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Molecular Dynamics™

PHOSPHORIMAGER® SI

USER'S GUIDE

for APPLE® MACINTOSH®
and MICROSOFT® WINDOWS NT™

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Part 1

Introduction and Procedures

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Chapter 1 Introduction to the PhosphorImager SI

The PhosphorImager SI scanning instrument is a compact laser-optical imager that uses innovative storage phosphor technology to detect and quantify radioactivity. It connects to the following computers:

- A Microsoft® Windows NT™-based computer containing ImageQuaNT™ and Scanner Control software.
- An Apple® Macintosh® computer containing image analysis software and Scanner Control software.

1.1 What You Can Do with the PhosphorImager SI

With the PhosphorImager SI you can:

- Use the Scanner Control software to scan a full 20 x 25 cm sample in approximately four minutes using 200µ resolution.
- Use image analysis software to analyze and manipulate the data generated by the scans.
- Create image files that can be transferred to compatible graphics programs on Macintosh, Windows™, and Windows NT computers to generate image prints and reports.

1.2 The PhosphorImager SI Equipment

The PhosphorImager SI equipment includes the following hardware components (figure 1-1):

- PhosphorImager SI—scans and analyzes samples.
- Phosphor screen—collects the image.
- Exposure cassette—holds the screen in a light-tight environment.
- Image Eraser SF™—erases screens for reuse.

Additional accessories include a SCSI cable, a SCSI terminator, and a power cord.

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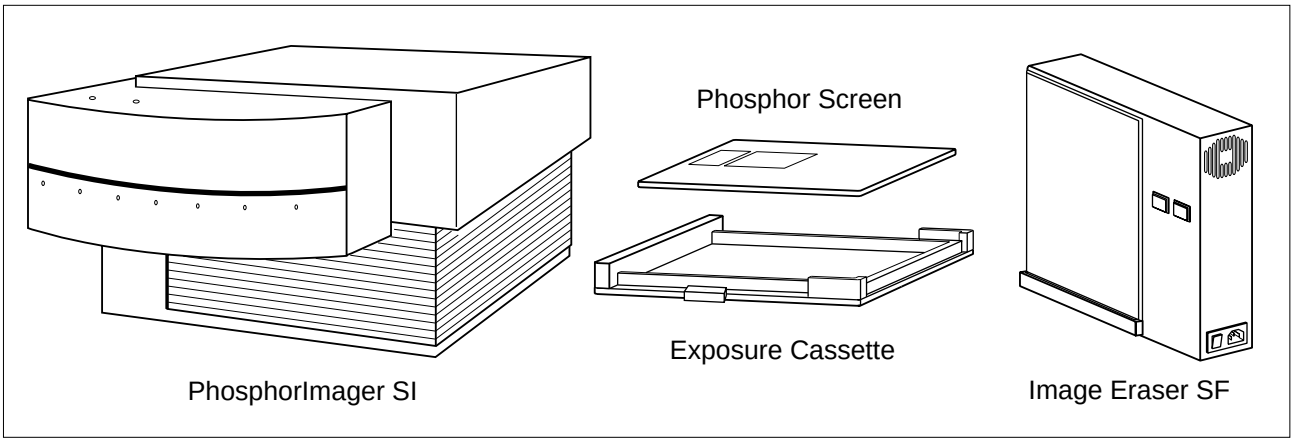


Figure 1-1. The PhosphorImager SI and accessories.

1.3 Advantages of Phosphor Screen Autoradiography

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 250 times that of film, depending on the isotope used and the sample type, such as membrane, gel, or TLC (thin-layer chromatography) plate.
- The linear dynamic range is 40 times better than film.
- Exposure takes place at room temperature.
- The phosphor screens are reusable.
- No chemicals, darkroom, or special treatment is required.
- Results can be analyzed qualitatively and quantitatively using image analysis software.

1.4 How the Phosphor Screen Works

Phosphors are compounds that absorb energy at one wavelength and re-emit energy at another. Storage phosphors release stored energy when stimulated by light of particular wavelengths. The Molecular Dynamics phosphor screens are composed of fine crystals of BaFBr:Eu^{+2} in an organic binder.

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When the phosphor screen is placed against the radioactive sample, high-energy radiation from the sample excites Eu^{+2} electrons to a state in which they move easily within the phosphor. The electrons become trapped in "F-centers"—believed to be empty sites in the $BaFBr^{-}$ crystal lattice. During the exposure process, Eu^{+2} is oxidized to Eu^{+3}, and BaFBr is reduced to $BaFBr^{-}$. These compounds remain oxidized and reduced after the phosphor screen is removed from the sample. In this way, the phosphor "stores" the energy from ionizing radiation. The screen retains more than 80% of the signal after three hours.

The PhosphorImager SI scans the screen, using a helium-neon laser that emits red light at 633 nm. Because the charged $BaFBr^{-}$ complexes absorb light in the 600-nm range, the screen absorbs energy from the laser, freeing electrons and reducing Eu^{+3} to Eu^{+2*}. As Eu^{+2*} returns to the ground state, it releases energy in the form of a blue light, which can be collected and measured. The light is proportional to the radioactivity in the sample.

1.5 How the PhosphorImager SI Works

The PhosphorImager SI scans and processes samples in the following sequence:

- The operator slides the exposed screen face down onto the PhosphorImager SI stage.
- The stage moves the screen past a helium-neon laser beam. At the same time, the galvanometer-controlled mirror directs the beam, moving it across the screen in a uniform fashion.
- When the laser beam hits a section of the phosphor screen that was exposed to radioactivity, the phosphor emits blue light as described in section 1.4.
- A fiber-optics bundle collects the blue light and passes it to a photomultiplier tube (PMT). The PMT converts the light to an electrical current, which varies with the intensity of the light hitting the PMT.
- The analog signal from the PMT is converted into digital information and stored on the computer hard disk.

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- The software maps the information to the appropriate pixel location on the computer screen to produce an image of the original radioactive sample.
- Image analysis software enables the operator to quantitate variations in the signal. The level of signal is proportional to the amount of radioactivity.

1.6 Overview of the Autoradiography Process

Autoradiography with the PhosphorImager SI is a six-step process:

1. Prepare the screen for exposure by cleaning and erasing it.
2. Place the sample in the exposure cassette.
3. Place the screen over the sample in the cassette and leave it for the amount of time necessary to expose the screen.
4. After removing the screen from the exposure cassette, place the screen in the PhosphorImager SI.
5. Initiate the scanning process using the Scanner Control software. The PhosphorImager SI scans the screen and produces a digitized image on the monitor.
6. Use image analysis software to obtain quantitative measurements.

1.7 Before You Begin: Protect Your Equipment

Before you use the storage phosphor screens and exposure cassette, learn how to protect them from damage by reviewing chapters 6 and 7 of this manual.

Caution **Do not use scintillants or enhancers on your sample. These compounds interfere with the proper operation of the phosphor screens.**

1.8 Important Safety Information

Sections 8.6 and 9.2 provide important information on the safety precautions you should follow when using the Image Eraser SF and the PhosphorImager SI.

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Chapter 2 Sample Exposure Procedures

This chapter provides step-by-step instructions for exposing the sample.

2.1 Clean the Phosphor Screen

When cleaning the phosphor screen, be sure to handle the screen on the edges or back only.

1. Use a soft cotton cloth and Kodak™ Intensifying Screen Cleaner to remove dust, foreign material, and static electricity.

2. Check for radioactive contamination. If any is found, clean the screen using a totally rinsable detergent.

Refer to chapter 6 for additional information on cleaning the phosphor screen.

2.2 Clean the Eraser

Warning **Because the Image Eraser SF uses high voltage, always turn the power off before cleaning the surface.**

1. Turn the power off by pressing down the end of the power switch that displays the "O" symbol. (The power switch is located on the right side of the Eraser, on the edge of the frame.)

2. Clean the surface of the Eraser with a damp cloth.

3. Check for radioactive contamination, then reclean the Eraser if necessary.

See chapter 8 for additional information on cleaning the Eraser.

2.3 Erase the Phosphor Screen

You use the Image Eraser SF to erase the phosphor screen.

1. Holding the screen by its edges, and with the white side facing the Eraser, place the bottom edge of the screen into the grooved

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screen holder (figure 2-1). A clip inside the holder secures the screen.

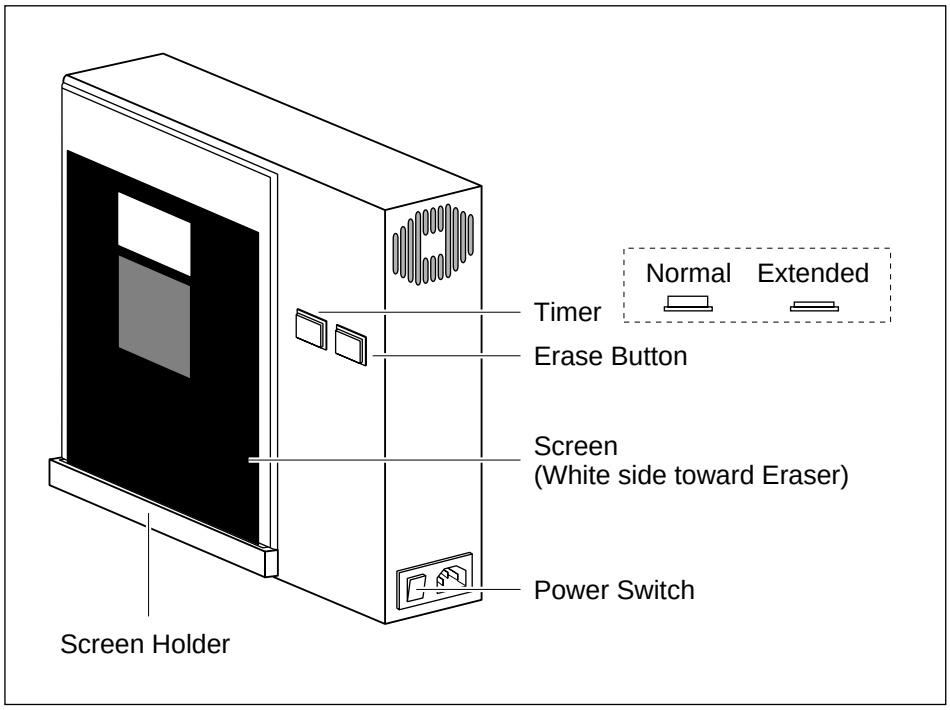


Figure 2-1. Erasing the phosphor screen.

2. Set the erase time: Leave the timer set to **Normal** for typical samples; use the **Extended** setting (button pressed in) if the background or residual image is high (for example, a saturated image with a reading of 10,000 counts). The Normal setting takes three minutes; the Extended setting takes six minutes.

3. Press the **Erase** button.

See chapter 8 for additional information on using the Eraser.

2.4 Place the Sample in the Exposure Cassette

Caution **Do not put uncovered wet gels in the exposure cassette. See section 6.4.2 for information on exposing wet gels.**

1. Clean the plastic surface inside the exposure cassette with a damp cloth and remove any radioactive contamination.

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2. Place the sample **right side up** on the grid. Placing the sample toward the top of the grid minimizes scan time.

3. Make a note of—

   - The coordinates of the **upper left** and **lower right** corners of your sample (figure 2-2).

   - The total number of squares partly or fully covered.

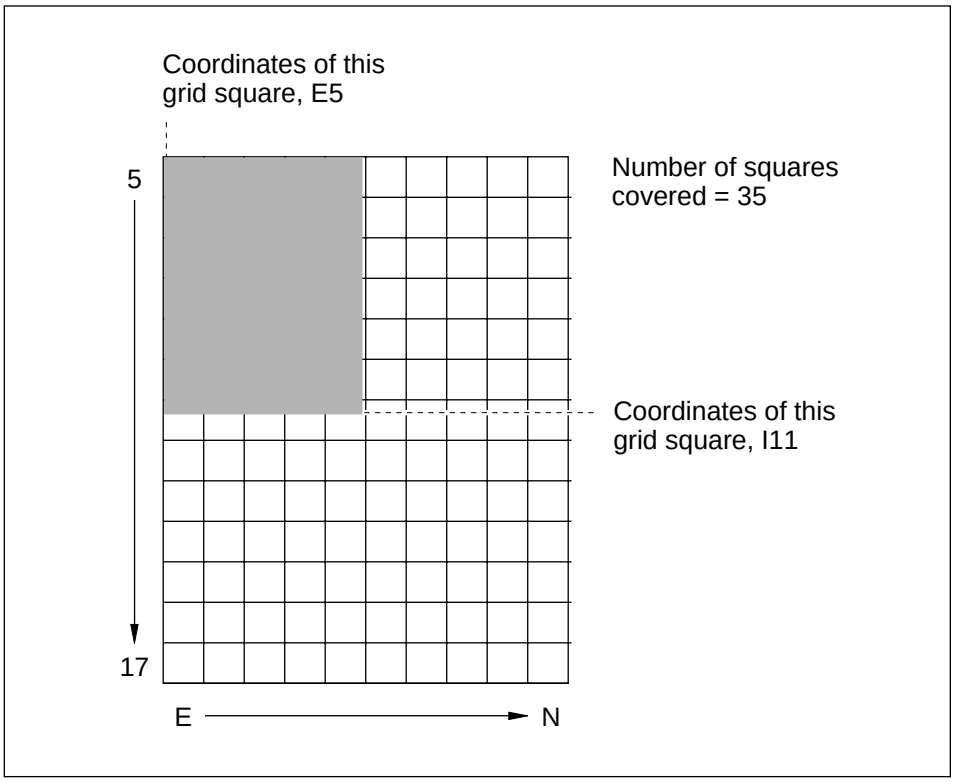


Figure 2-2. Finding the coordinates of the grid squares.

2.5 Place the Screen in the Exposure Cassette

Phosphor screen autoradiography is carried out at room temperature. Exposing the screen at sub-zero temperatures is inappropriate.

Important **Be sure to place the screen in the correct position when you first set it down. Because the screen is extremely sensitive, adjusting the position may result in a double image.**

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To place the screen in the exposure cassette—

1. Make sure the clamp on the cassette is 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. Place the edge of the screen into the groove.

   The **white** side of the screen should face **down,** toward the sample. The **top of the label** on the screen should be **toward the top** of your sample (figure 2-3).

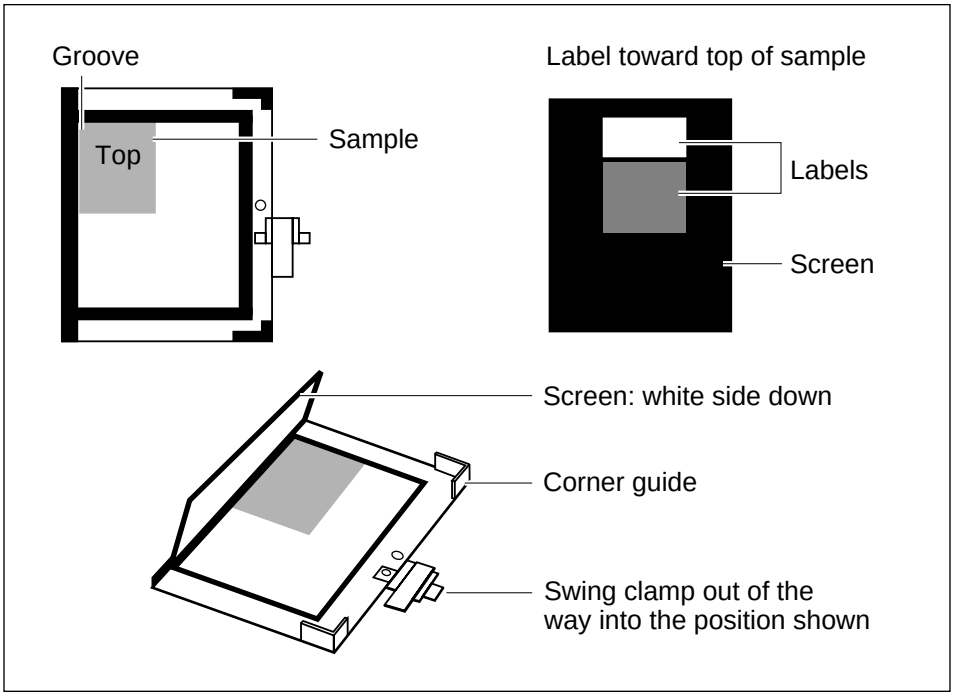


Figure 2-3. Loading the exposure cassette.

