PharosFX™
Molecular Imager® System

Hardware Instruction Manual

for Catalog Numbers

170-9450   **PharosFX System (532 nm)**

170-9460   **PharosFX Plus System (532 nm)**

170-7890   **External Laser (488 nm)**
170-7892   **External Laser (488 and 635 nm)**

This instrument is for Laboratory Use Only

BIO·RAD

Welcome

Dear Customer,

On behalf of Bio-Rad Laboratories, we would like to thank you for investing in the Molecular Imager PharosFX Product Family. This combination of systems allows you the highest level of flexibility for the best in isotopic and fluorescence imaging and we are sure that it will provide you with many years of high quality imaging.

One of the best ways to familiarize yourself with the capabilities of your new PharosFX system is to read this manual. In it, you will learn how to set up the system and operate all hardware components. It is also recommended that you read the accompanying software manual, to familiarize yourself with general acquisition functions and data analysis. After reading this manual, please keep it close to your PharosFX system so that it can be conveniently referred to.

Your PharosFX system is protected by a comprehensive instrument warranty agreement. Please read this warranty (Appendix 3) thoroughly, so that you fully understand the coverage it provides and are aware of your rights and responsibilities. One of the responsibilities of system ownership is regular maintenance. Following the maintenance instructions provided with this manual will help to keep your system and peripherals functioning optimally and will protect your investment. Please also keep in mind that Bio-Rad offers a range of comprehensive service agreements that can be tailored to meet your specific needs.

Bio-Rad Laboratories is dedicated to your total satisfaction and would be pleased to answer any questions or concerns that you may have.

How to Contact Bio-Rad Laboratories

In the United States you can reach Bio-Rad Laboratories at the following numbers:

For general information

Toll free:	1-800- 4BIORAD
	1-800-424-6723
Fax:	1-510-741-5802
email:	lsg.techserv.us@bio-rad.com

For service or technical assistance
Toll free:	1-800-424-6723
Fax:	1-800-741-5802

Outside the United States contact your local Bio-Rad Laboratories office.

For information concerning Bio-Rad Laboratories and its products, visit our Worldwide Web site at **http://www.bio-rad.com**.

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Section 1
General Information

1.1 About this Manual

This manual provides instructions for installing, operating and maintaining the PharosFX system. This manual uses certain conventions to facilitate understanding of the text material and to assist operators in using the PharosFX system.

Notes, Cautions and Warnings

Notes, cautions and warnings are used to highlight certain operating procedures and recommendations.

A note indicates a special procedure, an exception to normal operation or something else of specific interest to the reader. Notes are preceded by the word "Note" in italics.

A caution precedes an operational step that could damage the instrument or destroy data unless the operator takes certain precautions. Cautions are located in the main text, are preceded by a **Caution**: statement and are accompanied by a "Caution Symbol" in the left margin.

A warning precedes an operating procedure that could cause injury to the operator if not followed correctly. Warnings are located in the main text, are preceded by a **Warning**: statement and are accompanied by a "Warning Symbol" in the left margin.

1.2 Safety Information

Your safety and the safety of others is very important to us. To help you make informed decisions about safety, we have provided comprehensive operating procedures and safety information in this manual and on labels affixed to instrumentation. This information will alert you to any potential hazards.

1.2.1 General Cautions

Caution: Always install the scan head locking screw before moving the PharosFX and avoid subjecting the PharosFX system to vibration. (See section 3.2.2)

Caution: After transport, always remove the scan head locking screw before supplying power to the PharosFX scanner. (See section 3.2.2)

Caution: Other than emission filter wheel access port, do not remove instrument covers. There are no user-serviceable parts inside. Refer all servicing to qualified Bio-Rad personnel or their agents. If you experience technical difficulties with the instrument, contact Bio-Rad to schedule a service appointment.

Caution: The instrument should not be modified or altered in any way other than moving or changing emission filters. Alteration of this instrument voids the manufacturer's warranty and may create a potential safety hazard for the user.

Caution: Bio-Rad is not responsible for any injury or damage caused by the use of this instrument for purposes other than that for which it is intended or by the modification of this instrument when not performed by qualified Bio-Rad personnel or an authorized agent.

1.2.2 General Warnings

Warning: There are hazardous voltages inside the PharosFX scanner. Do not attempt to defeat the door interlock or remove the instrument's cover. These are designed to prevent user injury.

1.2.3 Power Safety Information

The PharosFX system is designed and certified to meet both I.E.C. 61010 safety standards and Center for Devices, Radiological Health (CDRH) laser safety standards. Certified products are safe to use when operated in accordance with the instruction manual. This safety certification does not extend to uncertified equipment or accessories, even when connected to the PharosFX system.

This instrument and its accessories should not be altered or modified in any way. Alteration of the PharosFX or its accessories will void the manufacturer's warranty, void the I.E.C. 61010 and CDRH certification, and create a potential safety hazard for the user.

Bio-Rad Laboratories is not responsible for any injury or damage caused by the use of this instrument for purposes other than for which it is intended or by modifications of the instrument not performed by Bio-Rad or an authorized agent.

Figure 1.1 shows two serial number certification labels. These are found at the rear of the PharosFX system and the rear of the External Laser. This label provides manufacturing data about the instrument, its voltage settings and CDRH compliance information.
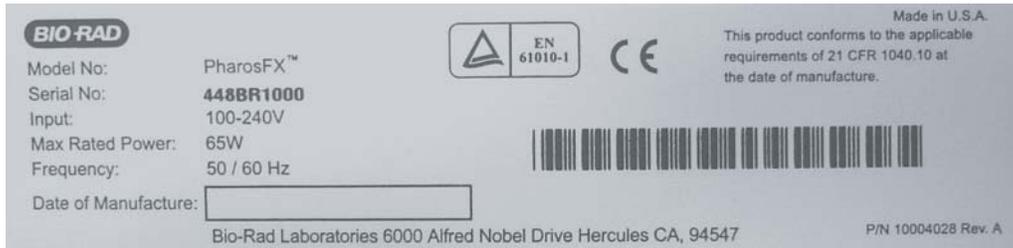

Fig. 1.1.a. Instrument serial number information on the rear of the PharosFX Scanner.

Note: For easy customer access, serial number information for the PharosFX scanner is also located on the right hand side of the overlay located behind the scanner door.
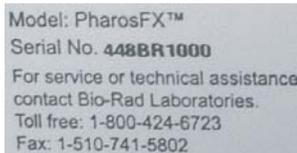
Fig. 1.1.b. Instrument serial number information on the rear of the External Laser.

1.2.4 Laser Safety Information

This instrument and its accessories are certified according to 21 CFR 1040 of the CDRH, as a Class I laser device and IEC / EN 60825-1+A1+A2 as a class 1 laser device. (Figure 1.1.a).

The laser contained within the PharosFX and PharossFX Plus scanning unit is configured with a laser that generates energy up to 15 milliwatts at 532 nm. The cover of each scanner has redundant interlocks and is designed to protect the user.

The optional External Laser Module contains an Argon-Ion laser that produces laser energy of up to 10 milliwatts at 488 nm. This External Laser Module can also be outfitted with an additional laser diode that produces laser energy of up to 10 milliwatts at 635 nm. The cover of this system is designed to protect the user at all times.

Warning: Do not remove the cover for any reason or defeat the interlock. Attempting to operate the unit with the cover removed may damage the instrument and expose the operator to energy from the laser.

Warning: Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous laser energy exposure.

Caution: The top cover should be removed by trained service personnel only. Do not attempt to operate the product with the cover removed. The PharosFX system should be serviced only by Bio-Rad or its trained representatives.

Laser warning labels (Figure 1.2) are located externally on the top cover and rear of the instrument and internally on the top surface of the mounting plate, at the rear right-hand corner and the top of the laser module.

Fig. 1.2. Laser warning label.

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1.2.5 Screen Eraser Safety Information

Warning: The Screen Eraser must be plugged into a grounded electrical outlet.

Section 2
Introduction

2.1 PharosFX System Capabilities

The PharosFX Product Family provides options for imaging and analyzing the following sample types:

Fluorescence (for PharosFX, and PharosFX-Plus Models)

The PharosFX detects almost any visibly excited fluorescent dye. The internal and optional external lasers allow optimal excitation of single-color or multi-color fluorescent samples. Computer controlled filter wheels with 6 available emission filter positions allow detection of many multi-color fluorescent signals. The system is also capable of performing sequential detection of multiple fluorescent dyes on the same gel or blot.

Colorimetric Detection

A Transilluminating Screen, made of white plastic, is provided with the sample tray for PharosFX and PharosFX Plus for documentation of gels stained with colorimetric stains, such as Coomassie and Silver Stain.

