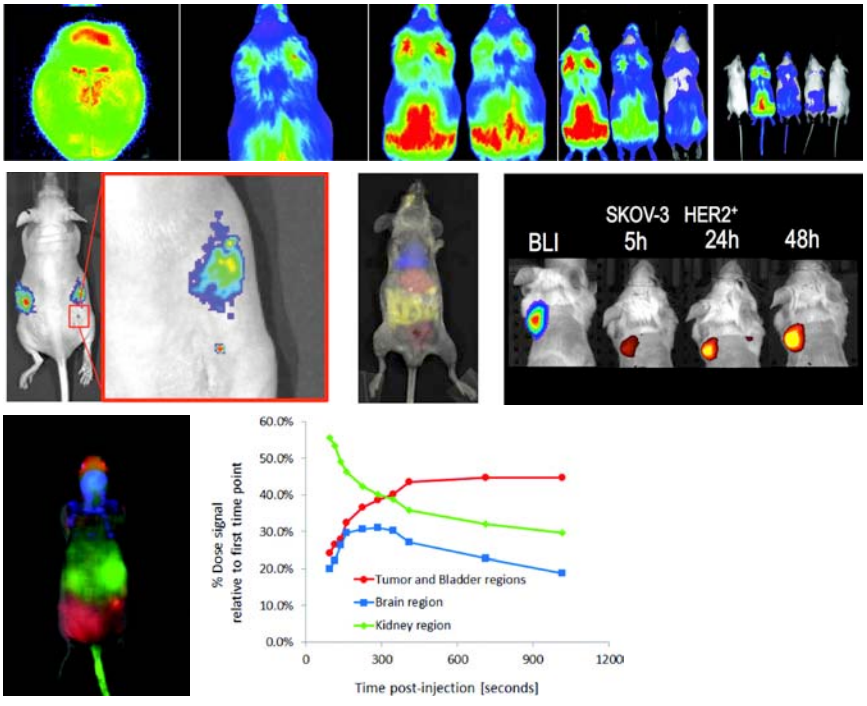
HARDWARE MANUAL

IVIS® Lumina Series III

April 2013



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1 Welcome

1.1 Introduction

The IVIS® Lumina Series III is a high-sensitivity, low noise imaging system enabling both fluorescent and bioluminescent imaging *in vivo* (Figure 1.1).

Figure 1.1 IVIS Lumina Series III

The IVIS Lumina Series III imaging system includes:

- A highly sensitive CCD camera.
- A light-tight imaging chamber with integrated fluorescence module.
- Fluorescence equipment:
  - 22-position excitation filter wheel with 19 equally spaced excitation filters covering the wavelength range from 410 to 790 nm
  - 8-position filter wheel with 7 emission filters spanning the wavelength range from 500 –865 nm
- Living Image® Software for automated image acquisition, post-processing, and data analysis.
- A Windows®-based computer system for data acquisition and analysis.

This manual explains how to operate and maintain the equipment, and provides guidelines for obtaining optimal bioluminescent and fluorescent images. Before using the IVIS Lumina Series III, please read this manual carefully to obtain safe, optimum performance and a maximum service life from the instrument. For instructions on using the Living Image software, please see the *Living Image® Software User Manual for IVIS Lumina Series III*.

1.2 Contact Information

If you have questions regarding this manual or the IVIS Lumina Series III, please contact PerkinElmer Technical Support.

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2 Important Safety Instructions

2.1 Safety Information

This manual provides safety information in the following formats:

CAUTION: A caution note indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury and/or mechanical damage. It is also used to alert you to unsafe practices. It reminds you that all safety instructions should be read and understood before installation, operation, maintenance, or repair of this instrument. When you see this symbol, pay particular attention to the safety information presented. Observance of safety precautions will help avoid actions that could damage or adversely affect the performance of the IVIS Lumina Series III. If the equipment is used in a manner not specified in this manual, the protection provided by the equipment may be impaired.

WARNING! Used when an action or condition may potentially cause serious personal injury or loss of life. Mechanical damage may also result.

VOLTAGE! Provides safety information about high voltage or risk of electric shock.

2.2 Instructions

Read, Retain, and Follow Instructions

Read and understand all the safety and operating instructions before you install, operate, or perform maintenance on the IVIS Lumina Series III. Makesure that you fully understand the following safety instructions, warnings, and disclaimers before proceeding to the rest of the manual.

Retain the safety and operating instructions for future reference.

Follow all operating and handling instructions. Failure to follow operating or handling instructions may void any warranty covering this product.

Heed Warnings

Abide by all warnings on the product and in the operating instructions. Failure to adhere to warnings or safety precautions may void any warranty covering the IVIS Lumina Series III.

2.3 Environmental Considerations for the System Components

Location for the IVIS Lumina Series III

Before the IVIS Lumina Series III is installed, consider the proper environment for the components. Install the equipment in an environment where:

- The temperature does not fluctuate widely and is maintained between 15-25 °C (59-77 °F).
- The humidity does not exceed 80%.
- No strong electric or magnetic fields exist.
- No vibrations are present.
- No corrosive gases are present.
- High amounts of dust are not present.
- No open flame is present.
- There is sufficient space behind the IVIS Lumina Series III equipment. A minimum space of four inches from the flat surface of the rear panel should be provided behind the IVIS Lumina Series III to provide unobstructed air flow and access to the main power on/off switch.
- The work space is level.

Heat

The system should be situated away from heat sources such as open flames, radiators, heat registers, stoves, and other heat-generating electrical equipment.

Water and Moisture

VOLTAGE! Do not use this product near water (for example, near a sink or wet room) due to risk of electric shock, electrical damage, and/or equipment failure.

2.4 Cleaning or Moving the System Components

Cleaning/Liquid Entry

VOLTAGE! Do not use liquid or aerosol cleaners and never spill liquid of any kind on any of the IVIS Lumina Series III components. Sprays and liquids that come into contact with the IVIS Lumina Series III hardware may result in damage to the system or electrocution. For more details on proper care of the system, see *Care and Maintenance* on page 36.

Moving the IVIS Lumina Series III

You can move the light source module on the laboratory bench within the extent of the fiber optic cable. Be careful not to bend the fiber optic cable (minimum radius of bending: 3 inches/7.6 cm).

CAUTION: The IVIS Lumina Series III is sensitive, scientific equipment and should not be moved by any user unless the system is located on the XWS-260 workstation. Due to the risk of potential damage, it is critical that only a trained PerkinElmer technician moves the IVIS Lumina Series III. If it is necessary to relocate the instrument, contact PerkinElmer Technical Support (see page 2). See Appendix A on page 40 for more details on moving the IVIS Lumina Series III on the XWS-260 workstation.

2.5 Power Considerations

Power Sources

The IVIS Lumina Series III is configured for the voltage requirements of the installation locality that was specified at the time of order. If the IVIS Lumina Series III is moved to another area, make sure that the same voltage requirements exist.

VOLTAGE! The IVIS Lumina Series III can operate at multiple voltages (100-240 VAC); however, you are not permitted to change the input voltage to any of the system components. Several internal modifications are required for voltage change. If the operating voltage must be changed, contact PerkinElmer Technical Support (see page 2).

Power Cord Protection

Power supply cords should be routed so that they are unlikely to be walked on or pinched by items placed upon or against them. Pay close attention to receptacles and to points of connection between cords and equipment.

Lightning and Power Line Surges

The IVIS Lumina Series III is supplied with a surge protector. All components should be connected to this device to protect against electrical transient events. Failure to isolate the camera from electrical transients may result in damage to the CCD camera.