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

5. Close the exposure cassette as follows:

   - Rotate the clamp counterclockwise until it stops at the pin (figure 2-4).

   - Flip the lever over to lock the screen into place.

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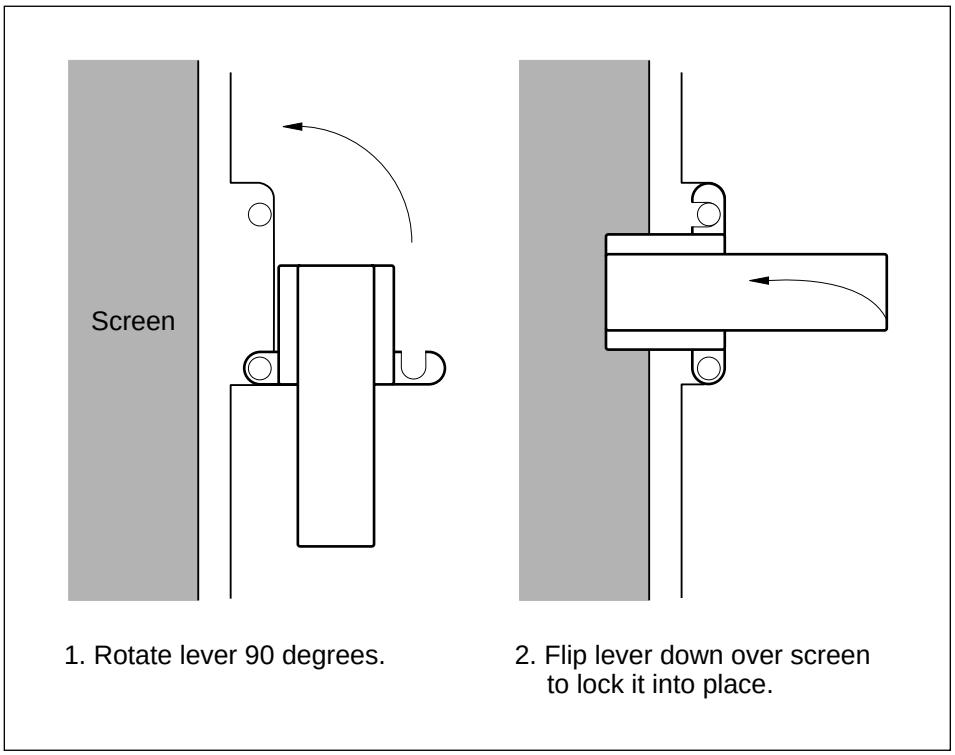


Figure 2-4. Closing the exposure cassette.

Exposure time. As a starting point, try using one-tenth of the exposure time you normally use for x-ray autoradiography.

Warm-up time. If the PhosphorImager SI has been turned off, plan to let it warm up for 20 to 30 minutes before removing the screen from the exposure cassette and initiating the scanning process. (See the instructions in chapter 3 to turn on the PhosphorImager SI.)

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Chapter 3 Scanner Setup Procedures

This chapter explains how to prepare the PhosphorImager SI and place the screen in the scanner.

Instructions for scanning the sample are located in chapter 4 for a Windows NT-based computer and in chapter 5 for a Macintosh computer.

3.1 Turn on the PhosphorImager SI

You must turn on the PhosphorImager SI and wait at least 10 seconds before you turn on the computer. To turn on the PhosphorImager SI, press the ON/OFF switch on the back of the instrument (figure 3-1). The power indicator light on the top of the PhosphorImager SI turns on. (If you have more than one instrument connected to the host computer, turn them all on before turning on the computer.)

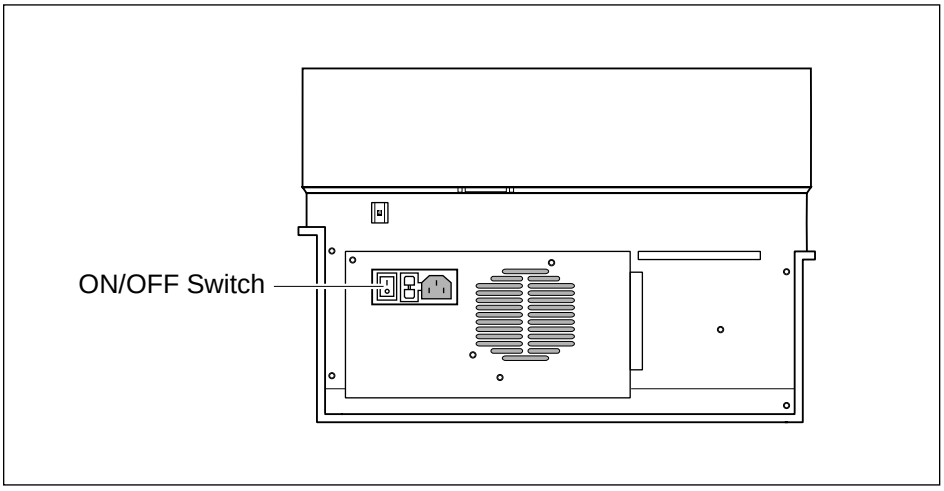


Figure 3-1. Back view of the PhosphorImager SI.

3.2 Turn on the Computer and Open Scanner Control

Use the following instructions to turn on the computer and open the Scanner Control software. (You must open the Scanner Control software to begin the instrument warmup.)

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3.2.1 Windows NT-Based Computer

Turn on the computer and monitor according to the manufacturer's directions. When directed, press **CTRL+ALT+DEL** to access the Windows NT Welcome window. If necessary, enter a user name and/or password and click on **OK.** After the Program Manager window opens, double-click on the MD Apps icon, then double-click on the Scanner Control SI icon in the MD Apps window. (See the *ImageQuaNT User's Guide* for more detailed instructions.)

3.2.2 Macintosh Computer

Turn on the computer and monitor according to the manufacturer's directions. Double-click on the hard disk icon to display the contents of the hard disk. Double-click on the Scanner Control SI icon or name in the window.

3.3 Warm Up the PhosphorImager SI

The PhosphorImager SI needs 20 to 30 minutes to warm up before you can scan a sample. The warm-up process does not begin until you open the Scanner Control window.

3.4 Remove the Screen from the Cassette

Important **When transferring the screen from the exposure cassette to the PhosphorImager SI, keep the screen phosphor-side down in subdued light. Exposure to light may erase part of the signal on the screen.**

1. Flip up the lever on the cassette (figure 3-2).

2. Rotate the clamp clockwise so that it no longer covers the screen.

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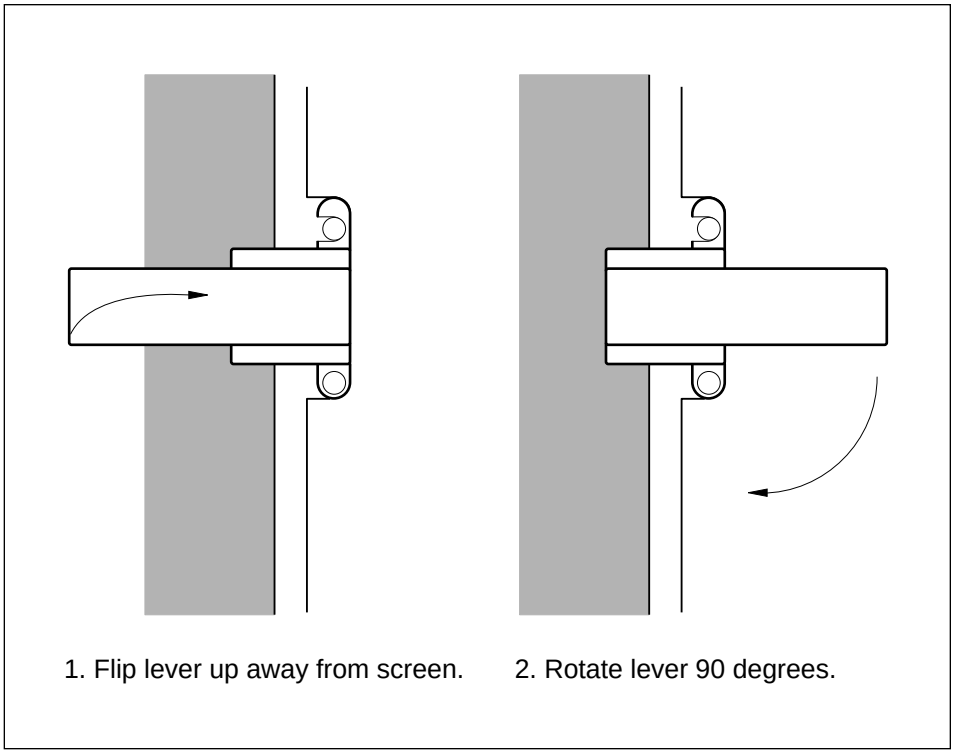


Figure 3-2. Opening the exposure cassette.

3. Remove the screen.

4. Immediately transfer the screen to the PhosphorImager SI, keeping the screen face down during the transfer. (Remember to remove your sample from the cassette.)

3.5 Load the Screen

When loading the screen hold the screen with the **top of the label** toward the **back** of the instrument.

1. Open the door of the PhosphorImager SI.

2. Holding the screen by its edges, press it up against the blue tab, rotating the tab out of the way, then slide the screen to the left and press it against the left rail (figure 3-3).

3. Make sure the screen is approximately level, then slide it into the machine, maintaining an upward pressure against the blue tab until the screen feels like it is sliding in straight (figure 3-3).

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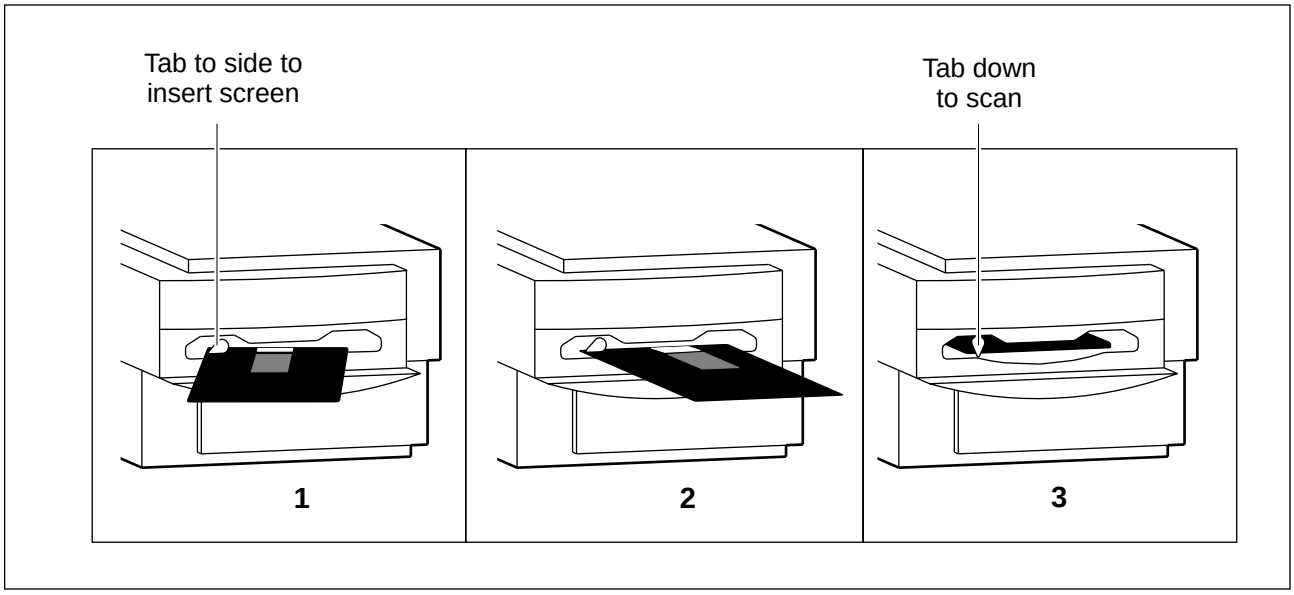


Figure 3-3. Inserting the phosphor screen into the PhosphorImager SI.

4. Push the screen into place until the tab drops down to its original position. (The door will not close unless the tab is down.)

5. Close the door of the PhosphorImager SI. The screen is ready to scan.

Important **Keep the door of the PhosphorImager SI closed during scanning. Opening the door during the scan will abort the scan and invalidate the data.**

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Chapter 4 Windows NT Scanning Procedures

This chapter provides step-by-step instructions for scanning a sample from a Windows NT-based computer. The instructions assume you have performed the steps in chapters 2 and 3. You use the Scanner Control software (figure 4-1) to initiate the scan process. You do not need to calibrate the PhosphorImager SI; calibration is automatic.

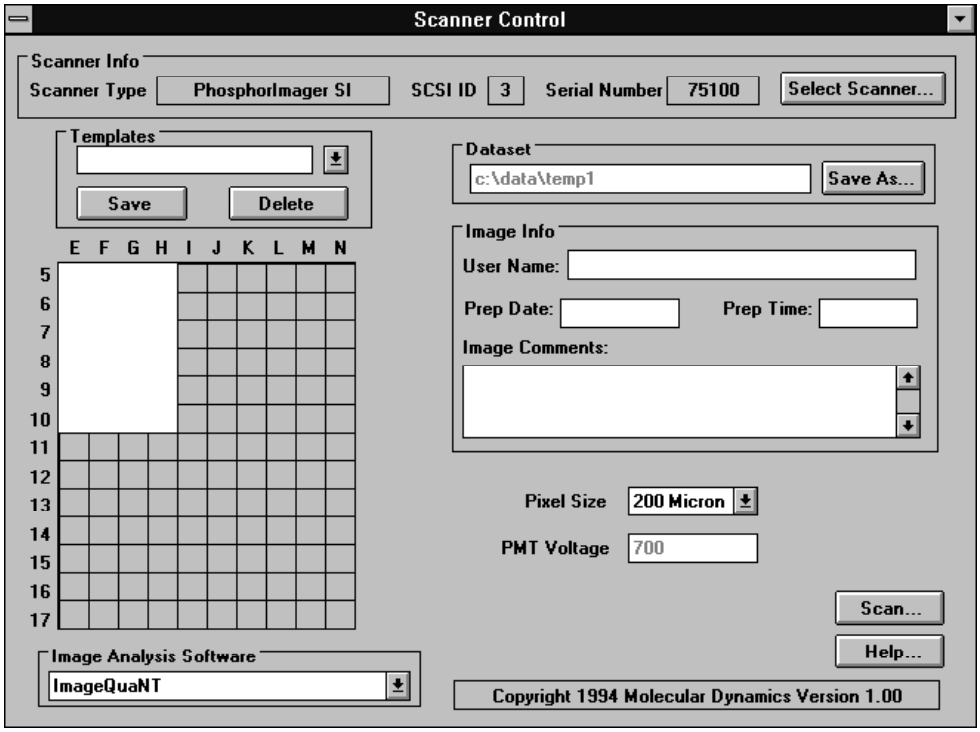


Figure 4-1. The Windows NT Scanner Control window.