Radioisotope Emissions (for PharosFX Plus Only)

Detects a broad range of isotopes, including ^{32}P, ^{33}P, ^{35}S, ^{14}C, and ^{3}H. The PharosFX uses storage phosphor screen technology that is at least ten times more sensitive to isotopic emission than x-ray film. The system is compatible with most available phosphor imaging screens based on the BaFBr:Eu storage phosphor chemistries

2.2 System Description

Fig. 2.1. A Typical PharosFX scanner and peripherals.

The PharosFX (Plus) imaging system consists of:

- Laser scanner

- Sample Tray set, including: glass sample tray, transilluminating screen, and sample holders

- Pre-installed filters for fluorescence detection: 640BP, 605BP, blank filter holder for custom filters. PharosFX Plus has an additional 390BP filter for phosphor screens

- External Laser Module is optional

(1) Laser Scanner

The PharosFX and PharosFX Plus have an internal laser that emits light at 532 nm only. All PharosFX units can scan at resolutions of 50, 100, 200, and 800 microns and have a linear dynamic range that extends over 4.8 orders of magnitude (1:65536). In contrast, x-ray film has a linear dynamic range that is limited to only 1.5 orders. The scanner also contains fully automated emission filter wheels with 6 filters. These combined features permit the PharosFX to image almost any fluorescent dye and to scan multi-color fluorescence applications, in addition to storage phosphor imaging with the PharosFX Plus.

(2) Sample Tray

The glass Sample Tray is used as a scanning platform for fluorescent gels or blots and K-type phosphor screens. Its spill resistant design exhibits very low background fluorescence and high chemical resistance.

Additional Multi-Sample trays can be purchased to accommodate thick agarose gels, gels within glass plates (1707819 Multi-Sample Tray II), metal backed storage phosphor screens,and microtiter plates (1707812 Multi-Sample Tray).
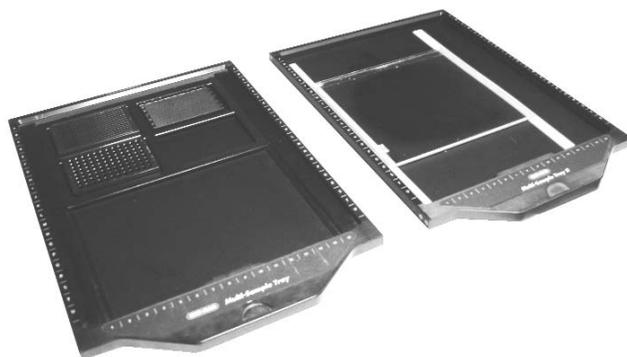

Fig. 2.2. a) Multi-Sample Tray, b) Multi-Sample Tray II

(3) Optional External Laser Module

The optional external laser module provides one or more additional excitation source(s). The current configurations for lasers are:

a)   488 nm Only

b)   488 nm and 635 nm

Fig. 2.3. External Laser Module

(4) Storage Phosphor Imaging Screens

Bio-Rad offers a range of storage phosphor screens to match different user requirements. Table 2.1 summarizes the key features of each screen. The screens are composed of a barium fluorobromide matrix doped with europium (BaFBr:Eu); they can be used with traditional autoradiography cassettes without a darkroom, are easy to handle and are used solely for the detection of isotopic emissions.

PharosFX Plus offers a variety of phosphor storage applications in addition to all the functionality of the PharosFX. All phosphor screens are reusable and unharmed by repeated exposure to radioactivity. Screens are sensitive to β particles and X-rays. All screens are flexible and easy to handle. Exposure takes place in standard X-ray cassettes. All phosphor screens require erasure prior to re-exposure, and their lifetime is extended when they are cared for properly.

Imaging Screen-K

This is a general-purpose screen designed for all common radioisotopic emitters, such as ^{32}P, ^{33}P, ^{35}S, and ^{14}C. These screens are available in 35 x 43 cm and 20 x 25 cm formats. Screens are guaranteed for 1 year.

Imaging Screen-K/Tritium

This is a special imaging screen available for imaging ^3H. These screens require special care and handling and are reusable only if cared for properly. Screens are 20 x 25 cm and are covered by a 6-month warranty.

Table 2.1. Imaging Screens Specifications and Recommended Applications

Screen Name	Application	Key Features	Sizes (cm)	Catalog Number
Imaging Screen-**K**	^{32}P, ^{33}P, ^{14}C, ^{35}S	• BaFBr:Eu formulation • Easy-to-use format • Compatible with standard X-ray cassettes • More durable	35 x 43 mounted 20 x 25 mounted	170-7841 170-7842 170-7843 170-7844
Imaging Screen-**K/Tritium**	^{3}H	• BaFBr:Eu formulation • Sensitive to weak ^{3}H signal • Easy-to-use format • Compatible with standard X-ray cassettes	20 x 25	170-7845

(5) Sample Exposure Cassettes

The sample exposure cassettes ensures that a close contact is made between the sample and imaging screen. The cassettes contain a grid marked exposure area, to which the sample is mounted. This allows the sample to be firmly pressed against the imaging screen to generate a high quality image.

Fig. 2.4. Sample exposure cassette and phosphor screen

(6) Screen Eraser

The screen eraser removes any residual signal or background from the imaging screen. The complete erasure process "zeros" or "blanks" the screen to a basal level, which is critical for maximizing sensitivity, linear response, quantitative accuracy and image quality. The Screen Eraser-K is used with the K-type screens and any other commercially available phosphor screens which are based on the BaFBr:Eu chemistry (Fig. 2.5).

Fig. 2.5. Screen Eraser.

(7) Control and Analysis Software

Quantity One software is included with the PharosFX and PharosFX Plus systems. Quantity One permits user-friendly, application driven control of the scanning system and accurate 1-D analysis of the captured image data.

2.3 Theory of Operation

2.3.1 Fluorescence Detection Mechanism

The fluorescence process occurs when a molecule absorbs light of a certain wavelength and excites electrons to a transient higher energy state (Figure 2.6, step 1). When the electrons return to ground state, energy is released in the form of photon emission at a wavelength which is longer than the illumination source (step 2). For example, ethidium bromide (EtBr) absorbs light of 532 nm and emits light at 595 nm. Using optical filters, the emission wavelength can be separated from the excitation wavelength and detected using a photomultiplier tube.
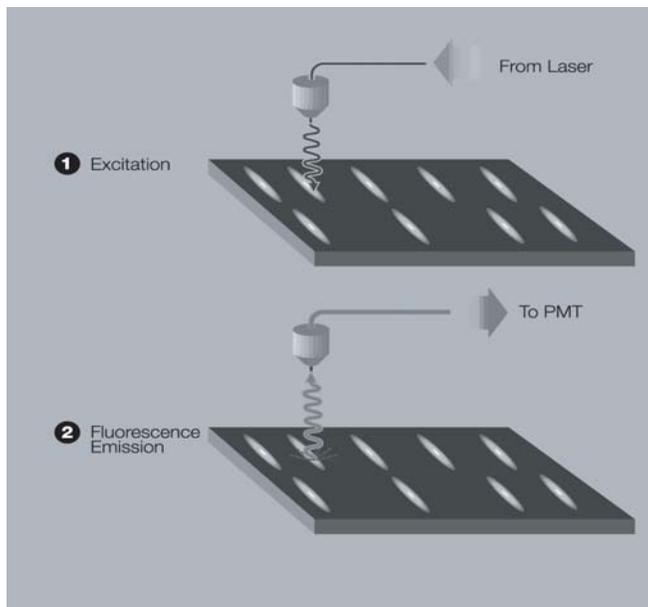
Fig. 2.6. The fluorescence detection mechanism.

2.3.2 Storage Phosphorescence Detection Mechanism

When a radioactive emission strikes the phosphor screen, phosphor oxidation occurs and a high-energy site is formed (Figure 2.3, step 1). When such an activated site is subsequently illuminated with certain wavelengths of visible light (step 2), the reduction reaction occurs. Trapped energy is released as photons that are in turn captured by a photomultiplier tube (step 3).
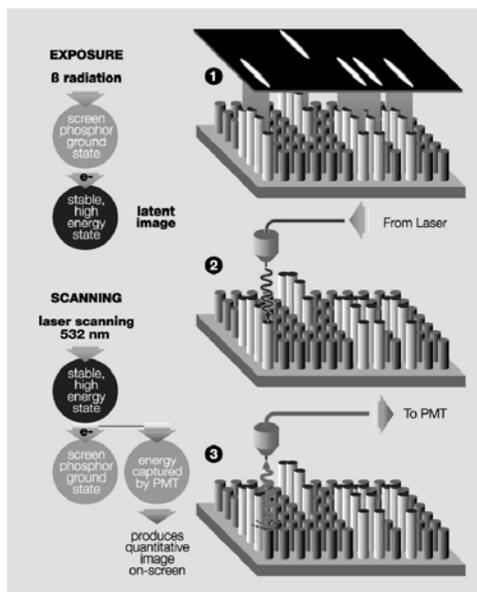
Fig. 2.7. The storage phosphor detection mechanism.