Power Outages

If the IVIS Lumina Series III experiences a loss of supply power, turn off the power switch for all components and do not restart the system until reliable power has been restored.

Overloading

WARNING! Do not overload wall outlets, extension cords, or integral convenience receptacles as this can result in a risk of fire or electric shock. See *Components and Specifications* on page 13 for more details on the power requirements of the equipment.

Facilities should be adequately wired according to local building codes.

2.6 Servicing

Refer all servicing to PerkinElmer Technical Support (see page 2). If the IVIS Lumina Series III is damaged and requires service, unplug the instrument from the outlet and contact PerkinElmer Technical Support. Servicing by anyone other than an authorized PerkinElmer representative voids the warranty covering the IVIS Lumina Series III.

2.7 Other Equipment

Use of any equipment other than that recommended by this manual has not been evaluated for safety and, therefore, is the sole responsibility of the user.

3 Warnings

3.1 Electrical Safety

VOLTAGE! DO NOT attempt to service the IVIS Lumina Series III yourself. Although there are no voltages in excess of 24V inside the imaging chamber, local line voltages (110VAC or 230VAC) are present in the lower electronics tray. The light source module may be user-serviced for line fuse and lamp replacement only. There are no other user serviceable electrical parts in the light source module with the exception of the line fuse. See *Fuse Replacement* on page 33 for instructions. Contact PerkinElmer Technical Support for other electrical service needs (see page 2).

WARNING! If necessary, wipe exterior surfaces of the light source module with a soft cloth. DO NOT use fluids to clean the exterior of the module. Do not allow fluids of any kind to enter the light source module under any circumstances. See *Care and Maintenance* on page 36 for instructions.

WARNING! When the power is on, DO NOT disconnect or reattach the electrical control cable that connects the fluorescence equipment (excitation filter assembly) to the light source module and the IVIS Lumina Series III (electronics tray). See System Components, page 45 for photographs of these components. Disconnecting or reconnecting the control cable when the system has electrical power will damage the system. Always turn off the switch on the front panel of the light source module and the rear-mounted ON/OFF switch on the IVIS Lumina Series III before you connect or disconnect any of these cable connections.

3.2 Eye Safety and Burn Hazard

The light source module and the connecting fiber optic cables produce intense light that can cause eye damage. The module uses a tungsten halogen lamp bulb that operates at a high temperature, which if exposed to a user's skin, could cause a burn.

WARNING! DO NOT operate the light source module or the fluorescence equipment without all of the fiber optic cables connected at both of their end connections.

WARNING! Do not attempt to replace the tungsten halogen lamp or lamp assembly in the light source module. Lamp replacement requires PerkinElmer Technical Support (see page 2) because access to the lamp could expose the user to dangerous voltages and the IVIS Lumina Series III will require recalibration after replacement of the halogen lamp or lamp assembly.

3.3 Mechanical Safety

The imaging chamber of the IVIS Lumina Series III is heavy and weighs 90 lbs. (41 kg).

IMPORTANT: The IVIS Lumina Series III may only be moved on the XWS-260 Workstation. See Appendix A on page 40 for instructions.

The IVIS Lumina Series III has many internal motorized components that can move atany time. The imaging stage can move when the door is open. Care should be taken to keep hands and equipment away from the sides of the platform when it is moving. Never place anything underneath the platform.

Do not attempt to put anything into the lens opening of the camera as there are optical components that can be compromised or damaged.

If the imaging chamber makes an unusual noise or appears to be jammed, turn off the power switch located on the back of the instrument.

WARNING! If the gas hoses become caught, kinked, or disconnected, do not operate the instrument. Over exposure to anesthesia gas may occur.

CAUTION: DO NOT touch or expose the four diffusing reflectors and the exposed emission filter to contaminants (see Figure 7.2 on page 27), as this may impair imaging performance. The reflectors' surfaces have been surface treated for optimum light diffusion.

CAUTION: The Living Image® software controls excitation filter selection. Do not manually turn the numbered knob on the Excitation Filter Wheel Assembly (see Figure 7.5 on page 28).

3.4 Chemical and Biological Safety

Normal operation may involve the use of test samples that are pathogenic, toxic, or radioactive. It is your responsibility to ensure that all necessary safety precautions are taken before such materials are used.

Dispose of all waste materials according to appropriate environmental health and safety guidelines.

It is your responsibility to decontaminate the IVIS Lumina Series III before requesting service by PerkinElmer Technical Support. Ask your laboratory safety officer to advise you about the level of containment required for your application and about the proper decontamination or sterilization procedures to follow.

Handle all infectious samples according to good laboratory procedures and methods to prevent the spread of disease.

3.5 Panels, Cover, and Modules

There are no user serviceable components in the lower electronics tray of the IVIS Lumina Series III. Do not remove the electronics tray from the IVIS Lumina Series III or the cover from the light source module unless you are instructed by and under the supervision of a PerkinElmer technical service representative.

4 Legal Notices

4.1 Introduction

This manual is provided to you by PerkinElmer Health Sciences, Inc. ("PEHS") on behalf of itself and its affiliates, Caliper Life Sciences, Inc. ("Caliper") and Xenogen Corporation ("Xenogen"). PEHS, Caliper and Xenogen are referred to collectively throughout this manual as "PerkinElmer". Section 4.2 below provides the standard limited warranty for the System and associated Living Image® Software.

4.2 Limited Warranty

a. PerkinElmer provides the following limited warranty for each new IVIS® Lumina Series III ("System") purchased from it as follows ("Limited Warranty"):

i. PerkinElmer warrants that This Limited Warranty for the System extends for a period of one (1) year following delivery to, and installation of, the System to the original customer, purchaser, or user ("Customer"), the System shall substantially conform to its published specifications existing at the time of purchase. This Limited Warranty and is not assignable or transferable to any successor, without the express written permission of PerkinElmer, which may be withheld in its sole discretion.

ii. During the Limited Warranty period, PerkinElmer will repair or replace, at PerkinElmer's sole option, any defective parts if such repair or replacement is needed because of System malfunction or failure during normal usage in accordance with the instructions in this manual. Repairs and replacements under the Limited Warranty will be made at PerkinElmer's expense. Parts replaced during this Limited Warranty period may be retained by PerkinElmer, at its sole option, and will be warranted for the longer of the remaining term of the original Limited Warranty period, or for thirty (30) days from the date of replacement. PerkinElmer's limit of liability under the Limited Warranty shall be the purchase price of the Imaging System. PerkinElmer shall not be liable for any other losses or damages. These remedies are the Customer's exclusive remedies for breach of warranty.

iii. No coverage or benefits shall be provided under this Limited Warranty if any of the following conditions apply:

a) The System has been subjected to abnormal use, abnormal conditions, unauthorized modifications (e.g., unauthorized installation of hardware or software), unauthorized repair or servicing, misuse, neglect, abuse, accident, alteration, any use inconsistent with or in contradiction to the instructions in this manual, or other acts which are not the fault of PerkinElmer.

b) PerkinElmer was not advised in writing by the Customer of the alleged defect or malfunction of the System within ten (10) days after the expiration of the applicable limited warranty period.

iv. If a problem develops during the Limited Warranty period, the Customer shall contact PerkinElmer Technical Support for assistance immediately following discovery of the problem.