4.1 Check Disk Space

The Scanner Control software automatically checks for sufficient disk space when you start the scan. However, you can check disk space manually before starting the scan. If necessary, you can then transfer or remove files to make room for your scan. To check disk space, use the formula in section 11.10 or draw the Scan area (section 4.4), then click on the **Help** button to display the amount of space needed.

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4.2 Use a Template

You can create a template that defines the Scan area, analysis software to use, pixel size, and scanner information. You can then use the template for subsequent scans of similar samples. To create or use a template, see section 11.9.

4.3 Select a Scanning Instrument

The top of the Scanner Control window displays the instrument currently accessed by the computer. If you want to switch to a different instrument—

1. Click on the **Select Scanner** button. The Select Scanner window opens (figure 4-2).

2. Click on the correct instrument to highlight it.

3. Click on **OK.** The new scanner is selected and the Select Scanner window closes.

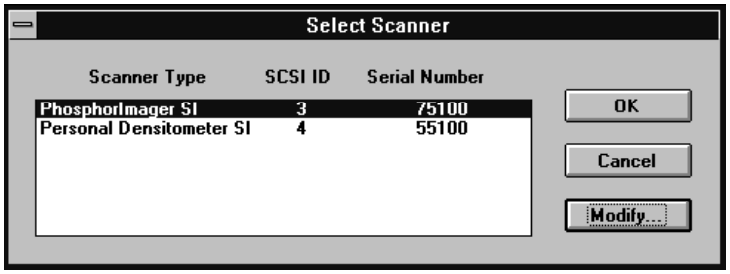


Figure 4-2. The Select Scanner window.

Note: If you need to change the serial number, use the Modify button. See section 10.1 for instructions.

4.4 Draw the Scan Area

The grid on the Scanner Control window represents the grid on the exposure cassette. The letter and number markings along the side and top of the grid correspond to the grid markings on the cassette.

The white rectangle on the grid designates the area you want to record (the Scan area). To designate a new Scan area—

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1. Place the pointer in the grid square corresponding to the upper left corner of the area you want to record.

2. Hold down the left mouse button and drag the pointer to the grid square corresponding to the lower right corner of the area you want to record.

3. Release the mouse button. The Save As window appears (figure 4-3).

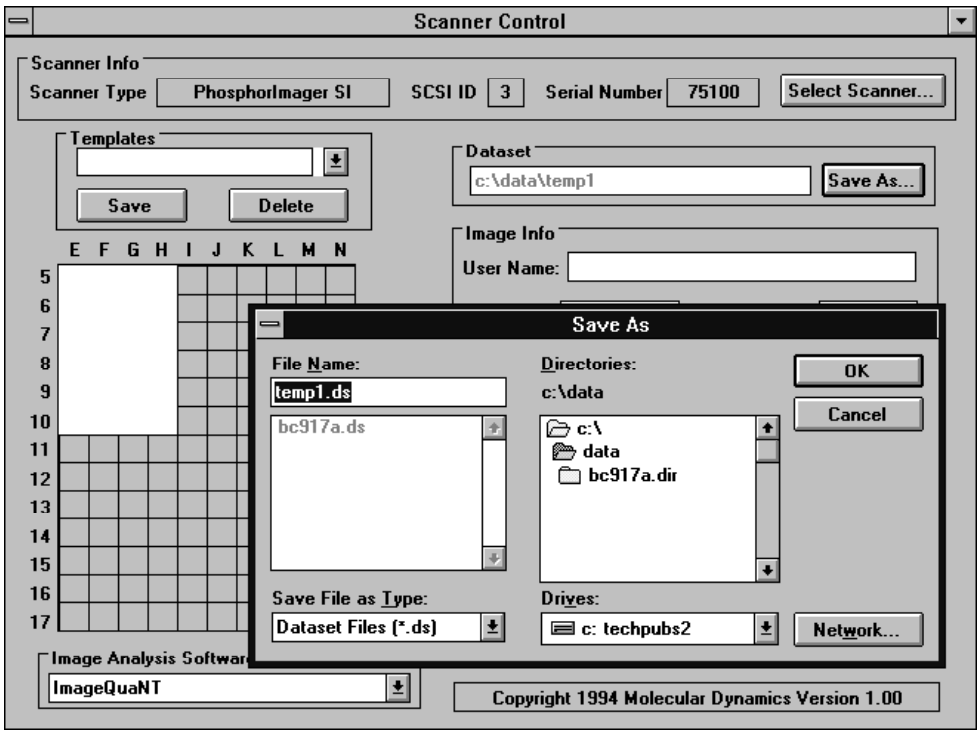


Figure 4-3. The Scanner Control window with a Scan area drawn and the Save As window displayed.

4.5 Name the Dataset

You use the Save As window to name the dataset—

1. Enter the dataset name in the File Name box. The software automatically adds the .DS extension or replaces any extension you add, except .GEL.

2. The default directory is DATA. If you want to change to a different directory, select the directory from the Directories box, or enter the entire path in the File Name box.

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3. Click on **OK** to accept the dataset name. The Save As window closes. The complete path to the dataset appears in the Dataset box on the Scanner Control window.

4.6 Select the Pixel Size

You select a pixel size to use when scanning the sample. To see the list of pixel sizes, click on the **Pixel Size** box, then select a pixel size from the list.

- For typical autoradiography samples, use the 200µ setting.
- For samples requiring high resolution—such as sequencing gels—use the 100µ setting.
- For samples requiring very high resolution—such as whole body samples—use the 50µ setting.

For more information about pixel sizes, see section 11.5.

4.7 Enter Image Information

In the Image Info area, enter a user name, preparation date and time, and comments. This information is for reference only. It does not affect the scan in any way.

4.8 Select the Image Analysis Software

Check that the **Image Analysis Software** box shows the analysis software you want to use at the end of your scan. A setting of **None** means the Scanner Control window remains open at the end of the scan so that you can perform another scan. If you select an application, the application opens and displays the image after the scan is complete.

To change the Image Analysis Software setting, click on the box. A list of available software appears. Click on the name of the software you want to use.

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4.9 Start the Scan

Ensure that the screen is in place, the instrument door is shut, and all the settings are correct in the Scanner Control window, then click on **Scan.** A Scan in Progress window appears. Click on the **Start** button to begin the scan and activate the Seconds Remaining counter. The PhosphorImager SI scans the sample and stores the image on the computer hard drive.

Note: If the message, "Not enough disk space to store image" appears when you start the scan, it means your hard disk is too full to accept the scan size or pixel size you selected. See the suggested solutions at the end of section 11.10.2.

Caution **Never turn off the PhosphorImager SI while a scan is in progress. You will abort the scan and not be able to retrieve your screen until you turn the instrument on.**

4.10 Remove the Screen from the PhosphorImager SI

To remove the screen from the PhosphorImager SI after the scan is finished, open the door, then rotate the blue tab. Next, grasp the front end of the screen and slide it toward you. Avoid touching the white phosphor screen.

Before you put the screen away for storage, clean and erase it as described in sections 2.2 and 2.3.

4.11 Analyze the Scan

The software you selected in the Image Analysis Software box appears on the screen and displays the scanned image. You can use the software to adjust the image display and make quantitative measurements of your scan. Refer to the image analysis software documentation for more information on analyzing your scan.

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Chapter 5 Macintosh Scanning Procedures

This chapter provides step-by-step instructions for scanning a sample from a Macintosh computer. The instructions assume you have performed the steps in chapters 2 and 3. You use the Scanner Control software (figure 5-1) to initiate the scan process. You do not need to calibrate the PhosphorImager SI; calibration is automatic.

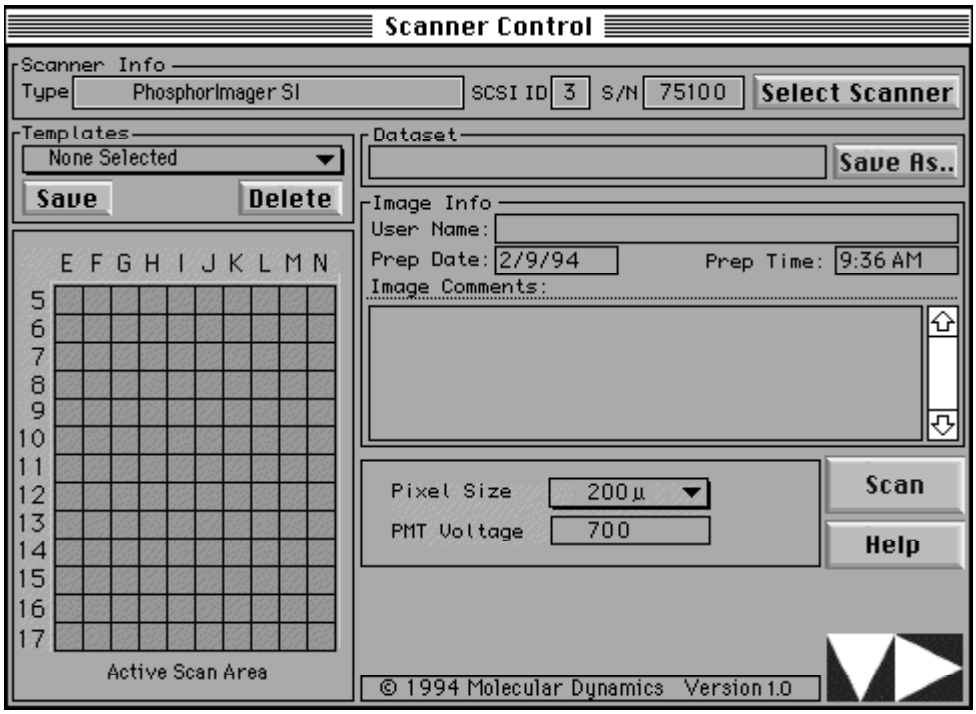


Figure 5-1. The Macintosh Scanner Control window.

5.1 Check Disk Space

The Scanner Control software automatically checks for sufficient disk space when you start the scan. However, you can check disk space manually before starting the scan. If necessary, you can then transfer or delete files to make room for your scan. To check disk space, use the formula in section 12.9 or draw the Scan area (section 4.4), then click on the **Help** button to display the amount of space needed.

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5.2 Use a Template

You can create a template that defines the Scan area, pixel size, and image information. You can then use the template for subsequent scans of similar samples. To create or use a template, see section 12.8.

5.3 Select a Scanning Instrument

The top of the Scanner Control window displays the instrument currently accessed by the computer. If you want to change to a different instrument—

1. Click on the **Select Scanner** button. The Select Scanner window opens (figure 5-2).

2. Click on the **SCSI** button. All the instruments connected on the SCSI bus are displayed in the window.

3. Click on the correct instrument to highlight it, then click on **OK** to close the Select Scanner window.

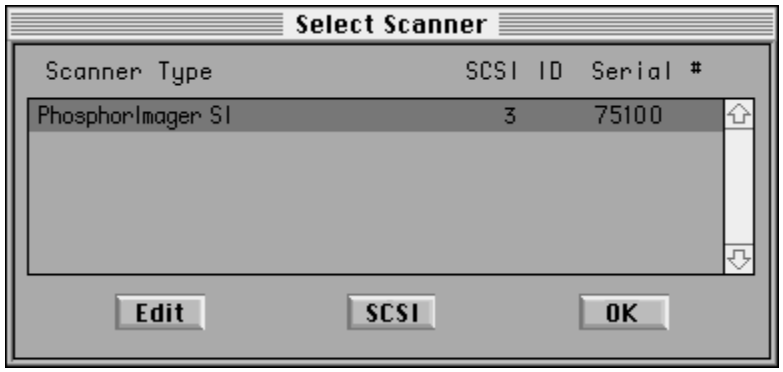


Figure 5-2. The Select Scanner window.

Note: If you need to change the serial number, use the Edit button. See section 10.2 for instructions.

5.4 Draw the Scan Area

The grid on the Scanner Control window represents the grid on the exposure cassette. The letter and number markings along the side and top of the grid correspond to the grid markings on the cassette.

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The rectangle on the grid designates the area you want to record (the Scan area). To designate a new Scan area—

1. Place the pointer in the grid square corresponding to the upper left corner of the area you want to record.

2. Hold down the mouse button and drag the pointer to the grid square corresponding to the lower right corner of the area you want to record.

3. Release the mouse button. The Save As window appears (figure 5-3).

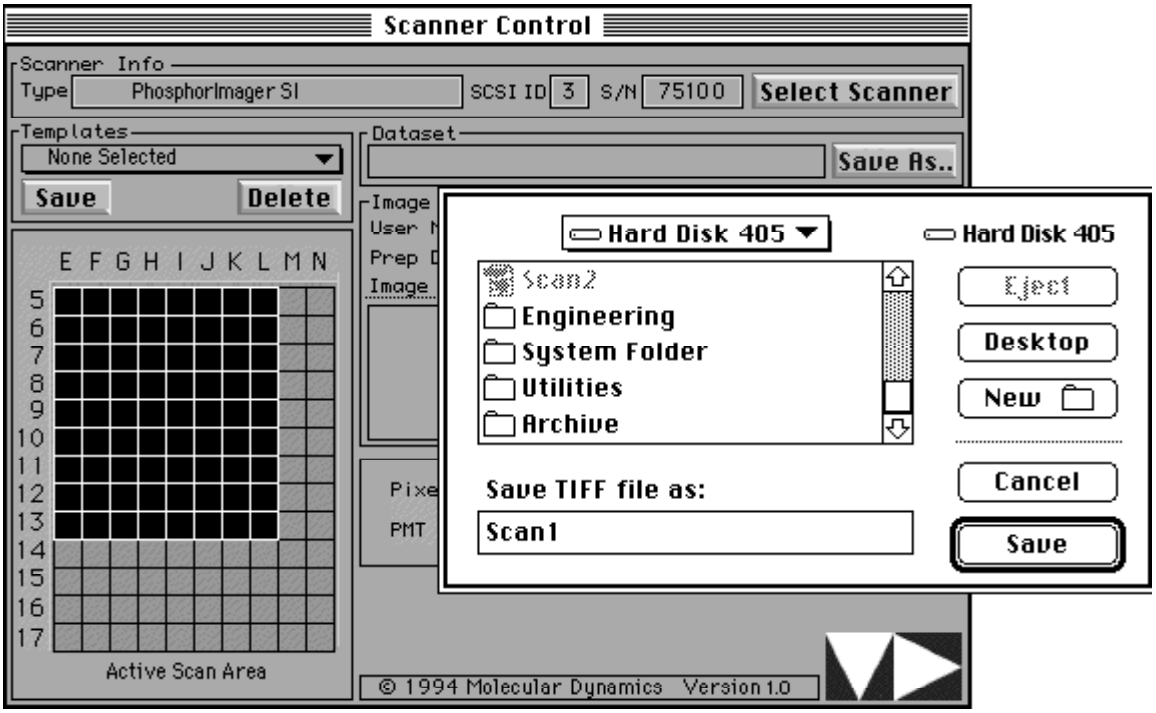


Figure 5-3. The Scanner Control window with a Scan area drawn and the Save As window displayed.