Note: Storage phosphor screens are reusable after erasure.

2.3.3 Data Processing and Analysis

Phosphorescence and fluorescence signals are captured as a 16-bit digital file. This file can then be analyzed and manipulated by the appropriate image analysis software for visualization and quantitation.

Traditionally the image is displayed in a two-dimensional format, where the darkness of each pixel is proportional to the signal intensity at that sample location (Figure 2.8, left). For the purpose of image analysis however, it is helpful and more accurate to think of the data as a three-dimensional structure, where the signal intensity at each pixel becomes the height or z-axis dimension (Figure 2.8, right). Sample spots or bands can also be visualized as peaks in a profile analysis along the length of a gel lane or perceived as topographic volumes when quantitating the total signal from a specific band or spot.
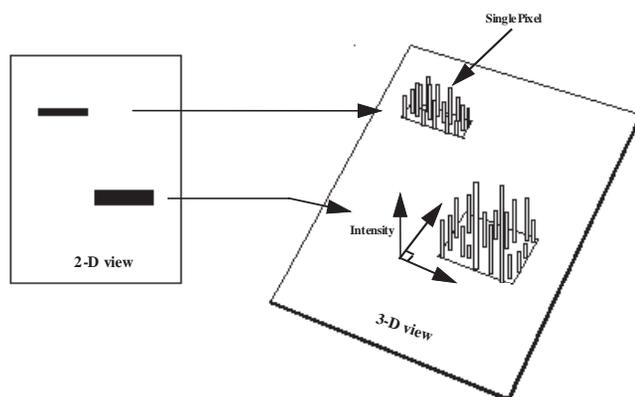
Fig. 2.8. Two and three-dimensional representations of a digitized image.

2.4 Overview of the Imaging Process

2.4.1 Steps in Fluorescence Imaging

The procedure for imaging fluorescent samples has four steps.

Step 1 involves thoroughly cleaning the sample tray with ethanol, using a clean soft cloth or lint free paper towel.

Step 2 involves placement of the sample onto the tray. Several accessories, such as gel holders, spacers and microtiter plate inserts are provided to optimize position of your sample on the tray.

In Step 3 the tray with sample loaded is pushed into the laser scanner and the sample imaged by direct fluorescence excitation. The host computer builds a digitized image of the sample by tracking each pixel's signal as the scanner head moves over the screen surface.

Step 4 Once the sample image is collected, it can then be reviewed and analyzed using an appropriate software package.

2.4.2 Steps in Storage Phosphorescence Imaging

Storage phosphor imaging is a simple four-part process. (Figure 2.9)

Step 1 involves erasing the reusable phosphor screen to remove any background or residual image. This normally takes 10 minutes. The screens should be erased to the background level of 100 counts or less.

Step 2 in the process involves placement of the prepared sample in an exposure cassette for subsequent close proximity exposure to the imaging screen. The captured signal generates a latent image of the sample, which is encoded in the number and pattern of charged phosphor crystals.

Step 3 involves placing the screen in a laser scanner. As each pixel of the screen is scanned, the electrons in charged areas of the latent image return to the ground state, releasing energy in the form of emitted photons of visible light. The emitted photons are collected and precisely counted by a photomultiplier tube, generating an intensity for each scanned pixel. This intensity is expressed in counts or pixel density units, which are analogous to the optical density of exposed x-ray film in autoradiography.

Step 4 is analogous to the procedure for imaging fluorescence samples, the resulting image can then be reviewed and analyzed using an appropriate software package.

Upon completion of the four-step process, the storage phosphor screen can be erased and the cycle repeated with a new sample.
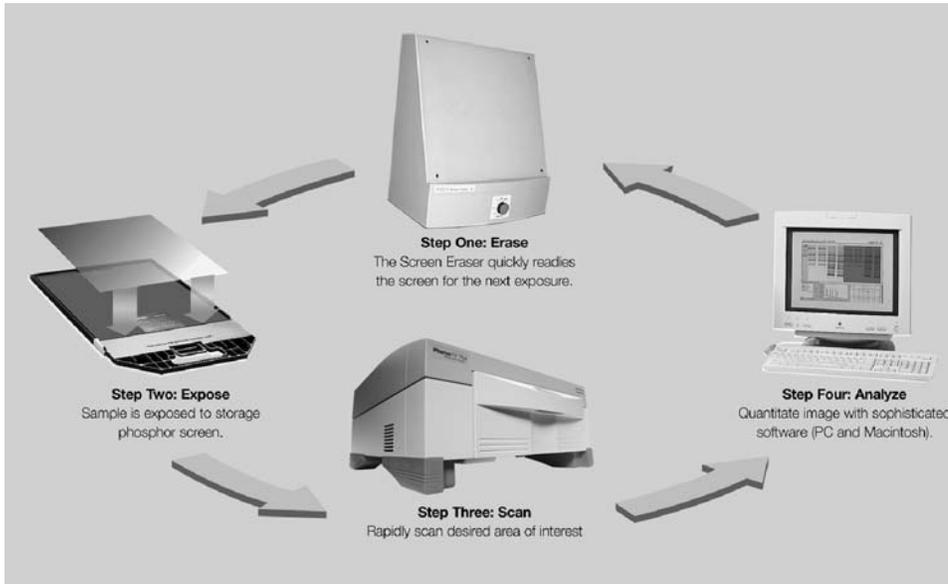
Fig.2.9. Phosphor Imaging work flow

Section 3
System Installation

3.1 Operating Requirements

3.1.1 System Location

The PharosFX scanners and all peripherals should be located in an area that is free of excessive dust or moisture, strong magnetic fields or ionizing radiation. It is also highly recommended that the ambient temperature be stable and within the range of 10°C to 32°C and the relative humidity not exceed 80%, noncondensing.

Laser Scanner and Host Computer

Warning: Care should be taken when lifting and moving the scanner to avoid personal injury. It is recommended that two people, one on each side of the instrument, lift the scanner from the bottom.

The scanner should be positioned on a level bench top with a minimum depth of 70 cm and a height clearance of 35 cm. The scanner is 59 cm wide and you should allow additional space for peripheral items such as screen erasers and exposure cassettes. You should also allow easy access to the scanner power switch, which is located on the right hand side of the unit.

The scanner should be placed where it can be easily connected to the host computer and where there is adequate room to insert the sample tray into the front of the instrument.

The maximum distance between the host computer and the scanner should be three meters and the system is supplied with a USB2 communication cable of this length.

Note: The host computer should be located at a workstation that minimizes operator fatigue.

External Laser Module

The External Laser Module has been designed for flexible placement. It can be located either to the left or right of the scanner, or placed directly below the scanner in an under-bench position. The maximum distance between the external laser and the scanner is 2 meters, as determined by the optical fiber that connects the two instruments. Once the External Laser has been installed and aligned it is not recommended to move it around to prevent any possible optical misalignment.

The External Laser Module has a weight of approximately 30 kg and to avoid personal injury the same precautions listed for the scanner should be taken when lifting the module. The external laser should only be placed on a surface capable of easily supporting its weight and located in a well ventilated area with 20 cm of clearance on the left hand side, top and rear of the unit. The unit has the following dimensions: 31 cm wide, 61 cm deep, 41 cm high.

Warning: Do not position the External Laser Module inside a cupboard or similar enclosure with poor airflow, as insufficient ventilation may cause permanent damage to the unit and present a hazard.

Screen Eraser

The Screen Eraser has no minimum clearance requirement and may even be wall mounted, using the mounting holes located in the unit's back plate.

All screen erasers must be plugged into a grounded electrical outlet.

Sample Exposure Cassettes

The exposure cassettes do not require any power and can be placed in any convenient location where radioactive samples are normally handled. If desired, the exposure peripherals can be stacked or placed directly on top or under the laser scanner to conserve bench space.

3.1.2 AC Power Requirements

The scanner and all powered peripherals including the host computer should be connected to a stable grounded power outlet on a circuit free of electrical noise. In addition, a high quality electrical surge suppressor/line filter with a 10 Amp or higher rating should be used to avoid damage from AC fluctuations. Only a grounded 3-pin power cord should be used to connect power.

The scanner is designed for input voltages of 100–240 VAC at 50-–60 Hz, and requires no voltage setting or fuse change before operation.

The screen eraser is configured for 100–120 VAC, 50–60 Hz or 200–240 VAC, 50–60 Hz operation. Please ensure that your eraser is configured to the appropriate voltage and fuse settings before operation by checking the identification and settings label next to the power input.