b. The following Limited Warranty provided by PerkinElmer is the sole and exclusive warranty applicable to the Living Image® software ("Software"):

v. PerkinElmer warrants that such software will conform to PerkinElmer's program manuals current at the time of shipment to Customer when properly installed, provided, however, that PerkinElmer does not warrant that the operation of the software will be uninterrupted or error-free.

vi. Customer agrees that as PerkinElmer's sole liability and as Customer's sole remedy, PerkinElmer will provide services to correct documented and reproducible errors which PerkinElmer's diagnosis indicates are caused by a defect in an unaltered version of the Software.

c. THE FOREGOING LIMITED WARRANTY IS THE CUSTOMER'S SOLE AND EXCLUSIVE REMEDY AND IS IN LIEU OF ALL OTHER WARRANTIES, EXPRESS OR IMPLIED. PERKINELMER SHALL NOT BE LIABLE FOR SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING BUT NOT LIMITED TO LOSS OF ANTICIPATED BENEFITS OR PROFITS, LOSS OF SAVINGS OR REVENUE, PUNITIVE DAMAGES, LOSS OF USE OF THE SYSTEM OR ANY ASSOCIATED EQUIPMENT, COST OF CAPITAL, COST OF ANY SUBSTITUTE EQUIPMENT OR FACILITIES, DOWNTIME, THE CLAIMS OF ANY THIRD PARTIES, INCLUDING CUSTOMERS, AND INJURY TO PROPERTY, RESULTING FROM THE PURCHASE OR USE OF THESYSTEM OR ARISING FROM BREACH OF THE WARRANTY, BREACH OF CONTRACT, NEGLIGENCE, STRICT TORT, OR ANY OTHER LEGAL OR EQUITABLE THEORY, EVEN IF PERKINELMER KNEW OF THE LIKELIHOOD OF SUCH DAMAGES. PERKINELMER SHALL NOT BE LIABLE FOR DELAY IN RENDERING SERVICE UNDER THE LIMITED WARRANTY, OR LOSS OF USE DURING THE PERIOD THAT THE SYSTEM IS BEING REPAIRED. PERKINELMER DOES NOT REPRESENT OR WARRANT THAT THE EQUIPMENT OR SOFTWARE WILL BE FREE FROM DEFECTS, UNINTERRUPTED OR, ERROR-FREE.

d. Some countries, states or provinces donot allow the exclusion or limitation of implied warranties or the limitation of incidental or consequential damages for certain products or the limitation of liability for personal injury, so the above limitations and exclusions may be limited in their application to you. When any implied warranties are not allowed to be excluded in their entirety, they will be limited to the duration of the applicable written warranty. This Limited Warranty gives you specific legal rights which may vary depending on local law.

e. This Limited Warranty shall be governed by the laws of the Commonwealth of Massachusetts, U.S.A., excluding its conflicts of laws principles and excluding the United Nations Convention on Contracts for the International Sale of Goods.

4.3 Patents

The detection and imaging of light originating within mammals is the subject of several issued patents and pending patent applications in the United States and around the world, including U.S. Patent Numbers 5,650,135, 6,217,847, 6,649,143, 6,890,515, 6908605, 6916462, 6923951, 6939533, 7198774 and 7255851, and European Patent Commission Numbers EP0861093 and EP1016419, for which Xenogen Corporation is the exclusive licensor. The use of an IVIS® Imaging System for such applications requires a sublicense from Xenogen Corporation.

In addition, many of the hardware and software components of the Imaging System are the subject of various issued patents and pending patent applications owned by Xenogen, including: United States Patent Number 6,614,452 (Graphical User Interface for In Vivo Imaging); 6,775,567 (Improved Imaging Apparatus); 7113217 (Multi-view Imaging Systems), 7616985 (Method and Apparatus for 3-D Reconstruction of Light Emitting Sources), 7403812 (Method and Apparatus for Determining Target Depth, Brightness, and Size Within a Body Region), 6894289 (Fluorescence illumination assembly for an imaging apparatus), and 6919919 (Light calibration device for use in low level light imaging systems).

4.4 Trademarks

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4.5 Disclaimers

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b. **Use of the System.** This manual has been developed for use by properly trained individuals only and use by individuals who have not received proper training is not advised. Any changes or modifications of the System, not expressly approved by PerkinElmer, will void the Limited Warranty and any repair thereafter shall be charged to the Customer. Any movement of the System not performed by authorized personnel of PerkinElmer may void the Limited Warranty. Failure to operate the System in accordance with this manual is likely to cause safety hazards, personal injury, property damage, and/or other damages.

5 Components and Specifications

The IVIS® Lumina Series III is an imaging system that consists of a charged coupled device (CCD) camera that can image animal subjects (primarily mice) using bioluminescence or fluorescence modalities. IVIS Lumina Series III includes:

- A highly sensitive CCD camera.
- An imaging chamber with controlling electronics.
- A Fluorescence Module, including excitation and emission filters.

The system is controlled by a pre-configured computer that runs Living Image® software. There are no user-serviceable parts in the IVIS Lumina Series III. See Chapter 7 on page 24 for more details on the fluorescence equipment.

Figure 5.1 IVIS Lumina Series III Imaging System

VOLTAGE! IVIS Lumina Series III can operate at multiple voltages (100 - 240 VAC). However, users are not permitted to change the input voltage to any of the system components. Several internal modifications are required for voltage change. If you need to change the operating voltage, contact PerkinElmer Technical Support (see page 2).

IMPORTANT: If you modify the IVIS Lumina Series III in any way, without prior approval from PerkinElmer, all warranties that cover this product are void. In addition, the computer included with the IVIS Lumina Series III is specifically configured to run all system-related applications. Any modification of existing software or hardware voids all warranties.

If you have any questions, please contact PerkinElmer Technical Support (see page 2).

5.1 CCD Camera

The camera is a scientific grade, back-thinned, back-illuminated, large format CCD manufactured by Andor Technologies.

CCD Camera Features

- Low dark current
- Thermoelectrically cooled
- 16 bit CCD digitization
- Low-noise electronic readout for extremely low-background images

CCD Camera Specifications

CCD Camera	Specification
Sensor Type	Back illuminated with AR coating optimized for visible spectral region
CCD Format	1024 x 1024
Pixel Dimensions	13 x 13 µm
Quantum Efficiency	>90% at 500 - 700 nm >65% at 700 - 850 nm 92% at 600 nm
Readout Noise – Bin 1	<2 e^- RMS
Dark Charge	<0.02015 e^-/pixel/sec

5.2 Imaging Chamber

The imaging chamber is a highly specialized device consisting of the imaging chamber housing, a heated, movable platform, an auto focusing lens system with F/Stop control, a filter wheel, and sample illumination LEDs (Figure 5.2). All adjustable components are motorized and computer-controlled, including the illumination system.

The imaging chamber is *light tight*, so that no light penetrates from the outside. The interior of the imaging chamber is constructed from materials that are non-phosphorescent and non-fluorescent to prevent internal light contamination that could compromise sample measurements.

WARNING! Under no circumstances should you attempt to make any mechanical modifications to the imaging chamber.