5.5 Name the Dataset

You use the Save As window to name the dataset—

1. Enter the dataset name in the **Save TIFF file as** box.

2. If you want to change to a different folder, select the desired folder from the hard disk list.

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3. Click on **Save** to accept the dataset name. The Save As window closes. The dataset name appears in the Dataset box on the Scanner Control window.

5.6 Select the Pixel Size

You select a pixel size to use when scanning the sample. To see the list of pixel sizes, move the pointer onto the **Pixel Size** box and hold down the mouse button, then select a pixel size from the list.

- For typical autoradiography samples, use the 200µ setting.
- For samples requiring high resolution—such as sequencing gels—use the 100µ setting.
- For samples requiring very high resolution—such as whole body samples—use the 50µ setting.

For more information about pixel sizes, see section 12.4.

5.7 Enter Image Information

In the Image Info area, enter a user name, preparation date and time, and comments. This information is for reference only. It does not affect the scan in any way.

5.8 Start the Scan

Ensure that the screen is in place, the instrument door is shut, and all the settings are correct in the Scanner Control window, then click on **Scan.** The Start Scan window appears. Click on the **Start Scan** button to begin the scan. The PhosphorImager SI scans the sample and stores the image on the computer hard drive.

Note: If the message, "Not enough disk space to store image" appears when you start the scan, it means your hard disk is too full to accept the scan size or pixel size you selected. See the suggested solutions at the end of section 12.9.2.

Caution **Never turn off the PhosphorImager SI while a scan is in progress. You will abort the scan and not be able to retrieve your screen until you turn the instrument on.**

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5.9 Remove the Screen from the PhosphorImager SI

To remove the screen from the PhosphorImager SI after the scan is finished, open the door, then rotate the blue tab. Next, grasp the front end of the screen and slide it toward you. Avoid touching the white phosphor screen.

Before you put the screen away for storage, clean and erase it as described in sections 2.2 and 2.3.

5.10 Analyze the Scan

You can use the image analysis software to adjust the image display and make quantitative measurements of your scan. Refer to the image analysis software documentation for more information on analyzing your scan.

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Part 2

Instrument Reference Guide

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Chapter 6 Storage Phosphor Screen

The storage phosphor screen is 20 x 25 cm. It "stores" the energy from ionizing radiation and releases it when stimulated by light of appropriate wavelengths. Phosphor screens can be erased and reused.

6.1 Handle Screens

Handle screens on the black edges or back. It is important not to touch the white phosphor surface. Fingerprints contain oils that fluoresce and cause inaccurate readings.

In addition, be careful not to scratch or otherwise damage the surface. (Damaged portions of a screen are unusable.)

6.2 Clean Screens

To clean screens, use a soft cotton cloth with Kodak Intensifying Screen Cleaner. Follow the directions on the bottle. This cleaning procedure removes dust, fingerprints, static electricity, and mild radioactive contamination.

6.3 Erase Screens

Follow the guidelines below when using the Image Eraser SF to erase phosphor screens.

- To avoid contaminating the Eraser, make sure the screens are clean and free from radioactive contamination before placing the screens on the Eraser.
- To avoid contaminating the screens, make sure the Eraser surface is free from radioactive contamination before placing the screens on the Eraser.
- Erase screens immediately before exposure and after scanning them in the PhosphorImager SI. Cosmic radiation exposes screens left unused for long periods of time.
- Erase screens until the recorded signal is fully removed. See section 2.3 for step-by-step instructions on erasing screens. See chapter 8 for further information about using the Image Eraser SF.

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6.4 Expose Screens

When setting up screens for exposure, follow the guidelines below. For step-by-step procedures, see chapter 2.

6.4.1 Guidelines for Exposing Screens

- Use the exposure cassette to expose the screen to the sample. The exposure cassette seals out light and also keeps the sample snug against the phosphor screen during exposure.
- Place the screen on top of the sample, being careful to place it correctly the first time. Adjusting the position of the screen following initial placement may result in a ghost image.
- Do not expose the screen to a sample that may contaminate the screen, such as an uncovered wet gel.
- Do not expose screens to acetic acid vapors. Such vapors are harmful to screens and can contaminate the screen even if the sample is wrapped in plastic.
- Do not use scintillants or enhancers, such as PPO, Dupont EN^{3}HANCE™, and Amersham Amplify®. These products interfere with the proper operation of the screen.

6.4.2 Using Wet Gels

Caution **Storage phosphor screens should not be exposed to wet chemicals of any kind. The only exception is Kodak Intensifying Screen Cleaner or a totally rinsable detergent.**

To protect the screen and cassette from a contaminating sample, such as a wet gel, **separate the sample from the screen and cassette** with a thin piece of plastic wrap or mylar. Wrap the plastic wrap completely around the sample so that liquid cannot leak out. This precaution minimizes screen contamination with a minimum attenuation of the signal.

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6.5 Store Screens

Before storing screens, clean, decontaminate, and erase them, following the guidelines in sections 6.1 through 6.3. Store them—

- In a protective box or face down in the exposure cassette.
- At room temperature.
- Away from sources of radiation, such as strong beta or gamma emitters or x-ray machines.

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Chapter 7 Exposure Cassette

This chapter explains the cassette design and offers guidelines for protecting and maintaining the cassette. For step-by-step instructions on placing the samples and phosphor storage screens in the cassette, see sections 2.4 and 2.5 of this guide.

7.1 Cassette Design Features

The exposure cassette holds samples in place and seals out light. The cassette contains a rectangular piece of foam covered with easy-to-clean plastic. The foam is surrounded by a black gasket.

When you place the sample on top of the plastic and lock the screen into place, the compacted foam holds the sample against the screen, and the black gasket seals out light.

The grid on the foam allows you to identify the coordinates you will use when you scan. You scan only the exposed portion of your phosphor screen, minimizing the scan time as well as the size of the scan file.

7.2 Protect the Cassette

To protect the cassette from contamination and damage, observe the following precautions:

- 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 6.4.2 of this manual.
- Do not place sharp or heavy objects on top of the cassette foam. A crease or dent in the foam causes uneven pressure on the sample.

7.3 Clean the Cassette

To clean the outer surface of the cassette, use a damp cloth and mild detergent. Keep the foam inside the cassette dry. Immediately before placing the sample in the cassette, clean the plastic surface on the inside with a damp cloth and remove any radioactive contamination.

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Chapter 8 Image Eraser SF

The Image Eraser SF allows you to reuse the phosphor storage screens. By placing the screen on the Eraser and turning on the light, you remove the latent image stored on the screen.

8.1 Install the Eraser

To install the Image Eraser SF, simply attach the appropriate power cord supplied by Molecular Dynamics and plug in the unit.

Warning **Be sure to plug the cord into a properly grounded outlet.**

You can hang the Eraser on the wall. To do this, fasten two screws or hooks—spaced 10 inches apart—securely to the wall. Hang the Eraser on the screws or hooks (the rear of the enclosure contains slots for that purpose).

8.2 Operate the Eraser

To operate the Eraser, press the power switch to ON, set the timer for Normal or Extended, then press the Erase button (figure 8-1):

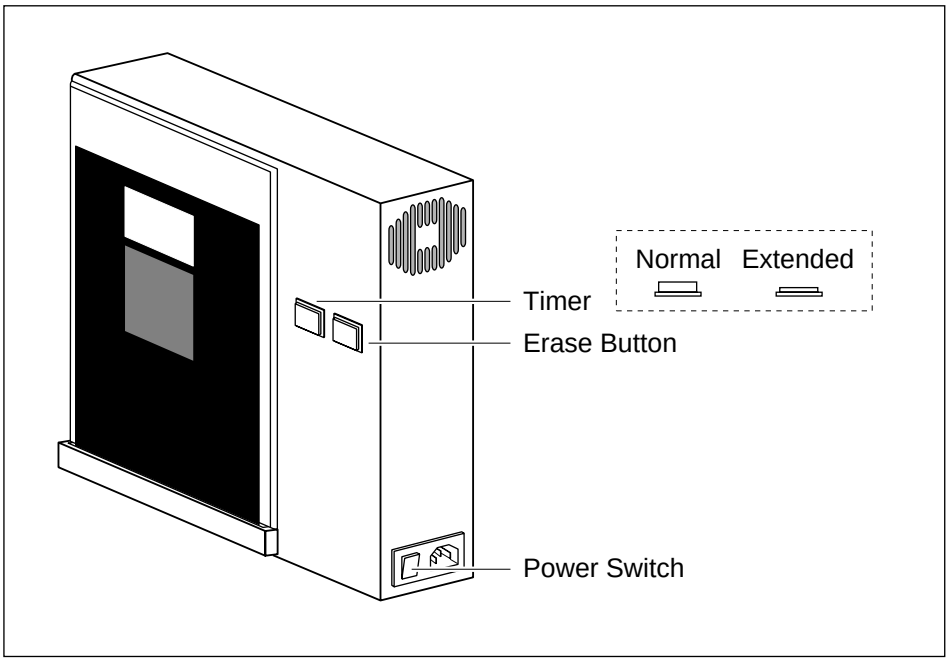


Figure 8-1. The Image Eraser SF.

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- The **power switch** is a rocker switch located on the right side of the Eraser frame. It must be pressed to the ON position to operate the Eraser.

- The **timer** allows you to set the exposure time. Leave the timer set to Normal for typical samples; set it to Extended (button pushed in) if the background or residual image is high (for example, a saturated image with a reading of 10,000 counts). The Normal setting takes three minutes; the Extended setting takes six minutes.

- The **Erase button,** located on the front of the Eraser, turns the light on for one erase cycle. Press the button to turn on the light. The light turns off automatically.

8.3 Clean

Warning **Clean the surface of the Image Eraser SF with the power turned off.**

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

To remove radioactive contamination, wipe the surface of the Eraser with a damp cloth moistened with a totally rinsable laboratory detergent.

Caution **Do not allow liquid to enter the Image Eraser SF. To avoid getting the inside of the Eraser wet, only use a damp cloth to clean the Eraser surface. Do not pour or spray liquid over the surface.**

8.4 Change Bulbs

The Image Eraser SF contains four fluorescent bulbs. When any one of the bulbs fails, replace it. Although the diffuser panel must be in place to erase screens, the Eraser has been designed to operate safely with the diffuser panel removed. If you are not sure which of the bulbs has failed, remove the diffuser panel and press the Erase button to test the bulbs. Turn off the Eraser, then replace the failed bulb and the diffuser panel.

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8.4.1 Replacement Bulbs

The Eraser uses warm white, compact fluorescent bulbs. To order replacement bulbs contact either—

- Molecular Dynamics (part number 0105-852).
- Your local distributor (see manufacturer part number on the bulb). For assistance in finding your local distributor, call Molecular Dynamics World Headquarters. (See section 13.2 for the numbers.)

8.4.2 Procedure for Changing Bulbs

1. Turn off and unplug the Image Eraser SF.
2. Using a crosspoint screwdriver, remove the two screws that hold the diffuser screen in place.
3. Lift off the diffuser screen.
4. To remove a bulb—
   - Grasp the bulb and press it into the foam pad (figure 8-2).
   - Swing the plug end of the bulb clear of the socket and pull it out of the foam pad.

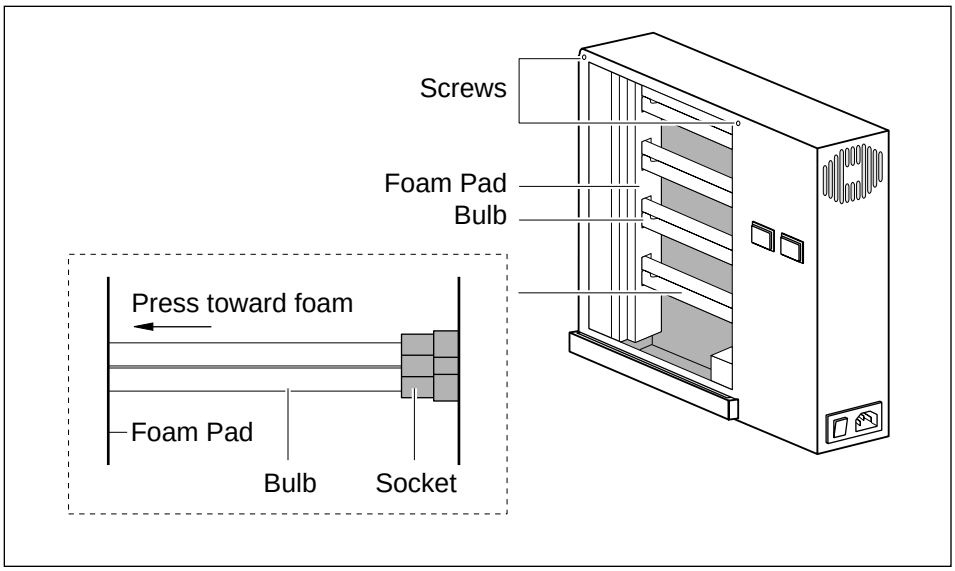


Figure 8-2. Removing a bulb from the Image Eraser SF.

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5. To install a new bulb—

   - Insert the curved end of the bulb into the foam padding, lining up the plug end with its socket.
   - Push the bulb into its socket.

6. Replace the front diffuser panel and the screws.

7. Plug in the Eraser and turn on the power.

8.5 Change the Fuse

The procedure for changing the fuse is similar to that used for the PhosphorImager SI.

1. Turn off the power and remove the power cord.

2. Look for the small vertical groove inside the left side of the power plug receptacle.

3. Place the end of a screwdriver into the groove and pry out the fuse holder (figure 8-3). When the fuse holder moves outward, grasp the holder and pull it all the way out.

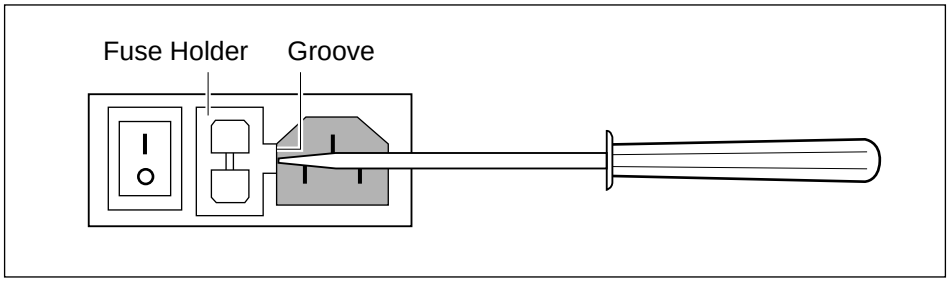


Figure 8-3. Opening the fuse box.

4. Remove the fuse.

5. Install a new fuse of the same type and rating as that shown on the label. (You can store an additional fuse in the drawer in the fuse holder.)

6. Return the fuse holder to its original location with the tab to the right.

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7. Press the fuse holder all the way in until it is flush with the surface.

8. Reconnect the power cord.

Replacing the fuse repeatedly could indicate a problem. Do not use the Image Eraser SF. Contact your service representative.