The External Laser Module must be configured for the proper voltage/current settings. This should be done by appropriate Bio-Rad Service personnel only, as damage could result if the settings are incorrect.

If the power setting on the Eraser or External Laser Module is incorrect, please contact your local Bio-Rad representative.

3.1.3 Host Computer Recommendations

The scanner is capable of producing large image files of high resolution; these can be up to 120 megabytes in size. To easily manipulate such large files, a powerful computer is required. The host computer MUST meet or exceed the specifications as detailed below.

Table 3.1 Host computer specifications

	Recommended PC	Recommended Mac
Processor	Pentium 333 MHz or better	Power PC G3 or better
RAM	≥256 MB	≥256 MB
Hard Drive	≥3 GB	≥3 GB
Monitor	17" 1024X768 res (required) (21" preferred)	17" 1024X768 res (required) (21" preferred)
Communications	USB 2	USB 2
Operating System	Windows 2000 or XP	OS 10.2.8 or better

It is recommended to have a storage device for large image files, such as a second hard drive or CDRW connected to your CPU.

Please refer to your software manual for detailed host computer system and software requirements. If the computer is not purchased from Bio-Rad, system compatibility is the responsibility of the user. Please check with your local Bio-Rad office regarding compatibility for your specific brand of computer.

3.2 Setting Up Your PharosFX System

3.2.1 Shipping Check

Inspect all shipping containers to ensure that you have received all ordered items and that no boxes are damaged. If items are either missing or damaged, report them to both the shipping company and Bio-Rad Laboratories immediately.

The PharosFX system should arrive complete with the following items:

Quantity	Item
1	PharosFX Scanner with pre-isntalled fluorescence and phosphor screen filters (PharosFX Plus only) and blank filter holders
1	Sample Tray with sample holders, and transilluminating plate
1	USB2 cable
1	Power Cord
1	Hardware Instruction Manual
1	Warranty Card
1	Quantity One software (single user license)

Remember to verify that any additional peripherals that you ordered with your PharosFX system have been received.

3.2.2 Unpacking

For the first-time installation please call Bio-Rad Technical Support (1-800 424 6723) to arrange a visit of a field service engineer.

Unpacking the Laser Scanner and Tray

Note: Explicit packing/unpacking instructions are located in a clear document envelope attached on the outside of the shipping container. Refer to these instructions at all times prior to unpacking or packing.

Unpack the laser scanner by following the steps below:

1. Cut the metal strap on the instrument packaging

2. Slide the cardboard cover off vertically

3. Remove the front and rear packaging

4. Grip the bottom of the scanner on both sides and place on the bench top.

Warning: Get a helper; a single person should not attempt to lift the scanner.

Warning: Always lift heavy objects with bent knees and a straight back to avoid back injury.

Caution: Do not supply power to the scanner until the system has been set up following the installation procedures and the scan head locking screw has been removed. Failure to remove the locking screw before starting the scanner may damage the instrument.

Note: Retain all packaging materials for future transport of the PharosFX system.

Unlocking the Scan Head

To protect the scanning mechanism during transport, the scanner uses a scan head locking screw. The screw, which is located at the rear left-hand side of the instrument, restrains the scan head during transport and must be removed before power is supplied to the scanner. If the screw is not removed the scanner may be damaged. To remove the locking screw, follow the procedure outlined below. Refer to figure 3.1 #1 through figure 3.1 #5.

1. Unscrew the metal locking screw by carefully rotating in a counter-clockwise direction. This will disengage the screw from the scan head.

2. Unscrew the black plastic screw guide by rotating in a counter-clockwise direction.

3. Remove the complete locking screw and guide assembly.

4. Remove the threaded sealing plug located in the storage hole directly below the locking port.

5. Insert the plug it into the locking port and tighten by clockwise rotation. Insert the locking screw and guide into the storage hole and tighten by clockwise rotation.

To lock the scan head for future transport; turn on the scanner, this will home the scanning head. With the power "ON" reverse the procedure listed above.

1.

2.

3.

4.
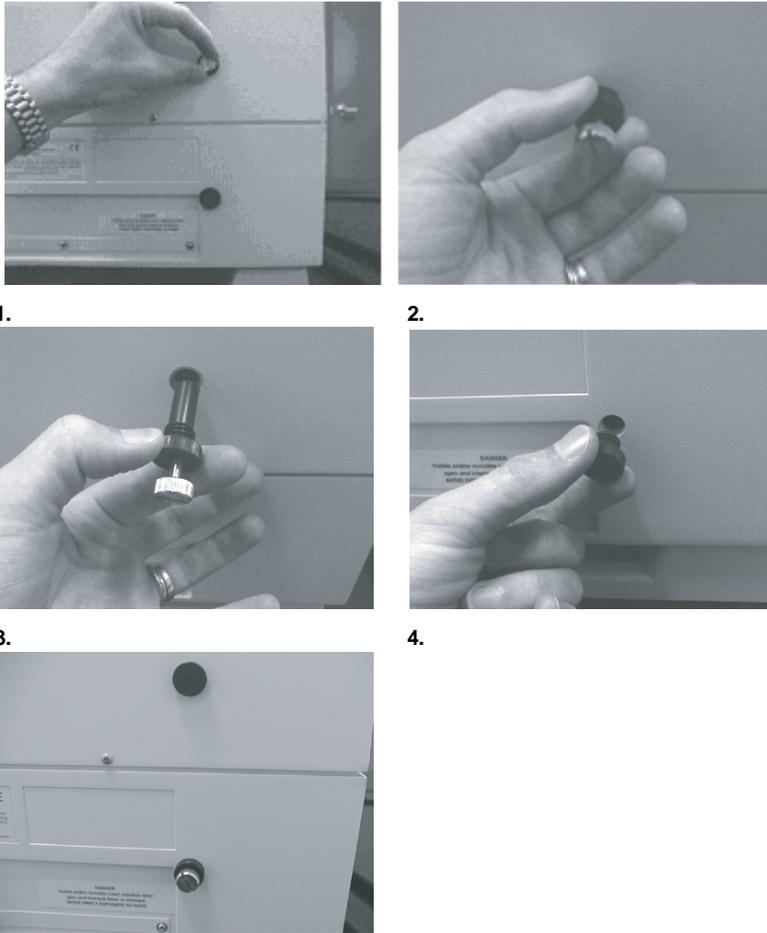
5.

Fig. 3.1. Steps for unlocking the scan head.

Unpacking the Imaging Screens

K-type imaging screens are shipped in a sealed cardboard box. The phosphor matrix is protected by a sheet of paper that must be removed prior to erasure and use.

Retain all packaging for screen storage.

3.2.3 Electrical and Communication Connections

Power

Insert the power cord into the power entry module on the rear panel of the scanner. The scanner uses a universal power supply and can be used with any voltage between 100 and 240 VAC.

For the screen eraser, confirm that the voltage setting on the power entry module is correctly configured for your country. If the eraser is not correctly configured contact your local Bio-Rad representative.

USB2 Connection

The laser scanner must be connected to the host computer via a USB2 interface (Figure 3.2). The USB2 link ports are located on the right-hand side of the rear of the scanner unit. The appropriate USB2 cable is included with the scanner.

Fig. 3.2. View of USB2 connection

3.2.4 Quantity One Software Installation

Please refer to your software instruction manual for comprehensive software installation procedures.

Section 4
Operating the PharosFX System

4.1 Starting the PharosFX Scanner

To turn on the PharosFX scanner, press the power switch located on the right side of the instrument. The LCD display on the front of the scanner displays internal diagnostic data and information relating to the instrument version (Figure 4.1). When the power is first turned on the LCD should display the sequence of messages shown below. This process takes approximately 40 seconds.

Start-up Display Sequence

1. Start Up
2. Main (Revision) v. x.xxx
3. Detector v. x.xxx
4. Laser v. x.xxx
5. Ready

When the **Ready** message is displayed, the host computer can communicate with the scanner.

Note: If any other messages are shown after 2 minutes, or if the scanner is inoperative or the scanner acquisition window cannot be opened on the host computer, please contact your Bio-Rad Technical Service Department for assistance (1-800-424-6723 press 2 for Technical Support).

It is recommended that the PharosFX scanner be allowed to warm-up for 15 minutes before use. It is generally recommended that the scanner be left on, unless it is not going to be used for a period of more than 48 hours.

Fig. 4.1. PharosFX LCD and control panel.

An LCD "Contrast" button is located on the control panel (Figure 4.1). This button can be used to adjust the brightness of the LCD display. The contrast function cycles so that holding the button down will cause the display to get lighter and then darker again.

The "On Line" button on the control panel should be depressed only in the unlikely event that the acquisition software cannot halt scanner operation.

