30 seconds, contact Technical Support. See Assistance in the preface for contact information.
- Check the instrument state in the Scanner Control window. If it is in the Sleep state, click **Initialize Scanner** to turn on the laser.
- If the problem still occurs, contact Technical Support. See Assistance in the preface for contact information.

(Typhoon 9400 and 9410 only) The blue laser module does not turn off when you turn off the instrument.

The blue laser module continues to run for several minutes after you turn off the instrument. After cooling the laser, the module should turn off. However, if the blue laser module does not turn off after several minutes, contact Technical Support. See Assistance in the preface for contact information.

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 ImageQuant Preview window remains unchanged for at least 3 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 start 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, start the scan again. If the scan aborts again, turn off the power switch on the right side of the Typhoon instrument, wait a few seconds, and then turn the instrument back on. If the error happens repeatedly, contact Technical Support. See Assistance in the preface for contact information.

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 right side of the Typhoon instrument. Wait a few seconds and turn it on again.
3. Start the Scanner Control software, reselect the parameters, and rescan.

If the problem occurs repeatedly, contact Technical Support. See Assistance in the preface for contact information.

(Typhoon 9400 and 9410 only) The instrument will not scan using the blue laser.

Make sure the blue laser module is plugged in, and the cables are connected to the instrument. If the instrument state is Ready or the instrument is scanning, all three lights on the front of the module are lit.

If the instrument still will not scan using the blue laser, contact Technical Support. See Assistance in the preface for contact information.

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 Typhoon instrument has been turned off, allow 30 minutes to warm up the instrument.
- The instrument could be damaged and no longer light tight. If so, do not continue to use the instrument. Contact Technical Support to arrange for repair. See Assistance in the preface for contact information.
- Dust, fingerprints, or other dirt could be on the screen, sample, or glass platen. Clean the glass platen. 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 (storage phosphor), 8.2 (fluorescence), or 11.2 (chemiluminescence).
- 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 could have high autofluorescence. Use a low-fluorescence material (section 8.1.3).
- The wrong emission filters could have been used for a fluorescent sample (section 9.3.3).

The image is incomplete.

- The selected scan area in the Scanner Control window could be incorrect. See sections 6.5.3 (storage phosphor), 9.2.3 (fluorescence), or 12.2.3 (chemiluminescence).
- The signal emitted by a chemiluminescent sample could have diminished before the instrument finished scanning.
- The scan was aborted before it was complete, or an error occurred during the scan.

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

A light leak could 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 Typhoon instrument has been turned off, allow 30 minutes to warm up the instrument.
- Diagonal streaks could indicate a light leak during scanning. Check for damaged panels on the instrument. Contact Technical Support. See Assistance in the preface for contact information.
- The glass platen could be scratched. If possible, scan the sample on another portion of the glass. Contact Technical Support to order replacement glass platen and arrange for a service call. See Assistance in the preface for contact information.
- The storage phosphor screen could be contaminated. See section 5.2.3 for details.

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

- Static electricity could have accumulated on the screen. Clean the GP and LE screens with an intensifying screen cleaner. Do not attempt to clean the tritium (TR) screen.
- The screen could 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 could 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 will generate 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 could 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 could 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 platen. If the fingerprints are on the gel, rinse the gel briefly in 0.1% Tween™ or SDS. Rinse the gel in distilled 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. Make sure you dissolve agarose completely before pouring the gel. Clean the glass platen of the Typhoon 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 could 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 chemiluminescent sample contains streaks or other artifacts.

- Make sure the sample is flat on the glass platen of the Typhoon instrument and no air bubbles are trapped under the sample.
- Refer to the manufacturer's documentation for any advice about sample preparation.

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

- The screen could have moved after the initial placement in the exposure cassette. 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 could have moved 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 could have moved after the initial placement on the glass platen of the instrument. If fluorescent material has come off onto the glass platen, carefully remove the sample and clean the glass. Then place the sample on the glass platen again and do not readjust the placement.
- Remove excess liquid from gels so that the gels do not move on the glass platen.

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

The sample could have moved after the initial placement on the glass platen of the instrument. If chemiluminescent material has come off onto the glass platen, carefully remove the sample and clean the glass. Then place the sample on the glass platen again and do not readjust the placement.

The image created using the blue laser shows major fluctuations, or the signal disappears in the middle of the scan.

The blue laser could be overheating. Make sure you have adequate room on all sides of the blue laser module for ventilation (section 2.1).