Figure 5.2 IVIS Lumina Series III – Imaging Chamber

Imaging Chamber Features

- Custom zero-background imaging chamber
- Eight position optical filter emission wheel with seven filters (See Table 5.1 on page 17.)
- 22-position excitation filter wheel with 19 narrow band pass filters (see Table 5.1 on page 17 for excitation filter specifications)
- High-efficiency lens assembly
- Sample illumination system
- F/Stop control
- Heated and regulated sample shelf temperature to reduce stress on an animal under anesthesia
- Gas anesthesia manifold, including gas delivery and exhaust plumbing
- Software-controlled field of view, F/Stop, focus, and optical filter wheels

Imaging Chamber Specifications

IVIS Lumina Series III Imaging Chamber	Description		
Power requirements	4.0 A max at 120 V	2.0 A max at 240 V	50-60 Hz
Dimensions	19″ x 28″ x 39″	48 cm x 71 cm x 100 cm	
Door opening dimensions	15″ x 20.25″	38 cm x 51 cm	
Weight	90 lbs	41 Kg	

5.3 Optics

Optics	Specification
Lens F/Stop	f/.95- f/16
Field of View	5 – 12 cm square

5.4 Optical Filter Wheel

A 22-position excitation filter wheel with 19 equally spaced narrow band filters is attached to the back of the imaging chamber (Table 5.1). An 8-position, computer-controlled optical filter wheel is located at the top of the imaging chamber in front of the imaging lens.

The filter wheel settings are selected in the Living Image software. See the *Living Image® User Manual – IVIS Lumina Series III* for instructions.

Table 5.1 IVIS® Lumina Series III Filters

Excitation Filters		Emission Filters	
Center (nm)	Passband (nm)	Center (nm)	Passband (nm)
420	20	520	40
440	20	570	40
460	20	620	40
480	20	670	40
500	20	710	40
520	20	790	40
540	20	845	40
560	20		
580	20		
600	20		
620	20		
640	20		
660	20		
680	20		
700	20		
720	20		
740	20		
760	20		
780	20		

Table 5.2 IVIS® Lumina LT Filters

Excitation Filters		Emission Filters	
Center (nm)	Passband (nm)	Center (nm)	Passband (nm)
430	30	515 – 575	60
465	30	575 – 650	75
500	30	695 – 770	75
535	30	810 – 875	65
570	30		
605	30		
640	30		
675	30		
710	30		
745	30		

5.5 Acquisition Computer

The computer contains an Intel family processor and Windows® operating system. Microsoft® Office is installed as well as the Living Image® software that controls the IVIS Lumina Series III.

The computer controls the IVIS Lumina Series III hardware, including the CCD camera. A printer can be connected to the computer.

Computer Features

- High speed Windows-based PC
- Microsoft Windows family operating system
- Living Image software installed. This software controls the IVIS Lumina, and displays and analyzes image data.
- CD-burner installed for data storage and transport
- Network ready
- 24" high-resolution flat screen monitor for image viewing
- Microsoft® Office installed

Computer Specifications

Computer	Description		
Power requirements	1.0 A at 120 V	0.5 A at 240 V	50-60 Hz
Dimensions	17.0″ D x 16.7″ H x 6.9″ W	43.4 cm D x 42.5 cm H x 17.5 cm W	
Weight	27.56 lbs	12.5 Kg	

Computer Monitor Specifications

Computer Monitor (Flat screen)	Description		
Power requirements	0.6 A at 120 V	0.35 A at 240 V	50-60 Hz
Dimensions with stand	10.13″ D x 15.5″ H x 22.03″ W	25.7 cm D x 39.48 cm H x 55.96 cm W	
Weight with stand	14.7 lbs	6.67 Kg	

5.6 Environmental Requirements

Environmental Requirements	Specification
Temperature	15 °C to 25 °C (50 °F to 78 °F)
Humidity	0% to 80% non-condensing
Type of use	Indoor
Imaging chamber shelf temperature	Ambient to 37 °C
Altitude rating	<2000 meters (6560 ft.)
Pollution degree	2
Installation category	II

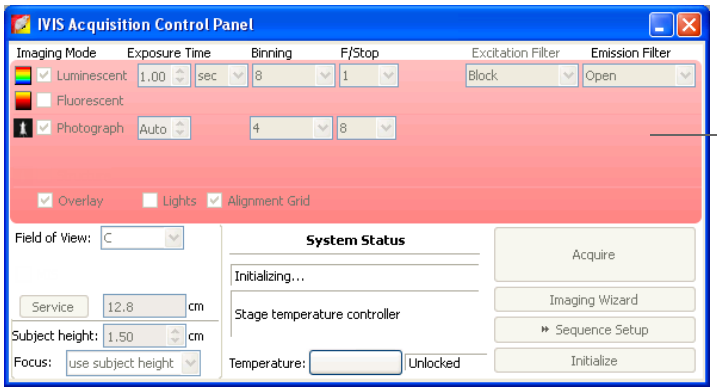
6 Basic Operation

6.1 Starting the IVIS Lumina Series III Imaging System

NOTE: All components of the IVIS Lumina Series III should be left on at all times. Periodically rebooting the computer is permissible and does not affect camera operation.

1. Plug the devices into the wall sockets in the new location.
2. Turn on the power surge protection devices.
3. Turn on the computer and monitor.
4. Turn on the IVIS Lumina Series III imaging chamber (the power switch is located on the back of the unit) and verify that the other componentssuch as the camera power supplyand fluorescence lamp are also turned to the On position.
5. Start the Living Image® software after the desktop screen is displayed.
6. Enter a User ID (up to three letters) when prompted, then click **Done**.
7. Click **Initialize** in the IVIS Acquisition Control Panel (Figure 6.2).

Figure 6.1 IVIS Acquisition Control Panel During Initialization

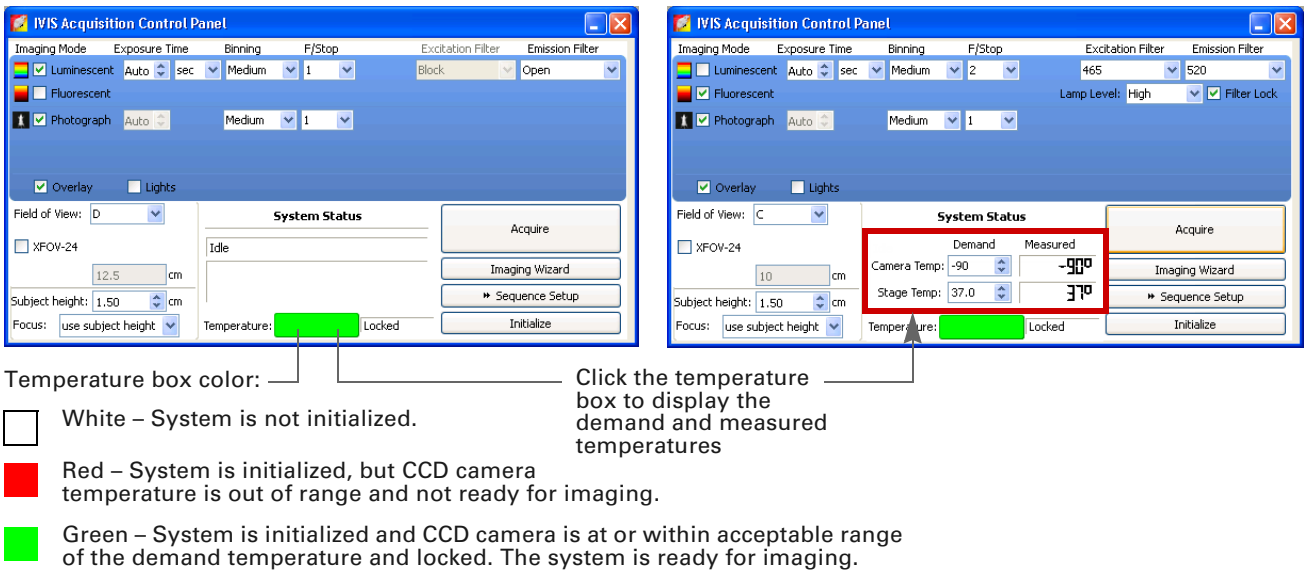


Allow the system to initialize. You will hear the motors move. The System Status box displays the current changes.