8.6 Important Safety Information

Warning **The Image Eraser SF uses high voltage.**

The Image Eraser SF has been carefully designed for safety. Because the Eraser uses high voltage, please follow the precautions below to ensure continued safe use of the equipment:

- Plug the power cord into a grounded outlet.
- Turn off the power before cleaning the Eraser.
- Never clean the surface of the Eraser with a damp sponge or cloth or with any type of liquid while the power switch is on. (Note that the power switch is not the same as the Erase switch.)
- Always unplug the Eraser before changing a bulb.
- When changing a bulb, do not remove covers other than the front diffuser panel. (There are no user-serviceable parts inside.)
- If the covers become damaged, do not use the Eraser. Return it to Molecular Dynamics for repair.
- Replacing the fuse repeatedly could indicate a problem, do not use the Eraser. Contact your service representative.

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Chapter 9 PhosphorImager SI

9.1 Instrument Care and Maintenance

The following sections provide guidelines on protecting the instrument, the power up and down sequences, instructions on moving and reinstalling the instrument, and instructions for changing a fuse.

9.1.1 Protecting Your Instrument

To protect your instrument—

- Install the instrument away from strong air currents. The airflow may carry dust or dirt particles into sensitive parts of the instrument.
- Do not place the instrument in direct sunlight or in any other very bright light. Bright light may cause excessive heat or compromise the light-tight operation of the instrument.
- In general, leave the PhosphorImager SI on. However, if it will be idle for more than a day—such as over the weekend—turn it off. You should not turn the instrument on and off for every scan.

9.1.2 Power Up and Power Down Sequences

The instrument(s) and computer should be turned on and off using the steps described below.

Power Up

Always power up the instrument and computer in the following order:

1. Turn on all the instruments and peripheral devices (if any).
2. Wait at least 10 seconds, then turn on the computer and the monitor.
3. Log in, if necessary.

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4. Open the Scanner Control window (which begins the warm-up process for all instruments) using one of the following methods:

   - Windows NT-based computer—Double-click on the MD Apps icon, then double-click on the Scanner Control SI icon.

   - Macintosh computer—Double-click on the Scanner Control SI icon or name.

5. Wait at least 20 minutes before scanning.

Power Down

Always power down the instrument(s) and computer in the following order:

1. Close the Scanner Control window and any other running applications using one of the following methods:

   - Windows NT-based computer—Double-click on the control menu button.

   - Macintosh computer—Highlight **Quit** on the File menu.

2. Shut down the computer using one of the following methods:

   - Windows NT-based computer—Select **Shutdown** on the Program Manager File menu.

   - Macintosh computer—Select **Shut Down** on the Special menu.

3. Turn off the computer and monitor.

4. Turn off all the instruments and peripheral devices (if any).

9.1.3 Moving and Reinstalling the Instrument

In preparation for moving the instrument, power down the instrument and computer using the instructions in section 9.1.2. Next, disconnect the power cord and the SCSI connection. When reinstalling, reconnect each item. Chapter 10 explains how to attach the SCSI devices.

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After you reconnect the hardware, power up the instrument and computer using the instructions in section 9.1.2. Make sure that the serial number is displayed in the Scanner Info area. If it is not, see section 10.1 (Windows NT) or section 10.2 (Macintosh) for instructions on reentering the serial number.

9.1.4 Changing the Fuse

The fuse is located in the back of the PhosphorImager SI next to the power outlet. For the type of fuse needed, see the label on the back of the instrument. To change the fuse—

1. Power down the instrument and computer using the instructions in section 9.1.2.

2. Remove the power cord from the back of the instrument.

3. Locate the small vertical groove inside the left side of the power plug receptacle.

4. Place the end of a screwdriver into the groove and pry out the fuse holder (figure 9-1). When the fuse holder moves outward, grasp the holder and pull it all the way out.

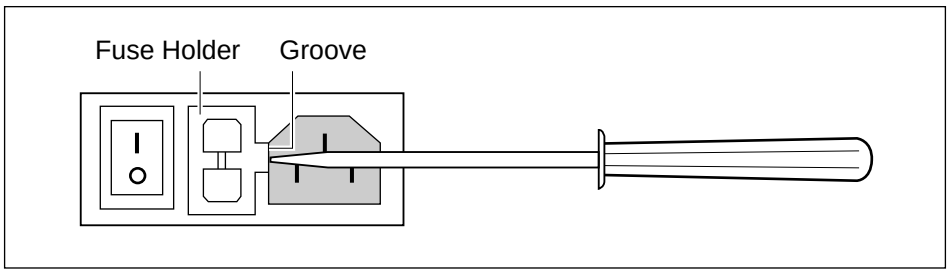


Figure 9-1. Opening the fuse box.

5. Remove the fuse.

6. Install a new fuse of the same type and rating as on the label. (You can store an additional fuse in the drawer in the fuse holder.)

7. Return the fuse holder to its original location with the tab to the right.

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8. Press the fuse holder all the way in until it is flush with the surface.

9. Reconnect the power cord.

10. Power up the instrument and computer using the instructions in section 9.1.2.

9.2 Important Safety Information

The PhosphorImager SI and its accessories have been carefully designed for safety. Please follow the precautions below to ensure continued safe use of the equipment.

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

9.2.1 Laser Light Warning Label

The label shown in figure 9-2 warns of exposure to laser light. It is located on the right side of the PhosphorImager SI near the back. The exact location of the label is shown later in figure 9-4.

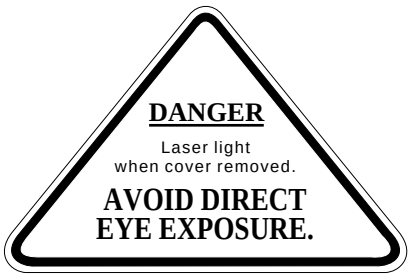


Figure 9-2. Warning label.

If the label becomes illegible for any reason, please contact Molecular Dynamics for a free replacement label.

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9.2.2 Safety Precautions

As an operator, you are completely protected from the laser beam. Nevertheless, HeNe laser power up to 5 mW can be accessed from within the interior of the instrument. For this reason, please observe the following precautions:

- Do not remove the top cover of the instrument.

  Because there are no user-serviceable components inside this instrument, you never need to remove the top cover.

- Contact Molecular Dynamics if the cover or door becomes damaged.

  Do not continue to use the instrument if the cover or door becomes damaged and the instrument is no longer light-tight. Contact Molecular Dynamics immediately to arrange for repair. See section 13.2 for the number of your Molecular Dynamics representative.

- Do not send your PhosphorImager SI elsewhere for service or attempt to service it yourself.

  To protect your warranty and ensure safe operation, the PhosphorImager SI should be serviced only by Molecular Dynamics or its representatives. If the PhosphorImager SI is not working correctly, please contact your Molecular Dynamics service representative.

- If the fuse must be replaced repeatedly, do not use the PhosphorImager SI. Contact your service representative.

When you call your representative, be prepared to give the serial number of your instrument. The serial number certification label, shown in figure 9-3, displays the model number, serial number, and CDRH compliance information. It is located on the back panel of the PhosphorImager SI. Figure 9-4 shows the exact location of this label.

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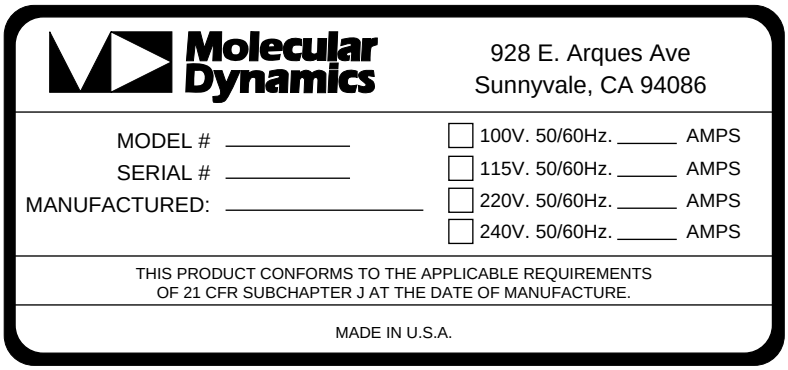


Figure 9-3. The serial number certification label.

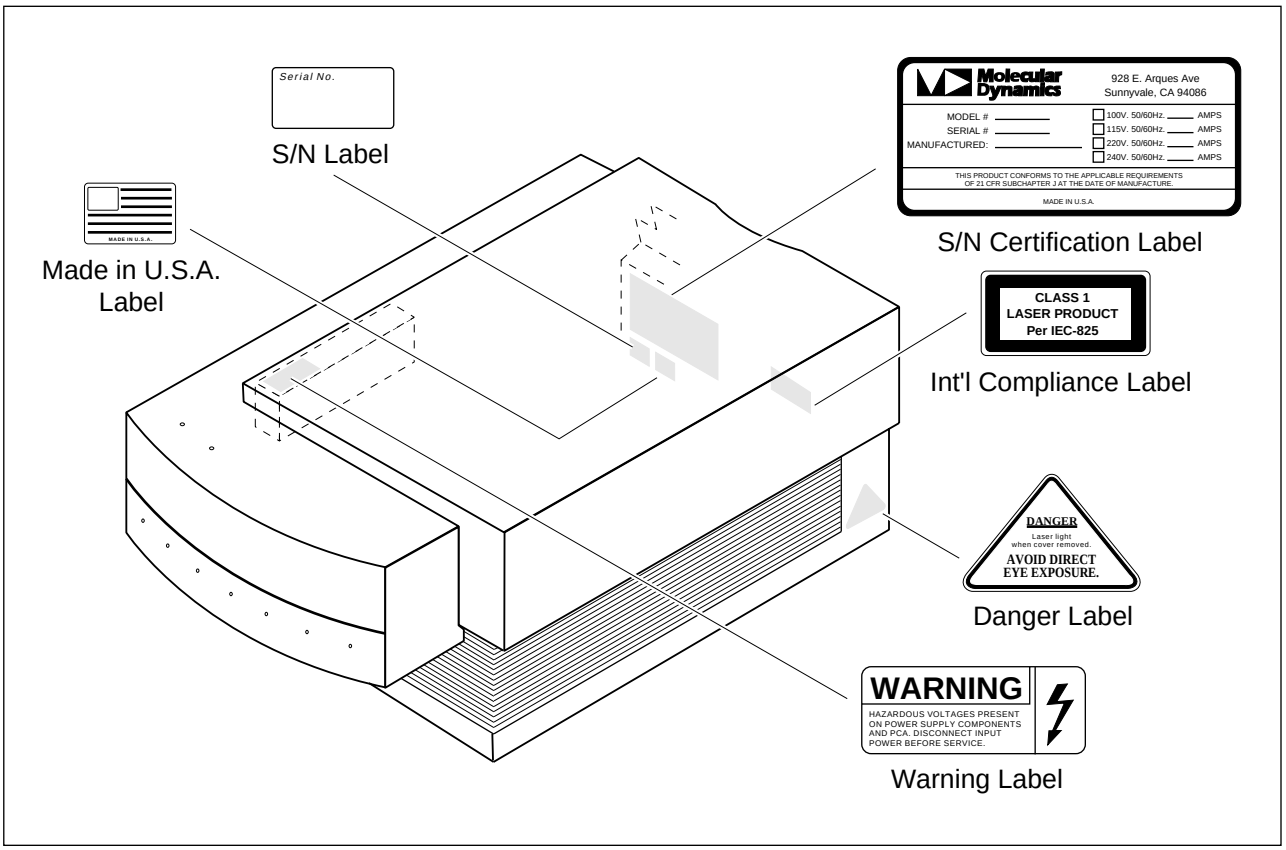


Figure 9-4. Location of important labels.

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Chapter 10 SCSI Ports

Two SCSI (Small Computer Systems Interface) ports are located on the back of the PhosphorImager SI. Use these ports to connect the instrument to the computer. Section 10.1 provides instructions for a Windows NT-based computer; section 10.2 provides instructions for a Macintosh computer.

10.1 Connect the Instrument to a Windows NT-Based Computer

A SCSI ID number identifies each device connected to the SCSI bus. SCSI IDs 0 and 7 are reserved for the computer. The PhosphorImager SI has been preset to SCSI ID 3. If your configuration includes a peripheral device, such as a disk drive, check the back of the hardware for the SCSI ID used by that device to ensure it uses a number that is different from those of the computer and the instrument. If it is not different, change the peripheral or instrument SCSI ID. SCSI peripherals supported by Molecular Dynamics include the Pinnacle Micro Optical™ magneto-optical disk drive, the SyQuest® disk drive, and the Archive Python™ DAT tape drive.

Each end of the SCSI chain must be terminated.The computer is at one end of the SCSI chain; the PhosphorImager SI is next, followed by a peripheral device. The terminator is built into the computer; however, you must add a terminator to the other end. Figure 10-1 shows a sample configuration.

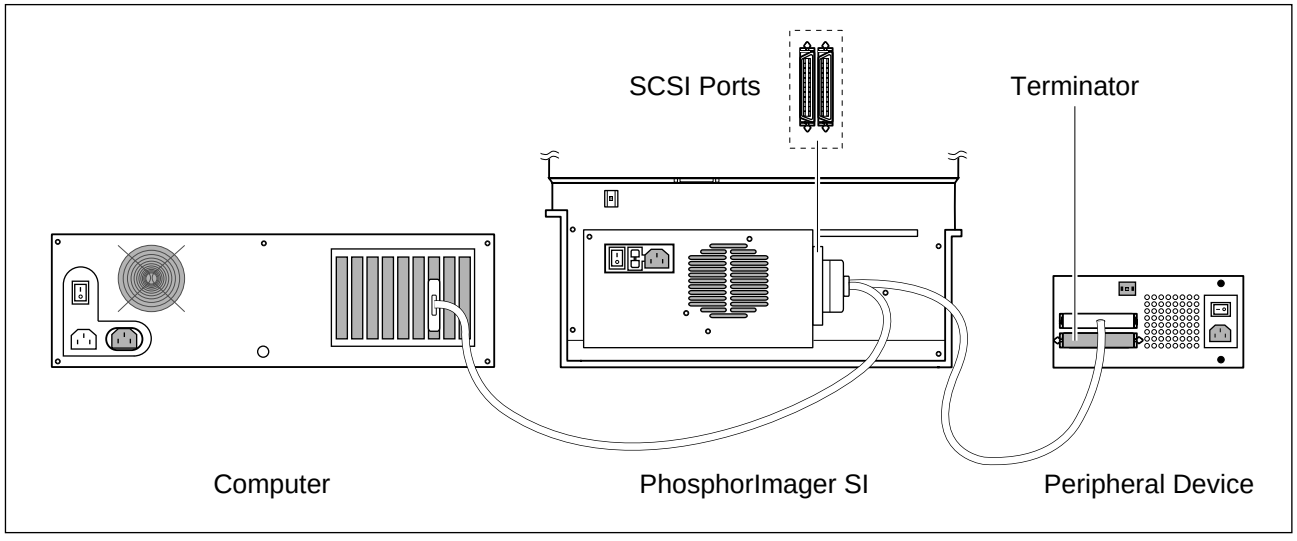


Figure 10-1. Connecting the PhosphorImager SI, computer, and peripheral.

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10.1.1 Installing the SCSI Adapter

To establish the connection between the instrument and the computer, a SCSI adapter card must be installed in the computer and the Windows NT device drivers must be loaded. If you have not installed the SCSI adapter card, use the instructions provided with the card. If you did not load the device drivers when you installed Windows NT, see the Windows NT documentation for instructions on adding them.

Use the following instructions to complete the installation:

1. Log into Windows NT as the Administrator.

2. In the Windows NT Program Manager window, double-click on the Main icon.

3. In the Main window, double-click on the Windows NT Setup icon.

4. In the Windows NT Setup window, open the **Options** menu and click on **Add/Remove SCSI Adapters.** The SCSI Adapter Setup window opens displaying the SCSI adapters currently installed.