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.
Exit	Closes the Scanner Control software.

Templates menu

Command	Description
Load	Displays a list of all the templates for the Typhoon 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 or chemiluminescence 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 Template(s) window, which lists all the templates, except the template displayed in the Scanner Control window and the Setup window (fluorescence and chemiluminescence only). Allows you to select and delete a template or templates.

Tray menu

Command	Description
Tray Editor	Displays the Tray Editor window, which allows you to define multiple scan areas for a scan and save the layout as a tray definition.

Help menu

Command	Description
User's Guide	Displays an electronic version (in PDF format) of the *Typhoon User's Guide*.
Quickstart	Displays an electronic version (in PDF format) of the *Typhoon Instrument QuickStart* card.
About	Displays the About Typhoon 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 parameters are used in the Scanner Control software:

Scanner Control window

Parameter	Description
Scanner Information area	Displays the model number, IP address, and serial number of the Typhoon instrument.
Instrument State area	Displays the current state of the instrument. The states are Ready, Warming Up, Sleep, Initialization, and Scanning. If Service appears, call Technical Support for service. See Assistance in the preface for contact information.
Instrument State area: Initialize Scanner button	Allows you to begin warming up the instrument before starting the scan. Resets the sleep time.
Template name	Displays the template name for the parameters displayed in the Scanner Control window and Setup window (fluorescence and chemiluminescence only). If no template is selected, the default parameters appear in the windows.

Parameter	Description
Scan area (grid)	If User Select is selected in the Tray area, displays the selected scan area (in white) and allows you to change the selected area by redrawing the white rectangle. If other than User Select is selected in the Tray area, displays the multiple scan areas for the selected tray definition.
Setup area: User Name	Displays the current user name.
Setup area: Acquisition Mode list	Allows you to select the scan acquisition mode. The choices are Storage Phosphor, Fluorescence, and Chemiluminescence.
Setup area: Tray area	Allows you to select a tray definition containing multiple scan areas. Allows you to select which predefined areas to scan.
Setup area: Phosphor Mode area: Best Sensitivity button	Available in the storage phosphor mode only. Allows you to use full laser power to achieve the best sensitivity.
Setup area: Phosphor Mode area: Best Resolution button	Available in the storage phosphor mode only. Allows you to lower the laser power to achieve greater resolution.
Setup area: Setup button	Available in the fluorescence and chemiluminescence modes. Depending on the selected acquisition mode, displays the Setup window for fluorescence or chemiluminescence.
Setup area: Options area: Orientation button	Click to display the eight sample orientation parameters. Allows you to select the parameter that matches how you placed the sample on the glass platen of the instrument or on the storage phosphor screen.
Setup area: Options area: Press Sample check box	Available in the fluorescence and chemiluminescence modes. Allows you to select that you want the sample pressed during scanning. (Pressing is automatic in the storage phosphor mode.)
Setup area: Options area: Pixel Size list	Displays a list of pixel size parameters. The selections are 1 000 microns, 500 microns, 200 microns, 100 microns, 50 microns, and 25 microns. (Typhoon 8610, 9210, and 9410 only) The 10 microns parameter is also available.

Parameter	Description
Focal Plane area	Available in the fluorescence and chemiluminescence modes. Allows you to change the focal plane of the laser and collection optics to match the sample type. You can select either Platen (sample is on glass platen) or +3 mm (sample is 3 mm higher than glass platen).
Setup area: User Comment box	Allows you to save comments (maximum 4 000 characters) with the image file.
Setup area: Image Analysis list	Allows you to select the image analysis software to open after scanning. Select None to leave Scanner Control active.
Setup area: DIGE File Naming Format	Allows you to use the DIGE file naming format for image file names.
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.

Fluorescence Setup window

Parameter	Description
Use check box	Allows you to select the number of scans to perform. Also displays scans that are linked.
Image box	Displays the order in which the scans will be performed by the Typhoon instrument.
Emission Filter list	Allows you to select the emission filters to use for each scan. After you select the emission filter, Scanner Control determines the most appropriate laser to use with the selected filter.
PMT Voltage list	Allows you to select the PMT voltage to use for each scan. Values range from 0 to 1 000 and are in 5-V increments.