The temperature square in the IVIS Acquisition Control Panel is red at startup and turns green when the operating temperature is reached. The control panel displays the current temperature (Figure 6.2).

When the temperature is locked at -90°C, as indicated by the green light in the control panel, the instrument is ready for operation. (For operating instructions, see the *Living Image® Software Manual for IVIS Lumina Series III*.)

Figure 6.2 IVIS Acquisition Control Panel in the Living Image Software



6.2 Restarting the System After a Power Outage

If the IVIS Lumina Series III experiences a loss of supply power, turn off the power switch on each component. Do not restart the system until reliable line power has been restored.

1. Turn on the computer
2. Turn on the imaging chamber.
3. Start the Living Image® software and click **Initialize IVIS System** in the IVIS Acquisition Control Panel.

6.3 Gas Plumbing

Anesthesia gas tubing is built into the IVIS Lumina Series III imaging chamber. On the back of the imaging chamber are 0.25" hose barbs that are marked "GAS IN" and "GAS OUT" (Figure 6.3).

Figure 6.3 External Gas Ports on Rear of Instrument

"GAS IN" means the direction of flow is intothat port. Similarly, the port labeled "GAS OUT" means that flow can be exhausted out of this port.

WARNING! Use only isoflurane with the IVIS Lumina Series III. DO NOT USE FLAMMABLE ANESTHESIA GAS.

CAUTION: It is recommended to use the XGI-8 Gas Anesthesia System when imaging small animals (Figure 6.4). The system supplies a controlled amount of isoflurane to the imaging chamber and continuously reduces the build-up of isoflurane in the chamber. If you want to use a gas other than the recommended isoflurane/oxygen gas mixture or pure air, contact PerkinElmer Technical Support (see page 2).

Figure 6.4 XGI-8 Gas Anesthesia Delivery System

NOTE: Be careful to use only tubing and other plumbing fixtures that do not fluoresce or phosphoresce (glow) in the imaging chamber. Contact PerkinElmer Technical Support for a list of acceptable materials (see page 2).

6.4 Door Operation

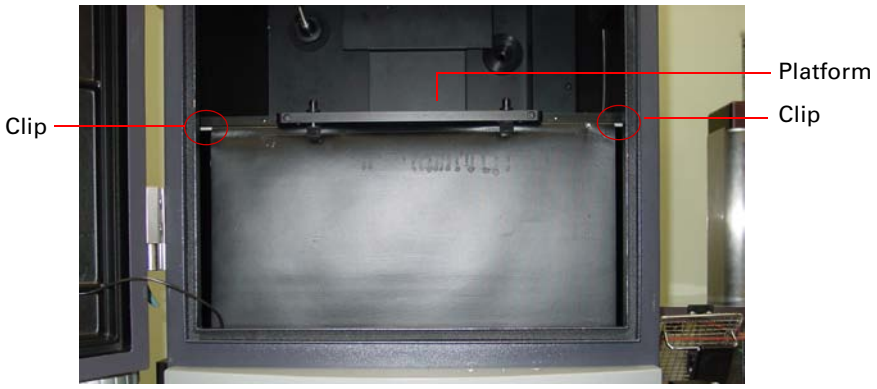
The IVIS Lumina Series III imaging chamber door has custom designed hinges, seals, and a four-point closure mechanism. The door is designed to provide a light-tight seal over numerous opening/closing cycles and should close easily without excessive handle turning resistance.

Stage Curtain

The stage curtain attached to the IVIS imaging chamber platform covers the empty space beneath the platform (Figure 6.5). The stage curtain serves as a reminder to not place anything below the platform. The curtain attaches to the platform by a bar that is held in place by clips at two locations. If it is necessary to access this area, the curtain can be easily removed and replaced.

To release the rod and curtain from the clips, pull the stage rod slightly forward. Do not allow the curtain to retract around the roller. To reattach the rod, push the rod back into the clips.

Figure 6.5 Stage Curtain



6.5 Imaging Basics

Black Paper

The imaging platform is a black anodized aluminum shelf with a special radiolucent insert. To protect this surface and to minimize the need to clean it, PerkinElmer recommends performing bioluminescence imaging on a high quality black paper. PerkinElmer has surveyed many types of paper and recommends Swarthmore, Artagain, Black, part no. 445-109, size 8.5 inch x 11 inch. This paper prevents illumination reflections and helps keep the stage clean.

Cleanable Lexan sheets are provided for use with fluorescent imaging as they produce less background than the black paper. The actual field of view pattern is provided on several of the Lexan sheets to aid in placing the subject in the center of the image. Instructions for mounting the field of view mats are provided with the mats and installation hardware.

Centering a Subject

It is recommended to confirm that the subject is centered on the stage before acquiring an image. Manual Focus adjusts the stage position to yield the optimal focus, but can also be used as a centering tool.

1. Place the subject on the center of the imaging stage and close the imaging chamber door.
2. Select the Manual Focus option from the Focus drop down menu of the Living Image control panel.

   The software displays a Manual Focus Window of the subject.
3. If the subject is not centered, open the door and reposition the subject. Click the Update button to refresh the image. Repeat this step until the subject is properly centered.
4. When the subject is centered properly, click the Done button on the Manual Focus Window to close the window.

See the *Living Image® Software User Manual for IVIS Lumina Series III* for detailed instructions.

Glowing Materials

Always keep in mind that nearly EVERYTHING glows (that is, has the potential to phosphoresce and contaminate the image). Most plastics, almostall tape, plants, paint, rodent food (mostly plants), mouse urine, and animal bedding have been found to glow.

Use caution when introducing materials into the IVISLumina Series III. It isadvisable to pre-screen all items by imaging them alone, before imaging them with samples under study. PerkinElmer recommends using non-powdered gloves when working with IVIS Lumina Series III equipment.

6.6 System Shut Down Procedure

It is not recommended to power cycle the IVIS Lumina Series III (turning the systemcomponents on and off). If it is necessary to shut down the imaging system for any reason, it is important to follow the procedure below.

1. Close the Living Image software and save any information of interest at the prompt.
2. Turn off the IVIS Lumina Series III imaging chamber using the power switch on the back of the unit.
3. Turn off the computer using the standard Windows® shut down procedure.
4. Turn off the power to the other system components and power surge protection devices.
5. If moving the system, unplug the devices from the wall.

If you have any problems during the shut down or start up procedure, please contact PerkinElmer Technical Support for assistance (see page 2).

7 Fluorescence Module

7.1 About the Fluorescence Module

The Fluorescence Module provides IVIS Lumina Series III with fluorescent imaging capability for *in vitro* or *in vivo* applications. The sensitive range of the IVIS Lumina Series III CCD camera sets the wavelength range for fluorescence applications, which is approximately 400-950 nm. As with bioluminescent imaging, wavelengths greater than 600 nm are preferred for *in vivo* fluorescent applications due to lower absorption in tissue. The Living Image software controls fluorescent image acquisition, including lamp power, level, and filter selection.