5. Click on **Add.** A Setup Warning message appears. Click on **OK.** The SCSI Adapter Option window appears.

6. Click on the **Adapter** box and select the **SCSI card adapter** from the list.

   Note: If the SCSI card is not listed, select Other, then insert the disk containing the Windows NT SCSI driver supplied by the manufacturer of the adapter card.

7. Click on **Install.** The SCSI Adapter Option window closes.

8. Click on **Close** to return to the Main window.

9. In the Main window, double-click on the Control Panel icon.

10. Double-click on the Devices icon to open the Devices window. All devices loaded with Windows NT are listed.

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11. Select **scsiscan** from the list, then click on **Startup** to open the Device Driver window.

12. Click on **Disable,** then click on **OK** to close the Device Driver window.

13. Click on **Close** to close the Devices window.

10.1.2 Connecting the SCSI Cable

Plug the SCSI cable into one of the SCSI ports on the PhosphorImager SI, then plug the other end into the SCSI port on the computer. If you are not adding a peripheral device, plug a terminator into the remaining SCSI port on the PhosphorImager SI. If you are adding a peripheral device, plug the SCSI cable into the remaining SCSI port on the instrument, then plug the other end into one of the SCSI ports on the peripheral. Plug a terminator into the second SCSI port on the peripheral. Ensure that the total length of all the SCSI cables does not exceed four meters.

If you need to change the SCSI ID on the instrument, use the push-buttons on the rear panel of the PhosphorImager SI to set the number. The upper pushbutton decreases the number; the lower pushbutton increases the number (figure 10-2).

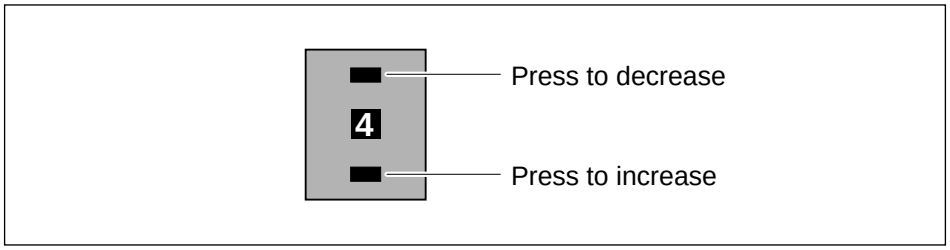


Figure 10-2. Setting the SCSI ID on the PhosphorImager SI.

10.1.3 Entering the Instrument Serial Number

To complete the SCSI connection, you must enter the serial number that is printed on the back of the instrument. See figures 9-3 and 9-4 for the location of the serial number. To enter the serial number—

1. Turn on the instrument and computer using the procedure in section 9.1.2.

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2. In the Scanner Control window, click on the **Select Scanner** button. The Select Scanner window appears.

3. Click on the **Modify** button. The Modify window appears.

4. Enter the serial number in the serial number box, then click on **OK** to close the Modify window.

5. Click on **OK** to close the Select Scanner window.

The Scanner Info area displays the scanner type, SCSI ID number, and serial number.

10.1.4 Installing a Peripheral Device

The instructions below explain how to install the Molecular Dynamics supported peripheral devices. For information about configuring your instrument for other peripherals, contact the peripheral manufacturer.

Note: You must have the SCSI adapter card installed in the computer and the Windows NT device drivers loaded. If you did not install the SCSI adapters and device drivers with Windows NT, you will need to add them. See the Windows NT documentation.

To install the device driver—

1. In the Main window, double-click on the Control Panel icon.

2. Double-click on the Devices icon to open the Devices window. All devices loaded with Windows NT are listed.

3. Select the device name from the list.

4. Click on the **Startup** button to open the Device Driver window.

5. Click on the **System** button in the list of startup types, then click on **OK** to return to the Devices window.

6. Click on **Clos**e to close the Devices window.

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10.1.5 Partitioning a Removable Disk

Before you can use a new removable disk, you must partition and format it. To do this—

1. Log into Windows NT as the Administrator.
2. Insert the disk into the disk drive.
3. Double-click on the Administrative Tools icon.
4. Double-click on the Disk Administrator icon. A window appears indicating either that this is the first time the Disk Administrator has been run or that something has changed.
5. Click on **OK** to continue. The Disk Administrator window appears. It contains at least two Disk icons.

   Disk 0 is the C: drive (single partition).
   Disk 1 is the M-O or SyQuest drive.

6. Click on the rectangle to the right of Disk 1. **Note:** It displays Free Space and either 281 MB or 105 MB depending on the type of disk. If the display does not say Free Space, there may be information on the disk.
7. Click on the **Partition** selection in the top menu.
8. Click on **Create.** The Create Partition window appears with either 281 MB or 105 MB displayed as the Create partition of size selection.
9. Click on **OK.**
10. Double-click on the control menu button () to close the Disk Administrator.
11. Click on **Yes** to save the changes.
12. Click on **OK** when the "Disks were updated successfully" message appears.
13. Double-click on the control menu button () to close the Administrative Tools window.

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14. Double-click on the File Manager icon.

15. Click on **Disk** in the main menu.

16. Click on **Format Disk.**

17. Click on the down arrow ( ) next to the Disk In list box in the Format Disk Window.

18. Click on **Drive D.**

19. Click on the **Quick Format** check box.

20. Click on **OK.**

21. Click on **Yes** to confirm that you want to format the disk. The Percent Complete window appears.

22. When the Format Complete window appears asking to format another disk, click on **No.**

23. Double-click on the control menu button ( ) to close the File Manager.

24. If you want to partition the second side of a 600-MB M-O disk, remove the disk, turn it over, and reinsert it into the drive. Repeat the procedure starting with step 3.

Note: Remove the disk from the disk drive if you will not be using it.

10.2 Connect the Instrument to a Macintosh Computer

A SCSI ID number identifies each device connected to the SCSI bus. SCSI IDs 0 and 7 are reserved for the computer. The PhosphorImager SI has been preset to SCSI ID 3. Ensure that the length of the SCSI cable does not exceed four meters.

Each end of the SCSI chain must be terminated. The terminator is built into the computer; however, you must add a terminator to the other end. Figure 10-3 shows a sample configuration.

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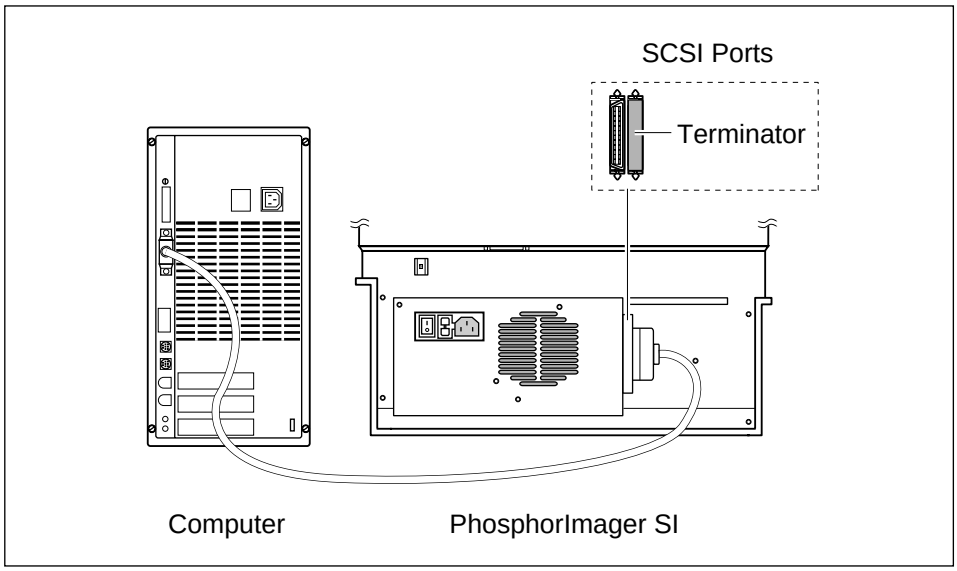


Figure 10-3. Connecting the PhosphorImager SI and Macintosh computer.

Plug the SCSI cable into the SCSI ports on the PhosphorImager SI, then plug the other end into the SCSI port on the Macintosh. Plug a terminator into the remaining SCSI port on the PhosphorImager SI.

After installing the PhosphorImager SI, you must enter the serial number that is printed on the back of the instrument. See figures 9-3 and 9-4 for the location of the serial number. To enter the serial number—

1. Turn on the instrument and computer using the procedure in section 9.1.2.

2. In the Scanner Control window, click on the **Select Scanner** button. The Select Scanner window appears.

3. Click on the **Edit** button. The Edit window appears.

4. Enter the serial number in the serial number box, then click on **OK** to close the Edit window.

5. Click on **OK** to close the Select Scanner window.

The Scanner Info area displays the scanner type, SCSI ID number, and serial number.

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Chapter 11 Windows NT Scanner Control

The Windows NT Scanner Control window (figure 11-1) has the following functions:

- **Dataset box**—Displays the name of the current dataset (section 11.1).
- **Help button**—Displays the disk space available on the computer hard disk (section 11.2).
- **Image Analysis Software box**—Selects the software to activate after scanning (section 11.3).
- **Image Info area**—Contains fields for entering user name, preparation date, preparation time, and comments pertaining to the sample (section 11.4).
- **Pixel Size box** —Establishes the pixel size for the scan (section 11.5).
- **Scan area (grid)**—Determines the scan size and location, then activates the Save As window (section 11.6).
- **Scan button**—Begins the scan (section 11.7).
- **Scanner Info area**—Displays the scanner type, SCSI ID, and serial number for the currently selected instrument (section 11.8).
- **Templates area**—Selects, saves, and deletes templates (section 11.9).

Each of these areas is discussed in the following sections. In addition, section 11.10 describes how to determine disk space requirements.

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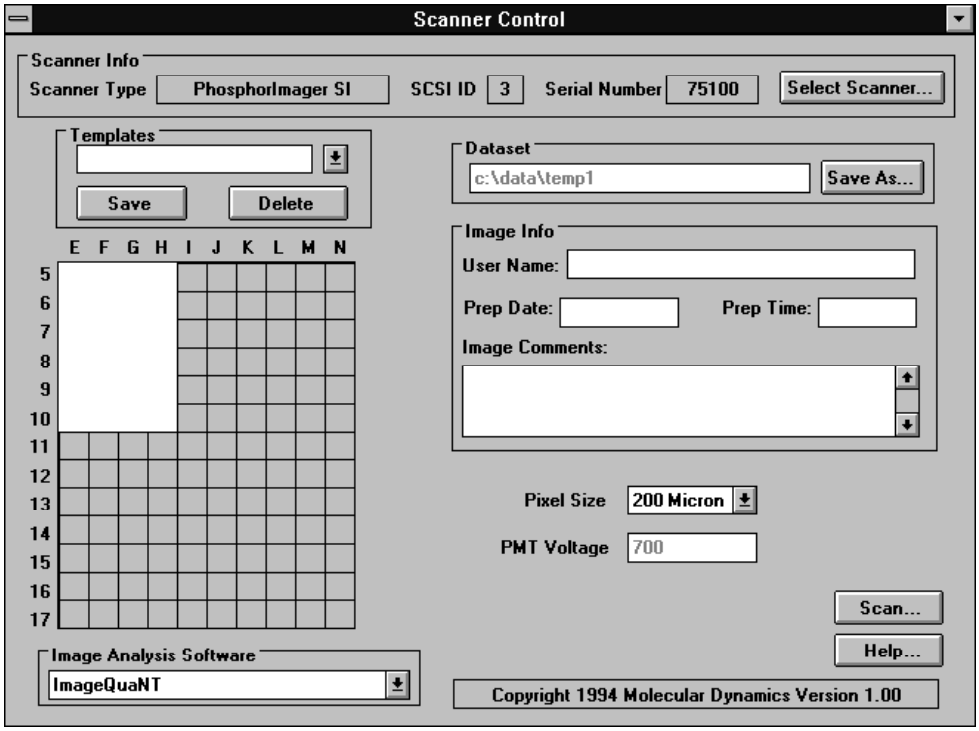


Figure 11-1. The Windows NT Scanner Control window.

11.1 Dataset Box

The Dataset box displays the name of the dataset (including the path) that you are about to create. The Save As button opens the Save As window so that you can create a new dataset without having to change the Scan area.

11.2 Help Button

You use the **Help** button to determine the amount of disk space available on the computer hard drive and the amount of disk space required to store the current Scan area at the selected pixel size.

11.3 Image Analysis Software Box

You use the Image Analysis Software box to indicate what software to open after the scan is complete. If you select None, the Scanner Control window remains open so that you can scan additional samples.

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11.4 Image Info Area

The Image Info area allows you to create notes to store with the image. To enter information, click on any box you want to use and type the information.

11.5 Pixel Size Box

Pixel size refers to the size of each individual "spot" (or picture element) that is recorded and, together with the thousands of other pixels, forms the image. The PhosphorImager SI allows you to select from three pixel sizes: 50µ pixel size (4000 data points/cm^2), 100µ pixel size (1000 data points/cm^2), and 200µ pixel size (250 data points/cm^2).

When scanning typical samples, you can save time and disk space by using the 200µ pixel setting. For example, at the 200µ setting, scan time is approximately five minutes, whereas a 100µ scan is approximately seven minutes. The file size at 200µ is approximately one-fourth that of a 100µ scan. The 50µ option gives you very high-resolution images, but the resulting image file size is approximately 16 times that of a 200µ scan and scan time is approximately 15.5 minutes.

Note: In the PhosphorImager SI, the digital file format is set at 16 bits. The digital format refers to the number of bits used to represent a pixel. The more bits-per-pixel, the higher the resolution. The instrument can distinguish between adjacent bands of similar signal intensity and can detect extremely faint bands. For transferring images to compatible graphics programs on Macintosh and DOS-based computers, you can convert 16-bit files to 8-bit files using the Convert 16-To-8 utility.

11.6 Scan Area

You identify the scan coordinates and enter a dataset name from the Scan area.

11.6.1 Selecting the Scan Area

The grid on the Scanner Control window (figure 11-1) represents the grid on the exposure cassette. You designate the area to scan by using the mouse to draw a white rectangle on the grid.

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When drawing the rectangle, select only those grid squares that were covered by the sample. Selecting an area larger than the sample increases the scan time and the size of the image file. Section 4.4 provides step-by-step instructions.

11.6.2 Entering a Dataset Name

You must enter a dataset name in the Save As window each time you create an image. If you draw a new Scan area, the Save As window opens automatically when you finish (figure 11-2). (If you want to use the existing Scan area, click on the Save As button in the Dataset area to open the Save As window.) Enter the name for the dataset in the File Name box.

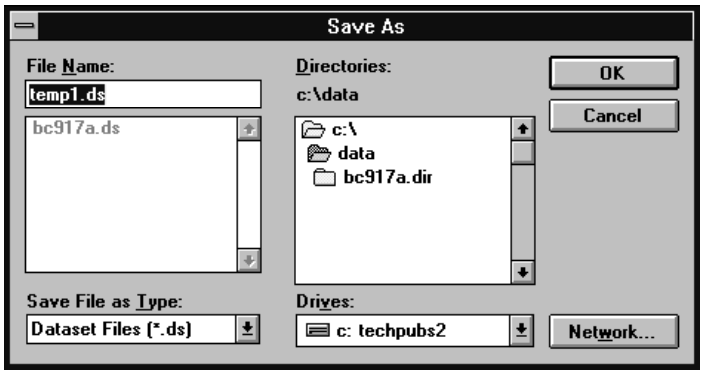


Figure 11-2. The Save As window.