4.2 Fluorescence Detection

4.2.1 Sample Preparation

Prepare fluorescent samples as recommended by published protocols. The following sample types can be imaged using PharosFX and PharosFX Plus.

Agarose Gel
Acrylamide Gel
Acrylamide Gel sandwiched between two glass plates
Acrylamide Gel on top of a single glass plate
Membranes and Blots
Hybridized Glass Plates
Microtiter Plates

Table 4.1

Sample Preparation and Accessories

Accessory	Use for	Preparation Notes
Sample Tray 170-7811	• Agarose Gels • Acrylamide Gels • Blots/Membranes • Colorimetric Gel Documentation • Unmounted Storage Phosphor Screens	• No gels thicker than 8 mm. • Gels should be wet. • Blots/Membranes should be moist. • Use Sample Holders (170-7813) to keep sample from moving during scan. • When performing Colorimetric Gel Documentation work, utilize the transilluminating screen supplied. • Will accept unmounted screens from many manufacturers including Bio-Rad, Kodak, MD and Fuji. • When working with 20 x 25 cm small format screens (170-7843) utilize the alignment overlay supplied with the Sample Tray.
Multi-Sample Tray 170-7812	• Mounted Screens (MD format) • Microtiter Plates	• Face MD screens upward inside the tray. • When scanning Microtiter Plates use the Microtiter Adaptor Assembly (170-7814). • Microtiter Adaptor Assembly accepts up to 8 microtiter plates. • Plates that can be scanned include 96, 384 and 1536 wells. • Make sure prior to preparing samples that the microtiter plate format can fit inside the scanner when used with the Multi-Sample Tray/Microtiter Adaptor Assembly.
Multi-Sample Tray II 170-7819	• Acrylamide gels sandwiched between glass plates. • Acrylamide gels sitting on glass with no top glass plate. • TLC plates	• Make certain that the thickness of the sample and the glass plates fits within the scanner prior to scanning. • The Multi Sample Tray II ships with three sets of non slip spacing strips. Use these to determine your optimal focus for Differential Display workscanning. • Utilize the Print 1:1 function to aid in any incision work for Differential Display processes.

Caution: When using the glass sample tray the maximum sample thickness must not exceed 8 mm. The sample tray cannot be inserted into the scanner with a sample thicker than 8 mm. The height of the side rail on the tray can be used as a guide to determine the maximum sample thickness, as the sample's height must not exceed that of the side rail. Thicker samples, up to 17 mm, can be imaged using the Multi-Sample Tray I (1707812) or Multi-Sample Tray II (1707819).

4.2.2 Inserting a Fluorescent Sample into the Scanner

1. Open the front door of the scanner.(Figure 4.2)

2. Pull out the glass sample tray and, if dirty, clean with a dry lint-free cloth.

3. Carefully place the prepared gels or blots onto the tray, making sure that the fluorescent signal is directed upwards. (Maximum sample thickness is 8mm.)

4. Note the coordinates of the sample on the tray for reference when selecting the desired scan area in the software. The grids with coordinates on the tray in the software match.

5. Slowly insert the tray with the sample all the way into the scanner, until the hold latch engages and you hear a click.

6. Close the front door of the scanner.

7. The sample can now be scanned using the acquisition software.

8. To remove the tray, simply wait until scanning is complete, open the scanner door and pull the tray out.

Note: For wet samples, use a clean, lint-free absorbent paper towel to remove excess liquid from around the sample. This will reduce the potential for the gel to slide on the sample tray and move during image collection.

Note: It is recommended that samples be placed against the edge of the tray to ensure straight alignment.

Note: Gel holders may also be placed at opposite corners of the sample to prevent movement during tray insertion.

Fig. 4.2. Steps for inserting a fluorescent sample into the scanner.

4.2.3 Scanning Fluorescent Samples

1. Open the scan window in the acquisition software. Under the **File** menu, choose PharosFX. In the acquisition window choose **Select** and use the layered menu to identify your application and sample strength. (i.e. for an ethidium bromide stained DNA sample of low concentration, select **Nucleic Acid Stain>Ethidium Bromide>Low Sample Intensity**).

2. The emission filters choice is specified through the software.

3. Choose the scan area that you would like to image.

4. Select the desired resolution setting. The highest resolution is 50 µm and the lowest is 800 µm.

5. Select **Acquire** to begin scanning. The scanning is performed sequentially. A single frame is scanned for a selected fluorophore.

Note: Refer to the Software Manual for detailed acquisition instructions.

Caution: Do NOT open the door of the scanner while the unit is scanning. This will terminate your scan and require you to rescan your fluorescent sample.

6. When the scanning is complete open the door of the PharosFX.

7. To remove the sample, simply pull out the tray and lift it off.

4.2.4 Inserting Additional Excitation and Emission Filters

PharosFX and PharosFX Plus are supplied pre-configured with emission filters that support most common fluorescence applications and as such, users should not routinely have to access the filter areas. If users wish to install additional filters and perform custom fluorescence experiments this can be easily done as the emission filter wheels can be accessed through the port in the scanner's front panel (Figure 4.3).

Fig. 4.3. Access port for emission filter cubes

Selecting Different Filter Positions

The filter wheel advance switch located on the front panel of the scanner can be used to rotate the two emission filter cubes (Figure 4.4). To activate the switch, simply depress once for each turn required.
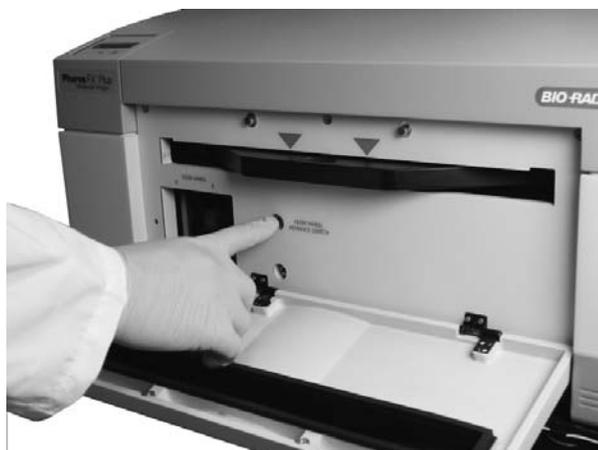
Fig. 4.4. Selecting a filter position using a filter wheel advance switch.

Caution: Do NOT hold down the filter wheel advance switch. To rotate the filters users should quickly press and release the button once. Users should allow the filters to move to the next position before activating the switch again.

Caution: Do NOT manually rotate the filters. This may result in misalignment of the excitation filter wheel and emission cubes. It may also cause permanent damage to the filter driver motors. Always use the advance filter wheel switch to change filter positions.

Adding or Changing an Emission Filter

1. Open the front door of the PharosFX scanner.

2. Unscrew the two black screws holding the emission filter port cover in place and remove the cover.

3. Depress and release the filter wheel advance switch until the desired emission filter location is presented. Filter cubes are identified by a wavelength and catalog number label, which should be clearly visible.

4. If a filter cube is to be removed from the slot do so by gently pulling the cube towards you

5. To install a new filter, hold the cube with the mounting slots on the left hand side. Align the cube's mounting slots with the guides located on the filter wheel and gently push the cube onto the guides.

6. Replace the filter port cover and tighten the two black retaining screws.

7. Note the filter position in order to configure the application and select it correctly in Quantity One.

Note: Do not operate the scanner with the filter port cover removed.

Fig.4.5. Emission filter port

Fig. 4.6. Adding a new emission filter.

The standard emission filter configurations are as follows:

Table 4.2

PharosFX Filter Wheel

532 only

Wheel	A	B
1	Blank	605 nm BP
2	Blank	Blank
3	640 nm BP	Blank
4	Blank	Blank

532 & 488

Wheel	A	B
1	Blank	605 nm BP
2	Blank	Blank
3	640 nm BP	530 nm BP
4	Blank	Blank

532, 488 & 635

Wheel	A	B
1	Blank	605 nm BP
2	Blank	695 nm BP
3	640 nm BP	530 nm BP
4	Blank	Blank

PharosFX Plus Filter Wheel

532 only

Wheel	A	B
1	Blank	605 nm BP
2	390 nm BP	Blank
3	640 nm BP	Blank
4	Blank	Blank

532 & 488

Wheel	A	B
1	Blank	605 nm BP
2	390 nm BP	Blank
3	640 nm BP	530 nm BP
4	Blank	Blank

532, 488 & 635

Wheel	A	B
1	Blank	605 nm BP
2	390 nm BP	695 nm BP
3	640 nm BP	530 nm BP
4	Blank	Blank

☐ filter can not be changed.