The IVIS Lumina Series III includes an extended Range (ER) illumination source which yields increased brightness in the near infrared (NIR) region, especially at wavelengths greater than 700nm. The excitation filter wheel has 22 positions that are filled with 19 narrow band filters spanning the range from 410 to 790 nm. The emission filter wheel has 8 positions with 7 broad band emission filters covering the range from 500 to 865 nm. A high service temperature fiber optic cable connects the illumination source to the input of the excitation filter wheel.

CAUTION: Only use the fiber optic cable that is provided with the IVIS Lumina Series III. Other fiber optic cables may be damaged by the heat from the lamp.

This chapter explains how to operate the fluorescence equipment with the IVIS Lumina Series III. It also provides important safety and maintenance information.

IMPORTANT: To ensure optimum and safe performance of the Fluorescence Module with maximum service life, read this chapter carefully before you use IVIS Lumina Series III with the Fluorescence Module.

You should also be familiar with and refer to the other chapters of this manual for the IVIS Lumina Series III.

For instructions on how to use the Living Image software that controls fluorescent image acquisition, see the *Living Image® Software Manual for IVIS Lumina Series III* that is provided with the software.

The *Schott Fostec DCR® III Direct Current Regulated Light Source User's Manual and Technical Reference* is a separate manual for the light source module. It provides additional useful information on the light source module and its safe operation.

7.2 Installation Requirements

The fluorescence equipment requires 90 - 260 VAC 50/60 Hz electrical power. The system automatically accepts the required voltage.

IMPORTANT: The fluorescence equipment operates at the same voltage as the IVIS Lumina Series III chamber and must not be used at other than its labeled voltage.

7.3 Specifications

Electrical Power and Fuses

Voltage Available	90 – 260 VAC, 50/60 Hz
Power Consumption	150 W

NOTE: See Chapter 5 on page 13 for more information on additional electrical power requirements of the IVIS Lumina Series III.

Environmental

Temperature	15-25° C
Humidity	0% to 80% Non-Condensing
Type of Use	Indoor (Pollution Degree 2)
Sound Level	61 dB <500 Hz
Altitude Ratin	<2000 meters (6560 feet)

Lamp and Fuse

Lamp	150 W tungsten halogen EKE bulb with an Extended Range (ER) coating to for increased brightness in the near infrared (NIR). Lamp voltage: 20V Color temperature: 2856 K°
Fuse	2.0 A, 5x20 mm, 250 VAC Slow Blow

Ventilation Requirements

Provide sufficient space (minimum 6 inches or 15 cm) behind the fan of the light source module so that airflow is unobstructed. Provide a similar minimum distance behind the IVIS Lumina Series III to enable fan cooling as well as adequate room for fiber optic cable routing.

Chemicals Required for Operation

No chemicals are required for the operation of the IVIS Lumina Series III or the fluorescence equipment. Other user supplied chemicals or materials may be required for your specific biological testing procedures.

Weight and Dimensions of the Fluorescence Light Source Module

Weight	4.94 lbs (2.24 kg)
Depth	8.61 inches (21.9 cm)
Width	7.27 inches (18.5 cm)
Height	4.6 inches (17.7 cm)

7.4 Description and Theory of Operation

System Components

The Fluorescence Module provides fluorescent imaging capability. You can conveniently switch between bioluminescent and fluorescent imaging applications.

Figure 7.1 shows the fluorescence equipment and Figure 7.2 depicts the interior of the imaging chamber (green indicates the excitation light).

Figure 7.1 IVIS Lumina Series III – Rear View

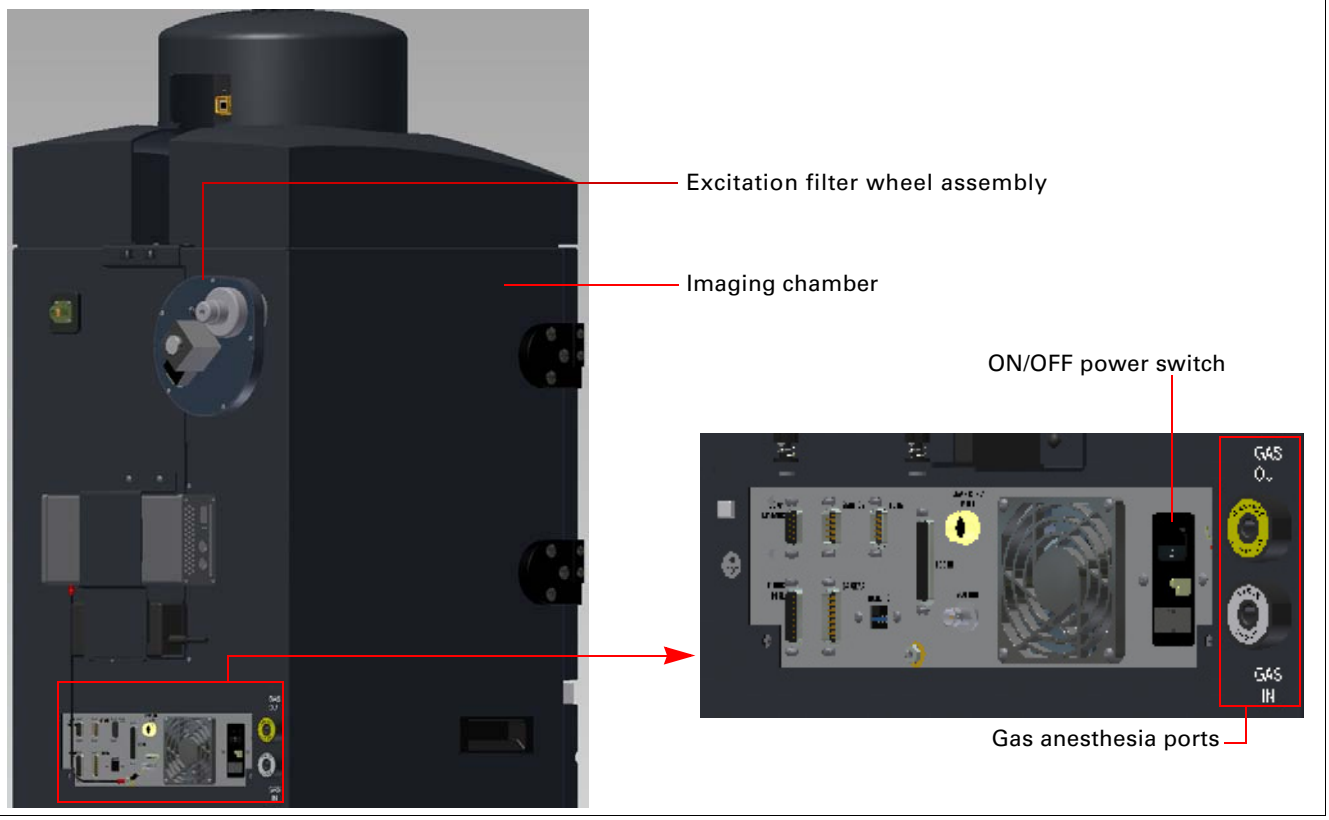
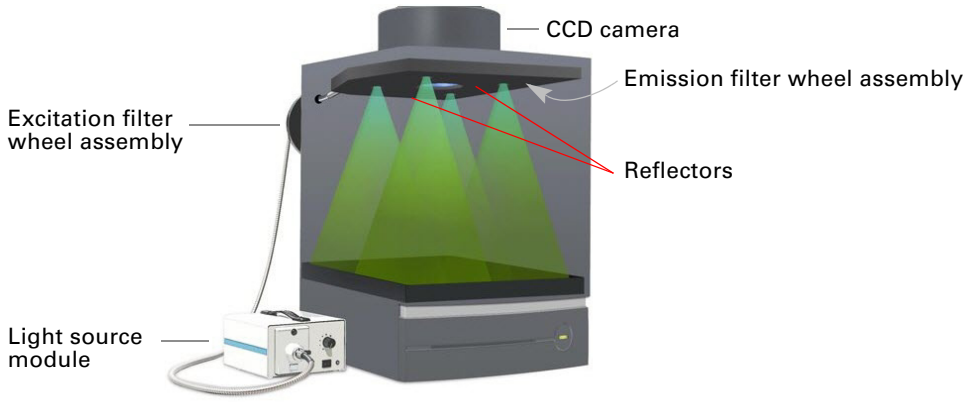