Parameter	Description
Laser list	Allows you to change the laser that Scanner Control selected as the most appropriate laser to use with the emission filter. You select either Green (532) or Red (633). (Typhoon 9400 and 9410 only) Two additional laser parameters appear in the list: Blue (457) and Blue (488).
Sensitivity list	Allows you to select the sensitivity (limit of detection) to use for each scan. Normal collects one data point from each pixel. Medium collects four data points from each pixel and averages the results. High collects eight data points from each pixel and averages the results.
Beamsplitters list	After you link the scans, displays the beamsplitter you should use for the linked scan. Allows you to change the selected beamsplitter.
Link With area: Scan buttons	Allows you to select the scans you want to link. Scanner Control displays brackets that connect the Use boxes of the linked scans.
Link With area: Auto-Link button	Allows you to link the scans automatically. Scanner Control links the optimal scans, displays brackets connecting the Use check boxes of the scans, and renumbers the Image boxes.
Link With area: UnLink All button	Allows you to remove the selected links. Scanner Control removes the brackets connecting the Use check boxes of the scans and renumbers the Image boxes.
Link With area: Auto Link Mode: Sensitivity	Allows you to decrease the cross talk between fluorochromes for linked scans.
Link With area: Auto Link Mode: Speed	Allows you to decrease the total scan time for the linked scans.
Disable Link Warning check box	Allows you to turn off the warning message that appears if you select nonoptimal links.
Cancel button	Closes the Fluorescence Setup window without saving any changes.
OK button	Saves the changes and closes the Fluorescence Setup window.

Chemiluminescence Setup window

Parameter	Description
Sensitivity list	Allows you to select the sensitivity (limit of detection) to use for each scan. Normal collects one data point from each pixel. Medium collects four data points from each pixel and averages the results. High collects eight data points from each pixel and averages the results.
Beamsplitters list	Allows you to select a beamsplitter if you want to perform a multichannel scan. If you are performing a single scan, leave the parameter at None.
PMT Voltage list	Allows you to select the PMT voltage to use. Values range from 0 to 1 000 and are in 5-V increments. If you selected None in the Beamsplitter list, you see one PMT Voltage list box. If you selected a beamsplitter, you see two PMT Voltage list boxes.
Cancel button	Closes the Chemiluminescence Setup window without saving any changes.
OK button	Saves the changes and closes the Chemiluminescence Setup window.

Tray Editor window

Parameter	Description
Tray area: Tray definition list	Displays the list of tray definitions and number of sample scan areas.
Tray area: New Tray button	Displays the New Tray window, which allows you to type a name for a new tray definition.
Tray area: Copy Tray button	Displays the Copy Tray window, which allows you to create a new tray definition from an existing one.
Tray area: Delete Tray button	Allows you to delete the selected tray definition from the Tray area.
Sample area: Sample definition list	Displays the sample scan areas for the selected tray definition.
Sample area: New Sample button	Displays the New Sample window, which allows you to select the scan area for each sample.
Sample area: Edit Sample button	Displays the Edit Sample window, which allows you to modify the scan area for the selected sample.
Sample area: Delete Sample	Allows you to delete the selected sample scan area.
Sample area: Disable (Hide in Scanner Control) check box	Allows you to hide the selected tray definition from the Tray list.
Preview button	Displays the Preview Sample Tray window, which allows you to view the sample scan areas using the scan grid.
Save Current Tray button	Allows you to save the tray definition.
Close button	Closes the Tray Editor window.

Multiple Sample Naming window

Parameter	Description
Sample file name list	Displays a list of the sample file names and the file sizes.
Edit Sample File Name	Displays the Save As window or the DIGE File Naming Format window, which allows you to type a new name for the selected file name.
Use Common Setting for All Samples: Folder box	Displays the name of the folder you selected for storing the new image files.
Use Common Setting for All Samples: Folder Browse button	Displays the Save folder to window, which allows you to select the folder in which you want to store the new image files.
Use Common Setting for All Samples: Folder Set button	Allows you to save the selected folder as the storage location for the new image files.
Use Common Setting for All Samples: Base File Name box	Allows you to type a base file name to use for the new image files. The software appends a number to the base file name to make each file unique.
Use Common Setting for All Samples: Base File Name Browse button	Displays a window, which allows you to select an existing file name to use as the base file name. The software appends a number to the base file name to make each file unique.
Use Common Setting for All Samples: Base File Name Set button	Allows you to save the base file name for the new image files.
Cancel button	Closes the Multiple Sample Naming window without saving the changes or starting the scan.
Scan button	Saves the changes, closes the Muliple Sample Naming window, and starts the scan.