Note: When a 488 Excitation Option is ordered an additional 530 Bandpass Filter is supplied (e.g. FITC and Cy2). When a 635 nm Excitation Option is ordered an additional 695 Bandpass Filter (e.g. Cy5) is supplied. The recommended filter positions are indicated in table 4.2 above.

4.3 Fluorescence Imaging Using the External Laser Module

The optional External Laser Module can be attached to PharosFX and PharosFX Plus to expand the system's range of laser excitation sources. The installation of the External Laser Module requires a field service engineer, please arrange the visit through Technical Support (1-800-424-6723 press 2 for Technical Support). The standard external module contains an Argon Ion laser, which is ideal for imaging fluorophores excited by 488 nm light. The External Laser Module can also be outfitted with an additional 635 nm laser, which further adds to the flexibility of the PharosFX imaging system.

The PharosFX Scanner detects when the External Laser Module is attached and the External Laser will be used whenever the appropriate application is selected.

Fig. 4.7. PharosFX External Laser Module connected to the scanner

4.3.1 External Laser Safety Information

The External Laser Module (Figure 4.7) is certified according to 21 CFR 1040 of the CDRH, as a Class I laser device. The laser contained within the External Laser Module produces 10 milliwatts at 488 nm. This laser can also be upgraded to include a 635 nm laser producing up to 10 milliwatts at 635 nm. The cover of the system is designed to protect the user and should not be removed.

Warning: Do not remove the cover for any reason or defeat the interlock. Attempting to operate the unit with the cover removed may damage the instrument and expose the operator to laser energy from the laser.

Warning: Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous laser energy exposure.

Caution: The cover should be removed by trained service personnel only. Do not attempt to operate the product with the cover removed. All PharosFX components should only be serviced by Bio-Rad or its trained representatives.

4.3.2 Fiber Optic Cable Alignment on the External Laser Module

The PharosFX system incorporates a motorized driver, which self-aligns the fiber-optic to optimize the illumination signal. Whenever a user selects an external laser source that is different than the last laser used the auto-alignment process is automatically performed.

Caution: Any movement of the metal sheathed fiber-optic cable will affect the efficiency of light transfer from the External Laser Module to the PharosFX or PharosFx Plus system. As such, it is recommended that the cable not be moved during image acquisition.

4.4 Phosphor Imaging Procedures

4.4.1 How to Prepare K-Type Imaging Screens

Fig. 4.8. K-type imaging screens.

All K-type imaging screens (K, K-HD, K/Tritium) are not erased prior to shipment from the factory and should be erased for 20 minutes prior to first use. Subsequent erasures should only take 10 minutes.

Note: Optimal image quality and sensitivity can only be achieved with a thoroughly erased screen.

Caution: The captured signal (latent image) stored on a K-type screen can be partially erased when the screen is exposed to fluorescent room light. Users should rapidly transfer the screen from the exposure cassette to the laser scanner. Some users choose to dim the room lights to prevent accidental erasure.

When screens are not in use, they should be placed in the original shipping box provided and placed in a dry and dark environment.

Caution: The phosphor surface of the screen is sensitive to damage from moisture, mishandling and improper use of solvents. For detailed instructions on how to maintain the K-type imaging screens, please refer to the Care and Maintenance section of this manual (Section 5.2.).

4.4.2 How to Erase Imaging Screens

The screen eraser (Figure 4.9) contains a series of bulbs that produce light that is a specific wavelength range. When illuminated with this light, the phosphor crystals in the screen discharge, returning to ground state. As a result, any screen background or residual signal is removed.

Fig. 4.9. Screen Eraser.

To erase the imaging screens, place the imaging screen against the front panel of the Eraser-K with the white phosphor side facing the white diffuser plate of the unit. Set the timer to the desired setting or to erase continuously set the timer to the HOLD position.

The erasure process typically requires only 10 minutes, assuming the previous sample has not charged the phosphor in the screen above 20,000 pixel density units. An erasing time guideline is shown in Table 4.3.

Note: The screen will not be damaged by extended erasure.

Note: Erasing the screen to the basal level is critical, since this has a direct effect on the sensitivity, linear response, quantitation, exposure time and image quality.

Erasure Guidelines

Table 4.3. Recommended erasure times.

Condition	Background (PD units)	Time
Before each exposure	1-20,000	10 minutes
After high dosage exposure	>20,000	20 minutes

4.4.3 How to Prepare Samples

The recommended phosphor screen exposure time can be estimated, based on one-tenth of the time it would normally take to visualize the sample on x-ray film.

Dry Radioactive Samples

Dry thin samples such as nitrocellulose membranes, dried gels or TLC sheets can be exposed directly to the imaging screen in the exposure cassette (Figure 4.10). Ensure that TLC plates are completely dry before placing them against the screen and always cover with plastic wrap to prevent flecks from contaminating the screen.

When imaging thick samples it is recommended that the screen is simply placed face down against the sample on a flat surface and the exposure is conducted in a light-tight drawer or other dark location.

Note: It is important when placing the screen against the sample, that the samples are correctly aligned the first time. Adjusting the screen after placement against the sample may result in a ghost image or double exposure. If the screen needs to be realigned it must first be erased.

Note: If you are exposing the screen to a frozen sample, the screen should first be sealed in a plastic bag as condensation may cause screen damage. After exposure the screen should be equilibrated to room temperature before the bag is opened and the screen removed.

Caution: Never expose screens to organic solvents or acetic acid vapors as these may cause screen damage even when the sample is covered with plastic wrap.

Caution: Samples should not contain either scintillants or enhancers, as these will interfere with the operation of the screen.
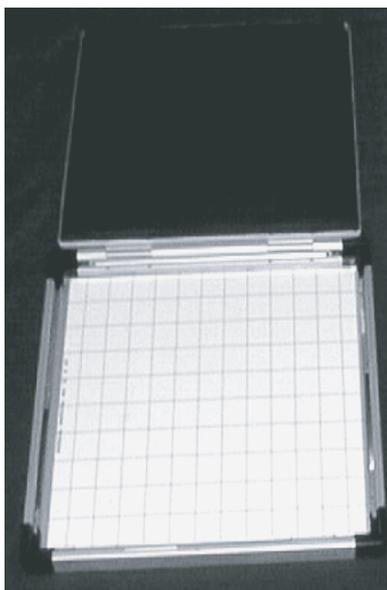
Fig. 4.10. Imaging Cassette.

A protective sheet should be placed between the sample and the screen to minimize the chance of radioactive contamination (Figure 4.11).

Fig. 11. Use of protective sheet in Imaging Cassette.

Wet Radioactive Samples

Precautions must be taken to prevent wet samples from contaminating the imaging screen. Wet thin samples should be completely enclosed in a heat-sealable bag and moist samples must be covered with plastic wrap before being exposed to the imaging screen.

Caution: Direct contact of a wet sample with the imaging screen may cause irreversible screen damage.

Caution: Alkaline denaturing gels must be neutralized before being wrapped and exposed to the screen.

Caution: Never expose the K/Tritium screens to wet samples, even if they are covered.

Plastic wraps will attenuate weak beta radiation signals according to the following table.

Table 4.4 Attenuation of radiation by various plastic wraps.

Wrap Type	^{32}P	^{14}C	^{3}H	Application
2 mm Seal-A-Meal Bag	16%	82%	>99.9%	Wet samples
0.5 mm Saran Wrap	6%	50%	99.7%	Moist samples

Note: When enclosing a sample in plastic wrap or a heat-sealable bag, make sure that there are no large air bubbles or surface wrinkles on the side of the sample that will come into contact with the screen. Air bubbles and wrinkles will prevent close contact with the screen, which may result in poor image quality and reduced sensitivity.

Note: Ensure that the external surface of the wrap or heat-seal bag is wiped dry to minimize potential screen contamination.

4.4.4 How to Use the Exposure Cassette

Exposure Cassette-K
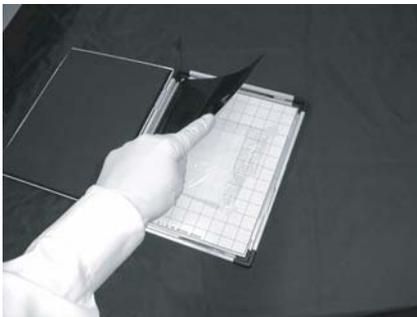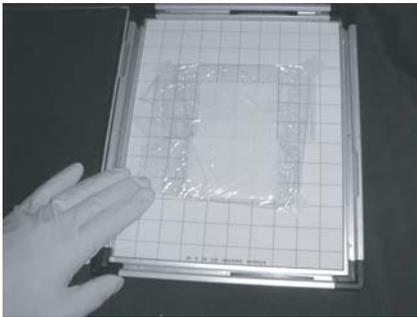
Fig. 4.12. Steps in use of the Exposure Cassette-K.