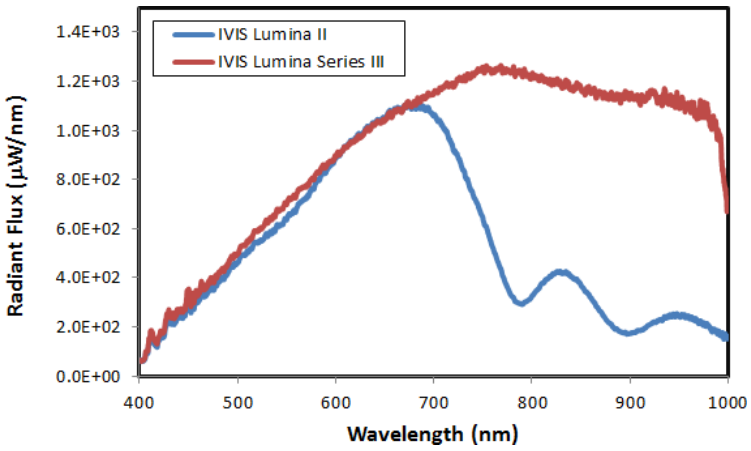
Figure 7.2 IVIS Lumina Series III and Fluorescence Equipment



The fluorescence light source module (Figure 7.3) provides the fluorescence excitation light. This light source consists of a 150-watt quartz tungsten halogen lamp with a dichroic reflector. Figure 7.4 shows the relative spectral radiance output ofthe lamp/ reflector combination and indicates emission throughout the IVIS Lumina wavelength range of 400-950 nm. The EKE-ERlamp provides improved light output in the 400-950nm range compared to the standard EKE lamp. Because of the higher temperature of the ER lamps, a high temperature fiber optic is used to deliver the light to the excitation filter wheel.

Figure 7.3 Fluorescence Light Source Module

Figure 7.4 Relative Spectral Radiance for the Quartz Halogen Lamp



The lamp intensity level is computer-controlled by the Living Image® software. The user can adjust the lamp output intensity bymeans of software to a low or high setting. The fluorescence light source module operates under software control; therefore manual adjustment of the front panel lamp potentiometer is disabled.

The illumination system of the IVIS® Lumina Series III is optimized for NIR imaging. The Extended NIR range (EKE-ER) 150W tungsten excitation light source allows increased power at wavelengths greater than 700 nm (red curve in Figure 7.4) compared to the previous illumination system (blue curve in Figure 7.4).

The lamp output is delivered to the excitation filter wheel assembly (Figure 7.5) on the back of the IVIS Lumina Series III imaging chamber. Figure 7.6 shows a cross section of the excitation filter wheel assembly. Light from the input fiber optic bundle passes through a collimating lens then travels through a 25 mm diameter excitation filter. Twenty-two (22) position filter wheel locations allow you to choose up to nineteen excitation filters. Light blocks are provided in the three unused filter slots. One of these positions is used during bioluminescent imaging to prevent external light from entering the imaging chamber. The 22 position excitation filter wheel is motor-controlled through the Living Image software

Figure 7.5 Excitation Filter Wheel Assembly

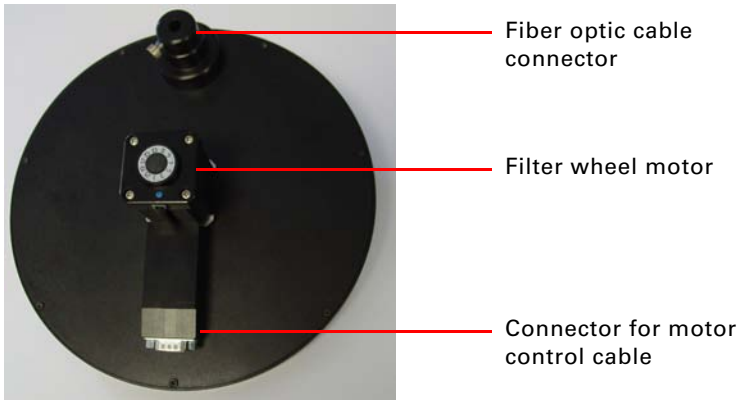
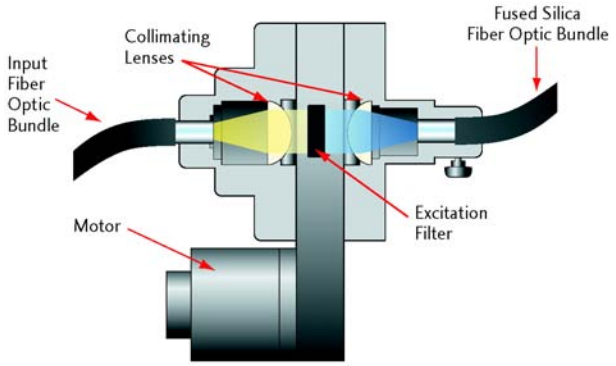


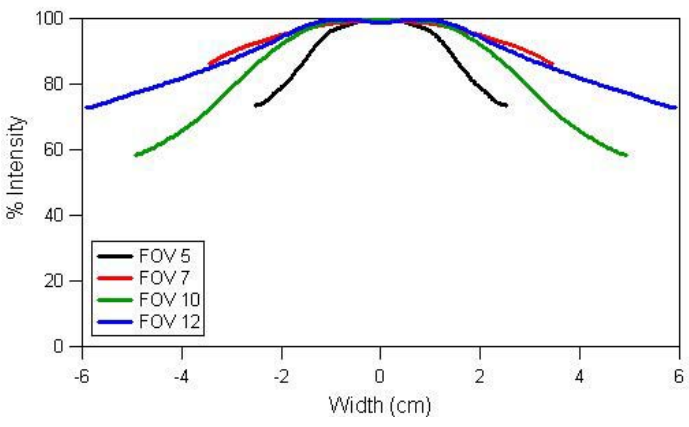
Figure 7.6 Excitation Filter Wheel – Cross Sectional Area



Following the excitation filter, a second lens focuses light into a one quarter inch fused silica fiber optic bundle inside the IVIS Lumina Series III imaging chamber. Fused silica (core and clad) fibers are used in this bundle to avoid the generation of auto-fluorescence in the fiber, as is the case with ordinary glass fibers.

The fused silica fiber bundle splits into four separate legs that deliver filtered light to four reflectors located on the ceiling of the imaging chamber (Figure 7.2 on page 27). Typical illumination profiles for stage locations A-D (fields of view 5-12 cm respectively) are shown in Figure 7.7. Note that the profiles for all the stage locations are peaked near their center. The non-uniformity of the illumination pattern is compensated for when units of efficiency are selected in the Living Image software (for more details, see the Living Image® Software User's Manual). When imaging 96-well plates, the lower stage positions (C and D) are recommended to minimize shadowing effects due to the off-axis illumination. Fluorescent emission from the target fluorophore is collected through an emission filter wheel located at the top of the imaging chamber and then focused into the CCD camera. The emission filter wheel contains eight openings. Users have the ability to choose up to seven emission filters (60 mm diameter), leaving one position open for bioluminescent imaging.