If you want to store the dataset in other than the default DATA directory on the current drive, select a new directory from the Directories box, select a new drive from the Drives list, or click on the Network button to select a shared directory on another workstation. Enter the name for the dataset.

Note: On a Windows NT-based computer, do not use the following characters: ." / \:<>+=;, and space. You can use the underline character. The dataset name can be up to 8 characters if you are using the FAT file system or up to 255 characters if you are using the NTFS file system.

When you click on **OK,** the Save As window closes and the directory path and dataset name appear in the Dataset box.

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11.7 Scan Button

After you enter all the information for the scan, click on the **Scan** button. The screen must be in the PhosphorImager SI and the door must be closed. A Scan in Progress window appears. Click on the **Start** button to begin the scan and activate the Seconds Remaining counter. The scan is saved under the file name entered in the Save As window. When the scan is finished, either the selected image analysis software opens or the Scanner Control window remains open for more scanning depending on your entry in the Image Analysis Software box.

11.8 Scanner Info Area

The Scanner Info area displays the instrument currently accessed by the Scanner Control. The scanner type, SCSI ID number, and serial number are displayed. If more than one instrument is connected to the host computer, you can switch to a different instrument.

To change instruments, click on the **Select Scanner** button, which opens the Select Scanner window (figure 11-3). Click on the name of the instrument you want to use.

To modify or enter the serial number, click on **Modify.** The Modify window opens. Enter or select the serial number of the instrument and click on **OK**.

Note: After installing the PhosphorImager SI, you must enter the serial number that is printed on the back of the instrument. See section 10.1 for instructions.

Click on **OK** to close the Select Scanner window. The Scanner Control window changes to reflect the new configuration of the instrument.

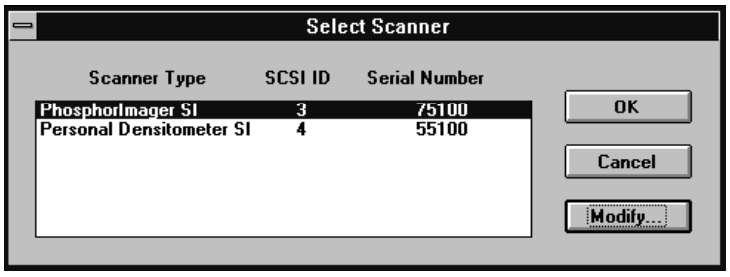


Figure 11-3. The Scanner Select window.

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11.9 Templates Area

A template contains settings for the area to scan, the pixel size, the image analysis software, and the scanner information. It does not include the dataset name.

11.9.1 Using a Template

To use an existing template, click on the down arrow ( ) in the Templates box, then click on the template in the list. The settings for the template appear in the Scanner Control window. You need only add the dataset name and, if desired, image information.

11.9.2 Creating a Template

To create a new template, you modify an existing template by changing the settings in the Scanner Control window, entering a new template name, and saving the template.

1. Click on the **Templates** box to display the list of templates, then select the template you want to modify.

2. Change the settings as follows:

   - In the **Scan area,** draw the rectangular area you want to record. (When the Save As window opens, click on the **Cancel** button because you do not want to specify a dataset name in the template.)

   - In the **Pixel Size** box, select the desired pixel size.

   - In the **Image Analysis Software** box, change the setting if necessary.

3. Double-click on the **Templates** box of the Scanner Control window and type the new name, then click on the **Save** button. The new template is saved under the new name and the old template remains unchanged.

   Instead of creating a new template, you can modify the existing template. To do this, click on **Save** without changing the template name. A message appears asking if you want to delete the original template and replace it with the new one, click on **Yes.**

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11.9.3 Deleting a Template

You can delete a template that is no longer useful.

1. In the Templates box, select the template you want to delete.

2. Click on the **Delete** button. The Delete Confirmation window opens and asks if you are sure you want to delete the selected template.

3. Click on **Yes** to delete the template and return to the Scanner Control window. (Click on **No** to close the window without deleting the template.)

11.10 Check Available Disk Space

The Scanner Control software automatically checks for sufficient disk space when you start the scan. If not enough space is available, you will see an error message that displays the maximum allowable dimensions. To create free space, you can either change the dimensions of the Scan area, change the pixel size, transfer files, or delete files.

11.10.1 Calculating Disk Space Needed

To calculate the amount of disk space you need for the scan, use the following formula:

(number of grid squares) x (disk space per grid square)

The number of grid squares is the number of grid squares covered by the white rectangle in the Scan area of the Scanner Control window.

The disk space per grid square of the scan depends on the pixel size you use:

- 50µ pixel size uses 315 KB of disk space per grid square. A full 20 cm x 25 cm image (130 grid squares) uses 40 MB.

- 100µ pixel size uses 79 KB of disk space per grid square. A full 20 cm x 25 cm image (130 grid squares) uses 10 MB.

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- 200µ pixel size uses 20 KB of disk space per grid square. A full 20 cm x 25 cm image (130 grid squares) uses 2.5 MB.

11.10.2 Displaying Available Disk Space

To display the disk space currently available on the computer hard drive, click on the **Help** button in the Scanner Control window. A window (figure 11-4) appears showing the disk space available in megabytes and the amount of space required to store the selected image size (Scan area) at the selected pixel size.

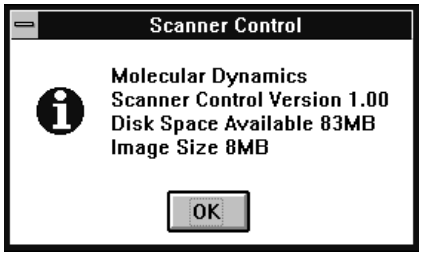


Figure 11-4. Displaying available disk space.

Compare the disk space available to the disk space needed for the scan. If not enough disk space is available you can—

- Move files to another workstation or a server if your workstation is connected to a network.
- Transfer files to a backup disk or tape.
- Delete some files to free up disk space.
- Decrease the total area to be scanned.
- Select a larger pixel size.

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Chapter 12 Macintosh Scanner Control

The Macintosh Scanner Control window (figure 12-1) has the following functions:

- **Dataset box**—Displays the name of the current dataset (section 12.1).
- **Help button**—Displays the disk space available on the computer hard disk (section 12.2).
- **Image Info area**—Contains fields for entering user name, preparation date, preparation time, and comments pertaining to the sample (section 12.3).
- **Pixel Size box**—Establishes the pixel size for the scan (section 12.4).
- **Scan area (grid)**—Determines the scan size and location, then activates the Save As window (section 12.5).
- **Scan button**—Begins the scan (section 12.6).
- **Scanner Info area**—Displays the scanner type, SCSI ID, and serial number for the currently selected instrument (section 12.7).
- **Templates area**—Selects, saves, and deletes templates (section 12.8).

Each of these areas is discussed in the following sections. In addition, section 12.9 describes how to determine disk space requirements.

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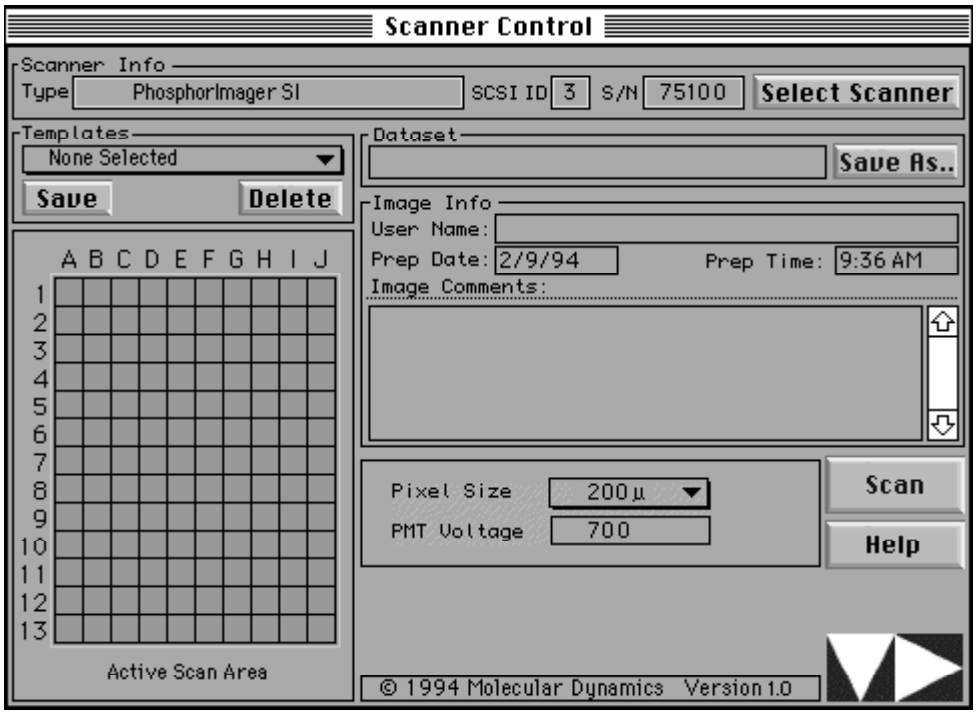


Figure 12-1. The Macintosh Scanner Control window.

12.1 Dataset Box

The Dataset box displays the name of the dataset that you are about to create. The Save As button opens the Save As window so that you can create a new dataset without changing the Scan area.

12.2 Help Button

You use the **Help** button to determine the amount of disk space available on the computer hard drive and the amount of disk space required to store the current Scan area at the selected pixel size.

12.3 Image Info Area

The Image Info area allows you to create notes to store with the image. To enter information, click on any box you want to use and type the information.

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12.4 Pixel Size Box

Pixel size refers to the size of each individual "spot" (or picture element) that is recorded and, together with the thousands of other pixels, forms the image. The PhosphorImager SI allows you to select from three pixel sizes: 50µ pixel size (4000 data points/cm^2), 100µ pixel size (1000 data points/cm^2), and 200µ pixel size (250 data points/cm^2).

When scanning typical samples, you can save time and disk space by using the 200µ pixel setting. For example, at the 200µ setting, scan time is approximately five minutes, whereas a 100µ scan is approximately seven minutes. The file size is approximately one-fourth that of a 100µ scan. The 50µ option gives you very high resolution images, but the resulting image file size is approximately 16 times that of a 200µ scan and scan time is approximately 15.5 minutes.

Note: In the PhosphorImager SI, the digital resolution is set at 16 bits. The digital format refers to the number of bits used to represent a pixel. The more bits-per-pixel, the higher the resolution. The instrument can distinguish between adjacent bands of similar signal intensity and can detect extremely faint bands.

12.5 Scan Area

You identify the scan coordinates and enter a dataset name from the Scan area.

12.5.1 Selecting the Scan Area

The grid on the Scanner Control window (figure 12-1) represents the grid on the exposure cassette. You designate the area to scan by using the mouse to draw a rectangle on the grid.

When drawing the rectangle, select only those grid squares that were covered by the sample. Selecting an area larger than the sample increases the scan time and the size of the image file. Section 5.4 provides step-by-step instructions.

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12.5.2 Entering a Dataset Name

You must enter a dataset name in the Save As window each time you create an image. If you draw a new Scan area, the Save As window opens automatically when you finish (figure 12-2). (If you want to use the existing Scan area, click on the **Save As** button in the Dataset area to open the Save As window.)

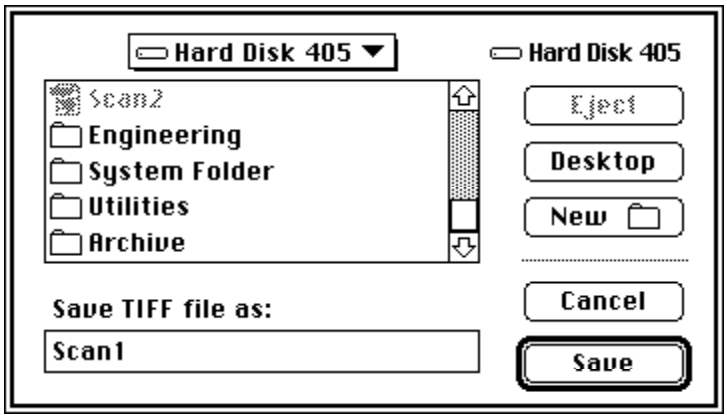


Figure 12-2. The Save As window.

If you want to store the dataset in other than the default folder, select a new folder from the hard disk list.

Enter the name for the dataset in the **Save TIFF file as** box. When you click on **Save,** the Save As window closes and the dataset name appears in the Dataset box.

12.6 Scan Button

After you enter all the information for the scan, click on the **Scan** button. The screen must be in the PhosphorImager SI and the door must be closed. The Start Scan window appears. Click on the **Start Scan** button to begin the scan. The scan is saved under the file name entered in the Save As window.

12.7 Scanner Info Area

The Scanner Info area displays the instrument currently accessed by the Scanner Control. The scanner type, SCSI ID number, and serial number are displayed. If more than one instrument is connected to the host computer, you can switch to a different instrument.

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To change instruments, click on the **Select Scanner** button, which opens the Select Scanner window (figure 12-3). Click on the **SCSI** button. The window changes to display all the instruments connected to the SCSI bus. Click on the instrument name you want to use.

To modify or enter the serial number, click on **Edit.** The Edit window opens. Enter or select the serial number of the instrument and click on **OK**.

Note: After installing the PhosphorImager SI, you must enter the serial number that is printed on the back of the instrument. See section 10.2 for instructions.

Click on **OK** to close the Select Scanner window. The Scanner Control window changes to reflect the new configuration of the instrument.

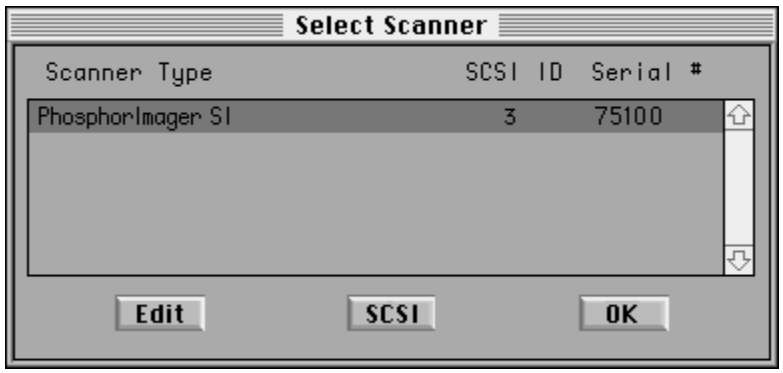


Figure 12-3. The Select Scanner window.

12.8 Templates Area

A template contains settings for the area to scan and the pixel size. It does not include the image information or dataset name.

12.8.1 Using a Template

To use an existing template, move the pointer on the Templates box and hold down the mouse button, then highlight the template in the list. The settings for the template appear in the Scanner Control window. You need only add the dataset name and, if desired, image information.

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12.8.2 Creating a Template

To create a new template, you modify an existing template by changing the settings in the Scanner Control window, entering a new template name, and saving the template.

1. Move the pointer onto the Templates box and hold down the mouse button to display the list of templates, then select the template you want to modify.