1. Push the two release buttons away from the edge of the cassette to open.

2. Use a clean cloth to wipe the internal surfaces of the cassette and remove any contamination.

3. Tape the prepared sample with the active surface facing upwards to the grid marked exposure pad, making sure to align the sample straight and in the correct orientation. It is recommended that plastic wrap is then taped in place over the sample.

4. Place the Imaging Screen-K directly on top the sample with the phosphor (white) side facing down onto the sample. It is recommended that the sample is at least 1 cm from all edges of the screen to prevent possible edge effects.

5. Make a note of the sample's upper-left and lower-right grid coordinates for later use in selecting the scan area.

6. Close the cassette and press until the release button catches and a snap sound is heard.

4.4.5 How to Scan the Imaging Screen

Inserting K-Type Screens into the Scanner

1. Open the scanner door on the front of the instrument.

2. Insert the sample tray if it is not already in the scanner.

3. Remove the imaging screen from the exposure cassette and quickly place it on the sample tray with the phosphor surface facing upwards.

Note: When transferring the screen from the exposure cassette to the scanner operator should minimize exposure to direct fluorescent light, as this may erase some of the collected signal. Some users choose to dim room lights during the transfer process.

Note: When scanning a small sized screen it is recommended that the appropriate location template be used. This template will correctly align the screen and will simplify the selection of scan coordinates. The template will also prevent the screen from moving out of position during insertion of the sample tray.

Note: Do not bend the screen as this may damage the phosphor material.

4. Insert the sample tray with screen completely into the scanner so that it engages the locking clip and you hear a click. (Figure 4.13)

5. Close the scanner door.

Fig. 4.13. Steps for inserting a phosphor screen into the scanner.

Scanning the Radioisotopic Samples

1. Open the scan window in the acquisition software. Under the **File** menu, choose PharosFX Plus. In the acquisition window choose **Select** and use the layered menu to identify your application. (i.e. for radioisotopes using the Imaging Screen-K, select **Radioisotopes/ K-Screen**).

2. Choose the scan area that you would like to image.

3. Select the desired resolution setting. The highest resolution is 50 μm and the lowest is 800 μm.

Note: Scanning phospor screens is a data destructive process so only one accurate scan can be performed per sample exposure.

4. Select **Acquire** to begin scanning.

Note: Refer to the Software Manual for detailed acquisition instructions.

Caution: Do NOT open the door of the scanner while the unit is scanning. This may terminate your scan prematurely and result in the loss of image data.

5. When the scanning is complete open the door of the PharosFX Plus.

6. To remove the imaging screen, simply pull out the sample tray and lift off the screen.

7. Once removed, imaging screens can be immediately erased and reused.

Section 5
Care and Maintenance

5.1 Scanner Maintenance

With regular use the PharosFX scanner should provide years of trouble-free operation, without the need for operator maintenance. If you suspect that the PharosFX system requires servicing, please contact your local Bio-Rad office.

It is recommended that the casing of the scanner be periodically inspected to verify that no panels are loose or distorted.

Caution: Do not remove the cover from the PharosFX system, as this voids the warranty. There are no user-serviceable components in the scanning unit. Attempting to operate the product with the cover removed may damage the instrument and expose the operator to laser energy. The PharosFX system can only be serviced by Bio-Rad or its trained representatives.Use only mild, non-abrasive and water-based detergents to clean the external surface of the scanner.

5.2 Care for PharosFX Plus Accessories

5.2.1 General Care of Imaging Screens

Utmost care should be taken to ensure that the protective plastic covering over the phosphor matrix is not damaged. The phosphor crystals are hygroscopic and any holes, nicks or punctures in this environmental barrier will eventually cause damage to the phosphor and render that portion of the screen unusable. For the same reason the imaging screen should never be directly exposed to wet gels or chemicals. Use some water-impermeable media between a wet sample and the phosphor screen (e.g. "Saran Wrap™").

Never expose the screen to acids, acid vapors or other organic solvents.

Never bend the screen as this may damage the phosphor matrix.

5.2.2 Radioactive Contamination Check

If you suspect that a storage phosphor screen has been contaminated, follow the procedure outlined below to confirm this.

1. Clean (see Section 5.2.3) and erase the screen to background levels.

2. Check for complete screen erasure by scanning at 800 microns.

3. Place the screen in a dark area such as a lab drawer for 6 to 24 hours.

4. Re-scan the screen at 800 microns.

5. Use Quantity One software to check the screen background counts. If no areas of high signal (hot spots) are detected, erase the screen and use.

Any contamination will be visible as a localized region of high signal over background (hot spots). If there is contamination, clean the screen as described below and clean the grid of the exposure unit. Screen contamination can be minimized by using plastic wrap as a physical barrier to separate the phosphor screen from the radioactive sample.

5.2.3 Cleaning Imaging Screens

If contaminated, the screens should be cleaned to remove any radioactive contamination, sample residue or dust.

Caution: Do not use powdered detergents to clean the screen as undissolved particles may damage the screens coating.

Cleaning Protocol for Imaging Screen-K and Imaging Screen-HD

1. Handle the screen only by the edges. Avoid touching the screen coating with anything sharp, such as fingernails. The screen is coated with a thin layer of plastic and scratching this protective layer could damage the screen, making that area unusable.

2. Apply a small amount of cleaner to a soft, lint-free-cloth and gently wipe the screen. It is recommended to use Kodak Intensifying Screen cleaner and Antistatic Solution Catalog# 1064930 from Kodak.

3. With a dry section of the cloth, gently wipe the screen to remove any excess moisture. If the screen had radioactive contamination, properly discard the radioactive cloth.

4. Erase the screen to background before use.

5. Repeat the contamination check above.

Cleaning Protocol for the Imaging Screen K/Tritium

Caution: The phosphor matrix of the tritium screen has no protective barrier and should NOT be cleaned with or exposed to any liquids.

Any particulate matter should be gently removed with a soft, dry brush or gentle dry gas stream.

The screen should be used only with ^{3}H, any other isotope will contaminate the screen.

5.2.4 Storage of the Imaging Screens

Always erase the screen prior to storage. The surface of the screen should be completely protected. Do not place heavy objects on top of the imaging screen. With proper care the imaging screen should maintain its performance through years of use.

Always store the screen "flat" in an exposure cassette, in the original shipping box or storage bag.

5.3 Exposure Cassette Maintenance

These devices do not require regular maintenance other than regular cleaning to remove any residue or possible radioactive contamination. To clean, wipe with a lint-free paper towel moistened with a mild detergent solution such as Bio-Rad's Cleaning Concentrate (Catalog #161-0722) that has been diluted 1 in 20 with distilled water.

5.4 Screen Eraser Maintenance

The white plastic filter requires occasional cleaning to remove accumulated dust from its surface. To clean the filter, gently wipe with moist, lint-free paper or a soft cloth. Do not use abrasive cleaning solutions, as they will scratch the filter.

5.4.1 Changing Bulbs

To order replacement bulbs (Catalog #170-7869) contact either Bio-Rad Laboratories or your local distributor.

The Screen Eraser uses four 15-watt fluorescent light bulbs. To replace a light bulb, use the following procedure.

1. Determine the defective light bulb.

2. Turn off the eraser.

3. Wait five minutes for the bulbs to cool.

4. Unplug the power cable.

5. Open the four hex screws on the front of the eraser, using the hex tool stored on the rear panel of the unit.

6. Remove the white plastic filter.

7. Remove the foam on both sides and replace the defective bulb.

8. Reassemble by reversing steps 4 to 7.

Step 6.

Step 7a   **Step 7b**

Fig. 5. Screen Eraser bulb replacement steps.

Caution: Use only 15 W bulbs. Higher wattage bulbs can damage the imaging screens and weaker bulbs will not erase the screens effectively.

Section 6
Troubleshooting

6.1 Factors Affecting Image Quality

Fluorescent samples

Resolution

Any movement of the sample during scanning can cause a blurred image. Be sure that excess liquid around gel samples is removed and that the imager is level. Sample holders are supplied with the glass sample tray. These have suction feet that allow attachment to the glass and will hold samples in place.

Sample height will affect the resolution of the image. The optics of the scanner are designed to allow for some variation in sample thickness but samples greater than 3 mm thickness or on a substrate such as glass or plastic plates are optimally imaged using the optional Multi Sample Tray II. This tray comes with variety of spacers that allow the sample to be positioned at the optimal imaging height. See the Multi Sample Tray instructions for further details.