DIGE File Naming Format window

Parameter	Description
Dataset Name box	Displays the path and dataset name that will be used for the image files.
Edit button	Displays a window that allows you to type a new dataset name. The new dataset name appears in the Dataset Name box and all the Image boxes.
Image 1 box	Allows you to type a new file name for Image 1.
Image 2 box	Allows you to type a new file name for Image 2.
Image 3 box	Allows you to type a new file name for Image 3.
Image 4 box	Allows you to type a new file name for Image 4.
Standard button	Allows you to select one image to use as a standard.
NONE button	Allows you to select no image as a standard.
OK button	Saves the changes and closes the DIGE File Naming window.
Cancel button	Closes the DIGE File Naming window without saving any changes.

Appendix C 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.

Glossary

.dir—the folder that contains the individual image files for a multichannel dataset. The name of the .dir folder is the same as the .ds file name.

.ds—the file extension for a multichannel dataset.

.gel—the file extension of a single-channel image.

absorption—the energy transfer from a photon of light to a dye molecule.

acquisition mode—the type of scan. Choices are storage phosphor, fluorescence, and chemiluminescence.

band-pass filter—an optical filter that allows light of wavelengths between two specified wavelength cutoffs to pass through. The filter rejects most of the light with wavelengths shorter than the first cutoff or longer than the second cutoff.

channel—one image in a set of images that make up a multichannel image. For a chemiluminescent sample, two channels are created using a beamsplitter. For a fluorescent sample, two, three, or four channels are created using a combination of fluorescent dyes, emission filters, and beamsplitters.

chemiluminescence—the emission of light by a dye molecule as a result of a chemical reaction.

cross talk—the interference from one signal collected by the Typhoon instrument on another signal collected by the instrument when scanning a multilabel fluorescent image. Also referred to as cross contamination.

dataset—the files and folders that make up a multichannel image.

default template—the template and parameters that appear in the Scanner Control window when the software is first opened.

electrophoresis glass plate—a 3-mm-thick, low-fluorescence piece of glass used to create sandwich gels.

emission—the release of light from a dye molecule when an electron falls from an excited to a lower energy state.

emission spectrum—a plot of emitted light wavelength versus relative intensity of emitted light.

excitation—the absorption of light energy by a molecule so that an electron is boosted to a higher-level energy state.

excitation spectrum—a plot of incident light wavelength versus relative intensity of emitted light.

fluorescence—the emission of light by a dye molecule as a result of absorption of laser light.

GP screen—general-purpose storage phosphor screen.

glass platen—the area of the Typhoon instrument on which you set the sample or storage phosphor screen.

grid coordinates—the markings on the exposure cassette and glass platen of the instrument that allow you to position the sample. The coordinates correspond to markings in the Scanner Control window.

LE screen—low-energy storage phosphor screen.

link—the process of creating two images with one scan. Uses emission filters and beamsplitters to separate and collect light of two different wavelengths.

long-pass filter—an optical filter that allows light of wavelengths longer than a specified cutoff to pass through. The filter rejects most of the light with wavelengths shorter than the cutoff.

multichannel image—a set of images that can be viewed as a composite (overlaid) or viewed as individual images (side by side). A chemiluminescent sample can be used to create a multichannel image consisting of two images. A fluorescent sample can be used to create multichannel images consisting of two, three, or four images. Each image is called a channel. Also referred to as a multilabel image.

photomultiplier tube (PMT)—an electro-optic device in the Typhoon instrument that converts light energy into electrical current and amplifies the current.

pixel size—the size of each individual picture element that is recorded and, together with the thousands of other pixels, forms the image.

quantitation—the process in which signal intensities of the sample and other values are calculated.

sample lid—the underside of the cover of the Typhoon instrument.

short-pass filter—an optical filter that allows light of wavelengths shorter than a specified cutoff to pass through. The filter rejects most of the light with wavelengths longer than the cutoff.

spectral separation—the software process that removes the unwanted signals present in each channel of a multichannel image.

Stokes shift—the difference, in wavelength, between the apex of the excitation spectrum (shorter wavelength, higher energy) and the apex of the emission spectrum (longer wavelength, lower energy).

storage phosphor screen autoradiography—the emission of light by a radioactive molecule, which is collected on a phosphor-coated screen instead of on film.

template—scan parameters that are saved in Scanner Control using a unique name. Templates can be saved for storage phosphor, fluorescence, and chemiluminescence.

TR screen—tritium storage phosphor screen.

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