2. Change the settings as follows:

   - In the **Scan area,** draw the rectangular area you want to record. (When the Save As window opens, click on the **Cancel** button because you do not want to specify a dataset name in the template.)

   - In the **Pixel Size** box, select the desired pixel size.

3. To save the template, click on the **Save** button. A window appears. Enter the name for the new template and click on **OK.** If you enter—

   - A **new name**—The new template is saved under the new name. The old template remains unchanged.

   - The **original template name**—The old template is over-written by the new template settings.

   If you entered an existing template name, a message appears asking if you want to replace the existing template—

   - If you want to delete the original template and replace it with the new template, click on **Yes**.

   - If you want to keep the original template, click on **No** to return to the Scanner Control window. Repeat this step.

12.8.3 Deleting a Template

You can delete a template that is no longer useful.

1. In the Templates box, select the template you want to delete.

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2. Click on the **Delete** button. The Delete Confirmation window opens and asks if you are sure you want to delete the selected template.

3. Click on **OK** to delete the template and return to the Scanner Control window. (Click on **Cancel** to close the window without deleting the template.)

12.9 Check Available Disk Space

The Scanner Control software automatically checks for sufficient disk space when you start the scan. If not enough space is available, you will see an error message that displays the maximum allowable dimensions. To create free space, you can either change the dimensions of the Scan area, change the pixel size, transfer files, or delete files.

12.9.1 Calculating Disk Space Needed

To calculate the amount of disk space you need for the scan, use the following formula:

(number of grid squares) x (disk space per grid square)

The number of grid squares is the number of grid squares covered by the rectangle in the Scan area of the Scanner Control window.

The disk space per grid square of the scan depends on the pixel size you want to use:

- 50µ pixel size uses 315 KB of disk space per grid square. A full 20 cm x 25 cm image (130 grid squares) uses 40 MB.

- 100µ pixel size uses 79 KB of disk space per grid square. A full 20 cm x 25 cm image (130 grid squares) uses 10 MB.

- 200µ pixel size uses 20 KB of disk space per grid square. A full 20 cm x 25 cm image (130 grid squares) uses 2.5 MB.

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12.9.2 Displaying Available Disk Space

To display the disk space currently available on the computer hard drive, click on the **Help** button in the Scanner Control window. A window (figure 12-4) appears showing the disk space available in kilobytes and the amount of space required to store the selected image size (Scan area) at the selected pixel size.

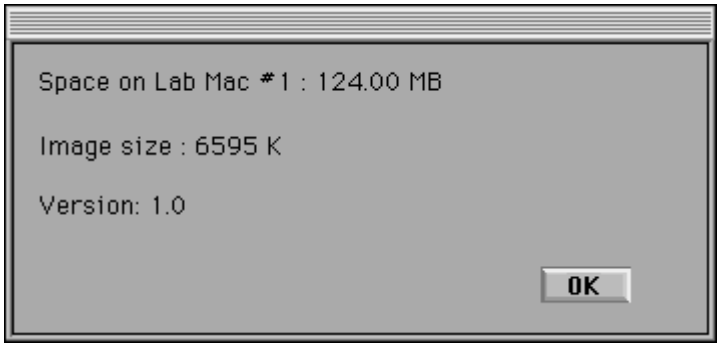


Figure 12-4. Displaying available disk space.

Compare the disk space available to the disk space needed for the scan. If not enough disk space is available you can—

- Move files to another workstation or server if your workstation is connected to a network.
- Transfer files to a backup disk or tape.
- Delete some files to free up disk space.
- Decrease the total area to be scanned.
- Select a larger pixel size.

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Chapter 13 Troubleshooting and Assistance

If you are having problems with your PhosphorImager SI, use the troubleshooting section below to locate the description that matches your problem. If you cannot find a solution, call Molecular Dynamics for assistance (see section 13.2 for locations and phone numbers).

13.1 Troubleshooting

Problems, in bold print below, are followed by possible causes and solutions.

The PhosphorImager SI Will Not Turn On

- The instrument may be unplugged.
- The wall outlet may be faulty. Test the outlet or try another one.
- The fuse may have blown. To change the fuse, see section 9.1.4.

The PhosphorImager SI Is On But Cannot Communicate

- The PhosphorImager SI may be set to a "bad" SCSI address or another SCSI device may have the same address as the PhosphorImager SI. Change the address on the PhosphorImager SI or on the conflicting SCSI device.
- The last peripheral on the SCSI bus may not be properly terminated. Add the terminator as discussed in section 10.1 (Windows NT) or section 10.2 (Macintosh).
- The SCSI cable may not be properly plugged into all peripherals. Check the connections to make sure they are tight.
- The serial number entered in the Scanner Control window may be wrong or missing. Enter the number from the back of the instrument. See section 10.1 (Windows NT) or section 10.2 (Macintosh) for instructions.
- The SCSI cables may be incorrect or too long.

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The Instrument Will Not Scan

- The phosphor screen may not be in place.
- The door may not be closed.

High Background or Inaccurate Readings

- The instrument may not have been warmed up before the sample was scanned.
- The phosphor screen may not have been erased immediately before you exposed it to the gel.
- The PhosphorImager SI may be damaged and no longer light-tight. If so, do not continue to use the PhosphorImager SI. Contact your Molecular Dynamics representative to arrange for repair.
- The instrument may be in an area with strong lights. Move the instrument to a darker area.

Incomplete Image

The scanning coordinates entered in the Scanner Control window may be incorrect. See section 4.4 (Windows NT) and section 5.4 (Macintosh) for instructions.

Loss of Signal at the Edges

A light leak may have occurred during exposure. Be sure to expose screens in the exposure cassette.

Loss of Resolution

The sample may not be placed directly against the screen. Use the exposure cassette. Cover a wet gel with plastic wrap or mylar.

Streaks or Other Oddities on the Image

- Diagonal streaks may indicate a light leak during scanning. Check for damaged panels on the PhosphorImager SI.
- Static electricity may have collected on the screen. Clean the screen with Kodak Intensifying Screen Cleaner before using it.

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- The screen may be contaminated with radioactive material. (The source of this contamination could also be the surfaces of the Eraser or the exposure cassette.) Clean the screens, exposure cassette, and Eraser surfaces (see sections 2.1, 2.2, and 2.3).

- The screen may have been exposed to too much light between the time you removed it from the exposure cassette and the time you inserted it into the PhosphorImager SI for scanning. Keep the screen face down in subdued light until it is placed in the PhosphorImager SI for reading.

- The screen may not have been erased immediately before scanning. (Cosmic radiation will expose screens left unused for long periods of time.)

- The screen may not have been completely erased. Select the Extended setting and erase again (see section 2.3).

- The screen may 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 may be on the screen. Clean the screen with Kodak Intensifying Screen Cleaner.

- The instrument may not have been warmed up before the screen was scanned.

Ghost Image

- The screen may have been adjusted after initial placement. Always position the screen correctly on the first try and do not readjust the placement. If readjustment is absolutely necessary, be sure to erase the screen completely before placing it in the exposure cassette again.

- The sample may have shifted when the screen was inserted into the cassette. Erase the screen and re-expose it to the sample.

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13.2 Assistance

When calling for assistance, be prepared to supply the serial number of your instrument. The serial number is located on the back panel (see figures 9-3 and 9-4) or in the Scanner Info area of the Scanner Control window.

United States
Molecular Dynamics World Headquarters, Sunnyvale, CA.
Telephone 1-800-743-7782 or 408-773-1222, Fax 408-773-8343.
Ask for Technical Support.

England
Molecular Dynamics Ltd., Kemsing.
Telephone (0732) 762565, Fax (0732) 763422.

Germany
Molecular Dynamics GmbH, Krefeld.
Telephone 02151-8387.0, Fax 02151-8387.40.

France
Molecular Dynamics S.A., Paris.
Telephone (1) 60 86 65 13, Fax (1) 60 86 65 33.

Japan
Molecular Dynamics Japan, Inc., Tokyo.
Telephone (03) 3976 - 9692, Fax (03) 3976 - 8845.

Australia
Molecular Dynamics Pty. Ltd., Kew East, VIC.
Telephone (03) 810 - 9572, Fax (03) 859 - 7253.

Other Countries
Please call your Molecular Dynamics distributor or representative. If you need the name and number of your representative, please contact Molecular Dynamics World Headquarters at 408-773-1222, FAX 408-773-8343.

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Part 3

Appendices

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Appendix A Specifications

Standard Units of Computer Memory

- Bit = the basic on/off unit of information (shorthand for Binary digIT)
- 1 Byte = eight bits
- 1 Kilobyte (KB) = 2^{10} or 1,024 bytes
- 1 Megabyte (MB) = 2^{20} or 1,048,576 bytes

Pixel Size

- 50µ pixel = 4000 data points/cm^2
- 100µ pixel = 1000 data points/cm^2
- 200µ pixel = 250 data points/cm^2

Digital File Format

- 16-bit data file

Disk Space per Grid Square of Scan

- 50µ pixel size uses 315 KB disk space per grid square. A full 20 cm x 25 cm image (130 grid squares) uses 40 MB.
- 100µ pixel size uses 79 KB disk space per grid square. A full 20 cm x 25 cm image (130 grid squares) uses 10 MB.
- 200µ pixel size uses 20 KB disk space per grid square. A full 20 cm x 25 cm image (130 grid squares) uses 2.5 MB.

Disk Space Required for the Scan

The amount of disk space you will need for your scan is:

(number of grid squares) x (disk space per grid square)

The number of grid squares is the area on the sample you want to scan. For example, scanning an area from coordinates F7 to M15 is (8 x 9) or 72 grid squares.

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Disk Space Available

To find the disk space available for your scan, select **Help** from the Scanner Control window. A window opens displaying the available disk space.

Performance Specifications

The following are the performance specifications for the PhosphorImager SI:

- Uniformity

  ±5% over 18 x 23 cm area

- Linear Dynamic Range

  Four orders of magnitude (1–10,000)

- Detection Threshold

  ^{14}C - 1.1 dpm/mm^2/hr
  ^{35}S - 1.1 dpm/mm^2/hr
  ^{32}P - 0.17 dpm/mm^2/hr
  ^{125}I - 0.23 dpm/mm^2/hr

- Spatial Resolution (CTF = 0.33)

  For ^{14}C autoradiography: ≥1.9 line pairs/mm
  For focused x-ray exposure: ≥12.1 line pairs/mm

- Scan Times (20 x 25 cm)

  ~5 minutes @ 200µ pixel
  ~7 minutes @ 100µ pixel
  ~15.5 minutes @ 50µ pixel

Windows NT-Based Computer

The Windows NT-based computer must meet or exceed the following specifications:

486-66 MHz computer, preferably with built-in Local Bus graphics
Keyboard and Mouse

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1 MB VRAM
16 MB of RAM, 32 MB of RAM recommended
340-MB hard disk, or larger
EtherLink® III 3C509 card, recommended but not required
Adaptec® 1520 SCSI card for peripheral devices
15-inch color monitor, 1024 x 768 capable
3.5-inch floppy drive
Windows NT v3.1 (build 528 or higher)
Excel 4.0 or higher
MS DOS recommended

Macintosh Computer

The Macintosh computer must meet or exceed the following specifications:

Quadra™ 650 computer with keyboard, mouse, and monitor
16 MB of RAM
230-MB hard disk
1 MB VRAM

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Appendix B Warranty Statement

Molecular Dynamics PhosphorImager SI
U.S.A. only, 2/94

Note: This warranty varies outside the United States. If you are located outside the United States, please contact your representative for the exact terms of your warranty.

Molecular Dynamics warrants to the Customer that the Molecular Dynamics PhosphorImager SI ("the Instrument") purchased by the Customer will be free from defects in material and workmanship and will meet its performance specifications for a period of one year (365 days) from the date of shipment from the manufacturing site. This warranty covers parts, labor, and shipping costs if the instrument is returned to the factory. Labor and travel for on-site repair during the warranty period is covered.

Customers are encouraged to have a Molecular Dynamics service engineer replace or repair technically complex components of the Instrument. Molecular Dynamics warrants to the Customer that only parts installed by a Molecular Dynamics service engineer, including any engineering change initiated by Molecular Dynamics, will be free from defects in workmanship for a period of one year (365 days). During the one-year period, this warranty covers parts and labor when the Instrument is returned to the factory. All Customer-installed parts supplied by Molecular Dynamics are warranted only to be free from defects in workmanship.

When the Instrument is returned to the factory for repair under warranty, the Customer must call Molecular Dynamics for a return authorization number and packing information. All Instruments must be packed for return according to Molecular Dynamics specifications. Molecular Dynamics will not be responsible for damage to an Instrument incurred during shipping if the Instrument was not packed according to Molecular Dynamics specifications. Molecular Dynamics will pay for air freight in both directions during the warranty period.

A list of parts that are considered to be Customer-installable consumables is attached. These parts are not covered by this warranty.

This warranty does not extend to any Instruments or parts thereof that have been subject to misuse, neglect or accident, or that have been modified by anyone other than Molecular Dynamics, or that

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have been affixed to any nonstandard accessory attachment or that have been used in violation of Molecular Dynamics instructions.

Because Molecular Dynamics instruments are fully configured with all software necessary for operation, service calls resulting from customer-installed software are not covered under this warranty agreement.

No agent, employee, or representative of Molecular Dynamics has any authority to bind Molecular Dynamics to any affirmation, representation, or warranty concerning the Instrument; and any affirmation, representation, or warranty made by any agent, employee or representative shall not be enforceable by the Customer.

This warranty is the sole and exclusive warranty as to the Instrument and extends only to the Customer and is expressly in lieu of any other expressed or implied warranties, including without limitation any implied warranty or merchantability or fitness for a particular purpose and of any other obligation on the part of Molecular Dynamics.

Molecular Dynamics shall not be liable for any incidental, special or consequential loss, damage, or expense directly or indirectly arising from the use of the Instrument. Molecular Dynamics makes no warranty whatsoever in regard to products or parts furnished by third parties, such being subject to the warranty of their respective manufacturers. Service under this warranty shall be requested by contacting the Molecular Dynamics service representative at 800-743-7782, or, outside the United States, by contacting your local representative. (If you need the name and number of your representative, please contact Molecular Dynamics at 408-773-1222; Fax 408-773-8343.)

A service contract is available on an annual basis. Information on the service contract may be obtained by contacting the Molecular Dynamics Service Department in Sunnyvale, California, or your local Molecular Dynamics representative.

Excluded from warranty:

Phosphor Screens
Eraser Lamps

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Appendix C Literature References

The following reprints and notes are available from Molecular Dynamics:

Johnston, R.F., S.C. Pickett, and D.L. Barker. 1990. Autoradiography Using Storage Phosphor Technology. *Electrophoresis II* 355-360.

Molecular Dynamics Technical Note #53, Storage Phosphor Screens.

Pickett, S.C., D.L. Barker, and R.F. Johnston. Molecular Dynamics Application Note #50, Quantitative Double-Label Autoradiography Using Storage Phosphor Imaging.

Pickett, S.C. Molecular Dynamics Application Note #51, Quantitative Southern Blot Analysis Using Storage Phosphor Technology.

Pickett, S.C., R. Scheuermann, and S. Bauer. Molecular Dynamics Application Note #53, Quantitative PCR Using Storage Phosphor Technology.

Pickett, S.C. Molecular Dynamics Technical Note #55, Evaluation of Storage Phosphor Imaging Systems.

Wygant, M.D., D.L. Barker, and J.W. Nelson. Molecular Dynamics Technical Note #52, Improving Quantitation in Autoradiography.

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