Sensitivity

Fluorescent intensity can vary greatly between samples and require changing the PMT voltage. Three standard PMT settings exist for all fluorescent applications, High, Medium, and Low Sample Intensity. These represent PMT voltages of 25, 35, and 45% of maximum respectively. The higher the PMT voltage, the greater the signal amplification. Therefore, with a sample that has a strong fluorescent signal, the PMT voltage should be lower than for a sample with a weak fluorescent signal. Empirical testing of the proper PMT setting is required to obtain the best quality image. If the preset PMT voltages are not satisfactory, a custom application can be made using a voltage other than what is available through the preset applications. Consult your software manual for details of creating a custom application.

Placing samples on substrates other than the provided sample trays can produce excessive background fluorescence. High background fluorescence will negatively impact sensitivity. Testing any other substrates with the same imager settings intended to be used for sample scanning will identify potential problems.

Radioactive samples

Resolution

Close contact of the sample with the active surface of the imaging screen is critical for producing the highest quality image. Remove excess layers of tape, air bubbles or wrinkles; these may produce only a very small gap, but this is sufficient to produce a fuzzy image. Samples that are over-exposed will result in images with low resolution. If this occurs, erase the screen and expose for a shorter time.

High background on a screen can cause decreased resolution of weak signals. Ensure that the screen is thoroughly erased before imaging your sample.

The phosphor screen is very sensitive to isotope emission, as such, place the screen evenly over the sample and do not move once it has been aligned, as this may result in a ghost or double image.

Sensitivity

Optimal sensitivity can only be achieved with a thoroughly erased screen. Close contact of the sample with the screen will also impact the imaging sensitivity. Ensure that the sample is pressed close to the screen surface.

6.2 Problem Solving Guide

Problem	Possible Cause	Solution
Scanner is not responding to host computer	• Scanner door is open • Scanner is not on-line • USB cable is not connected to scanner or computer • USB cable is defective • Scanner is not turned on	• Close Door • Press on-line button • Reconnect USB cable • Replace USB cable • Turn on scanner
Image is not visible on the monitor	• The 'Transform" function in the software is set too high • (radioisotope only) Insufficient exposure time • Area where sample was placed was not scanned	• Set to a lower maximum value • Expose sample for a longer time • Check location of sample and rescan. (radioisotopes) When imaging small screens ensure that the appropriate location template is used
Scans have image artifacts	• Radioactive contamination on the phosphor surface coating • Static electricity on phosphor screen • Phosphor screen may be scratched or damaged • Contamination of sample tray with fluorescent material • Imager is not level and sample moved during scan • Diagonal line on a phosphor screen image	• Check and clean using the protocols in this manual • Check and clean using the protocols in this manual • Visually inspect the screen • Clean sample tray with Bio-Rad cleaning solution, verify no residual contamination by scanning the sample tray. • Level imager, minimize liquid around sample and use sample holders to restrain sample from moving • The image was obtained by rescanning the screen.
Scans have speckled images.	• Dust on phosphor screen or fluorescent sample	• Insure powder free gloves are used for handling screens and samples.

Appendix 1
PharosFX System Specifications

System Technical Specifications	Specification
Linear dynamic range	1:65535
Pixel resolution	50, 100, 200 or 800 µm, selectable
Image resolution	2 line pairs/mm or 250 µm
Pixel density	16-bit (0–65,535)
Signal decay	^{32}P 50% retention in 24 hr
Scanning area	35 x 43 cm

Main Component Specifications

Laser Scanner	Specification
Dimensions	69 (D) x 59 (W) x 30.25 (H) cm
Construction	Molded plastic housing, Aluminum mounting plates
Excitation source	532nm Diode Pumped Solid State Laser 15 mW
Weight	30 kg
Electrical	
Maximum power	650 Watts
Input voltage range	90–260 VAC, 50–60 Hz
Fuses	No user-serviceable fuses
Operating Requirements	10–32°C, 30–80% humidity
Storage Requirements	0–60°C, 10–90% humidity

External Laser Module Specification

Dimensions	61 (deep) x 31 (wide) x 41 (high) cm
Construction	Welded 16 Gauge steel
Weight	30 kg
Electrical	
Input voltage range	100/120 VAC, 50–60 Hz
	220/240 VAC, 50–60 Hz
Fuses	6.3 Amp (100/120 VAC)
	3.15 Amp (220/240 VAC)
Excitation Source	
Option I	488 nM, 10 mW, Ar-ion Laser
Option II	488 nM, 10 mW, Ar-ion Laser and 635 nm, 10 mW Laser Diode
Operating Requirements	10–40°C, 0–90% humidity
Storage Requirements	0–50°C, 0–90% humidity

Screen Eraser-K	Specification
Dimensions	35 (D) x 48 (W) x 57 (H) cm
Construction	Molded plastic
Weight	8.6 kg
Electrical	
Input voltage range	100/120 VAC, 50–60 Hz
	220/240 VAC, 50–60 Hz
Fuses	6.3 Amp (100/120 VAC)
	3.15 Amp (220/240 VAC)
Illumination	4 x 15 Watt user-replaceable fluorescent bulbs
Operating Requirements	10–32°C, 30–80% humidity
Storage Requirements	0–60°C, 10–90% humidity

Appendix 2
PharosFX Warranty Information

The FX system and all peripheral items are warranted for a period of one year against defects in materials and workmanship. If any defects should occur during this period, Bio-Rad Laboratories will either replace or repair the defective parts free of charge. For the exact terms of warranty, please see the Instrument Warranty Card shipped with the instrument.

Defects caused by the following actions are specifically excluded:

1. Improper system operation or abuse.

2. Repair or modification of the system performed by anyone other than Bio-Rad Laboratories or its authorized agent.

3. Use of fittings or other spare parts not authorized by Bio-Rad Laboratories.

4. Inappropriate interfacing to external devices.

5. Use of inappropriate solvents, cleaning agents or samples.

6. Non system related facility problems such as power surges.

The one year warranty does not apply to the parts listed below:

1. Phosphor screens.

2. Fuses and lasers.

3. Support consumables.

4. Computers purchased outside of Bio-Rad Laboratories.

For inquiries and requests regarding system repair or service, contact your local Bio-Rad office or distributor (in the U.S., call Technical Service at 1-800-424-6723). Please have the following details available:

1. Instrument model and catalog number.

2. Serial number (label is behind scanner door).

3. Hardware and firmware version information.

4. Software version (in operating software, "About" box).

Appendix 3
Ordering Information

Accessories

Excitation Sources

Catalog #	Description
170-7890	**External Laser**, 488 nm, includes 170-9459 filter
170-7893	**635 nm External Laser Upgrade for 170-7890**, includes 170-7865 filter
170-7892	**External Lasers**, 488 nm and 635 nm, includes 170-9459 and 170-7865 filters

Filters

170-9459	**Filter 530nm BP**, for ECL Plus, AttoPhos, SYBR Green, Alexa Fluor 488, FITC, Cy2, and Pro-Q Emerald
170-7863	**Filter 555LP**, applications similar to 170-7866 and 170-7896 filters
170-7866	**Filter 605 nm BP**, for Flamingo, ethidium bromide, SYPRO Ruby, Alexa Fluor 555, and Cy3 dyes
170-7896	**Filter 640 nm BP**, for Texas Red, SYPRO Red, Nile Red, Radiant Red, propidium iodide
170-7865	**Filter 695 nm BP**, for Cy5 and Alexa Fluor 635 dyes
170-7867	**Blank Filter Holder**

Sample Handling

170-7811	**Glass Sample Tray**
170-7812	**Multi-Sample Tray I**, for small aluminum-mounted screens and microtiter plates
170-7819	**Multi-Sample Tray II**, for scanning gels mounted to glass plates;
170-7813	**Sample Holders**, for gels
170-7814	**Microplate Adaptor**, for Multi-Sample Tray I

Storage Phosphor screens

170-7845	**Imaging Screen-K (Kodak)/Tritium**, 20 x 25 cm
170-7843	**Imaging Screen-K (Kodak)**, 20 x 25 cm
170-7841	**Imaging Screen-K (Kodak)**, 35 x 43 cm
170-7861	**Exposure Cassette-K**, for 20 x 25 cm Kodak screen
170-7862	**Exposure Cassette-K**, for 35 x 43 cm Kodak screen
170-7809	**Eraser K-Screen**, 110/120 V
170-7806	**Eraser K-Screen**, 220/240 V
170-7869	**Replacement Bulb for Eraser K-Screen**

Catalog #	Description
Software	
170-9600	**The Discovery Series™ Quantity One 1-D Analysis Software**
170-9620	**PDQuest 2-D Gel Analysis Software Basic License**
170-9630	**PDQuest 2-D Gel Analysis Software Advanced License**
Miscellaneous	
931-0071	**3 meter USB cable**
161-0722	**Bio-Rad Cleaning Concentrate**
165-3414	**Gel Clip**

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