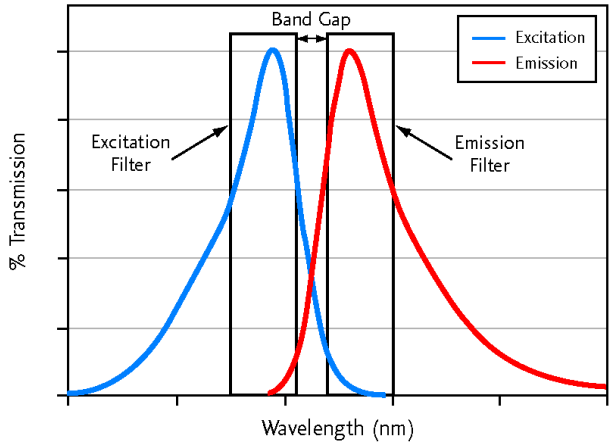
Figure 7.7 Typical Illumination Profiles for Stage Locations (FOV of 5, 7, 10 and 12 cm, respectively) Measured From the FOV Center



Understanding Filter Spectra

The use of high quality filters is essential for obtaining good signal-to-background levels (contrast) in fluorescence measurements, particularly in a high sensitivity instrument such as the IVIS Lumina Series III. Figure 7.8 shows typical excitation and emission fluorophore spectra, along with idealized excitation and emission filter transmission curves shown as rectangles. The excitation and emission filters are called *bandpass* filters; ideally they transmit all the wavelengths within the bandpass region and block (absorb or reflect) all wavelengths outside the bandpass. This spectral band is like a window, characterized by its central wavelength and its width at 50% peak transmission (full width half maximum, FWHM).

Figure 7.8 Typical Excitation and Emission Spectra for a Fluorescent Compound

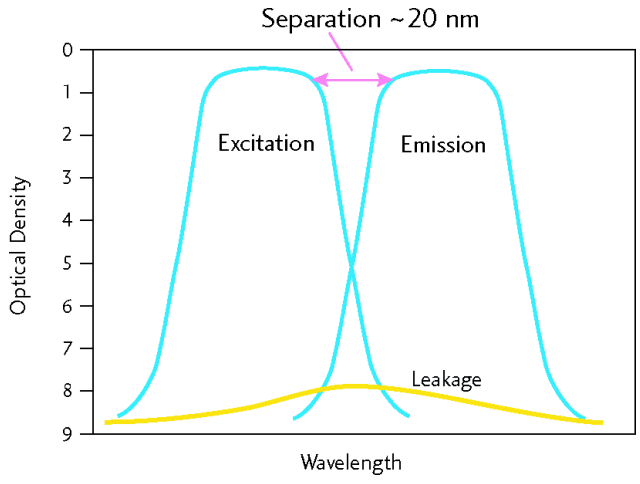


This figure includes two idealized bandpass filters that would be used with this fluorescent compound.

- Bandgap – Spacing between the transmission regions of the excitation and emission filters.
- Transmission – Fraction of light that passes though the filter bandpass region.
- Blockage – Light rejection in the non-transmitting region (or the region outside the band) of the filter spectrum.
- Leakage – Undesirable light that is not blocked properly by the filter and is detected by the camera.

Real filters have some leakage outside of the bandpass region and can also exhibit autofluorescence, depending on the materials used in the filter construction. More realistic filter transmission curves are shown in Figure 7.9.

Figure 7.9 Illustration of Typical Excitation and Emission Curves



The vertical axis in Figure 7.9 is optical density, defined as OD =-log(T) where T is thetransmission. An optical density of 0 indicates 100% transmission, whereas OD7 indicates a reduction of the transmission to $1x10^{-7}$. Typical transmission of a filter in the bandpass region is about 0.7 (OD0.15) and typical blocking outside of the bandpass region is about OD7. The band gap between the two filters is usually defined as the gap at 50% transmission (OD0.3). There is a slope in the transition region from bandpass to blocking, as indicated in Figure 7.9. A steep slope is required to avoid overlap between the two filters.

The fluorescence filters are high quality interference filters, constructed from alternating layers of dielectric films on a substrate of low auto-fluorescent glass. Care has been taken to minimize filter auto-fluorescence so that its level is below OD7. Filter passbands for the standard set are listed in Table 5.1 on page 17.

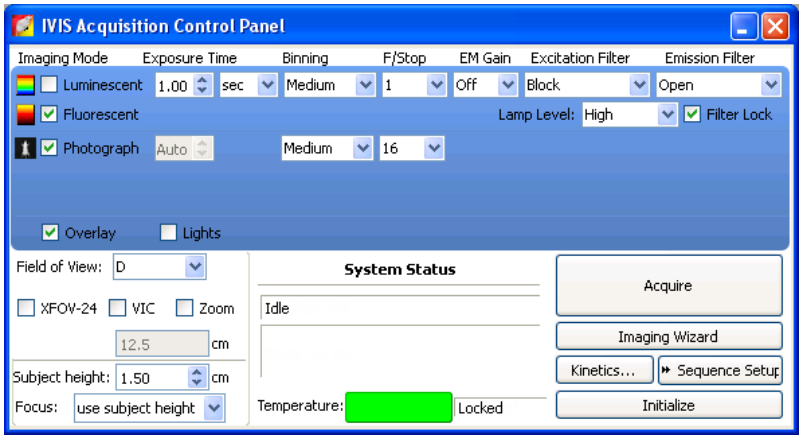
7.5 Fluorescent Imaging

NOTE: This chapter provides a quick start guide to acquiring fluorescent images. See the *Living Image® Software User Manual for IVIS Lumina Series III* for complete details.

IVIS Acquisition Control Panel

Acquiring fluorescent images using Living Image software is controlled through the IVIS Acquisition Control Panel (Figure 7.10). To acquire a fluorescent image, the user must check the **Fluorescent** box on the left side of the panel. Once selected, controls for the illumination lamp – **Fluor Lamp Level**, and **Filter Lock** – will appear in the top half of the panel. Checking the **Filter Lock** box ensures that the excitation and emission filters are properly paired. During image acquisition, the lamp is computer-controlled through Living Image software. The **Lamp Level** drop-down list controls the illumination intensity level of the lamp with options – **Off**, **Low**, **High**, and **Inspect**. The **Low** setting is approximately 18% of the**High** setting. **Inspect** turns on the illumination lamp, allowing the user to manually inspect the excitation lamp.

Figure 7.10 IVIS Acquisition Control Panel – Living Image Software



NOTE: Ensure that the correct filters selected from the Excitation Filter and Emission Filter drop-down lists before you select Inspect. The inspect operation automatically positions the filters that are selected from the drop-down lists before turning on the lamp. Changing the filter selection in the drop-down lists will have no effect until another inspect operation is performed.

Acquiring Fluorescent Images

1. If it is not already on, start the acquisition computer and Living Image® software.

   The control panel appears (Figure 7.10).
2. Click **Initialize IVIS system**.

   After initialization, the **Temperature** box in the center of the panel should be green, indicating that the CCD camera is adequately cooled. (Allow 10 to 15 minutes for the camera to reach the proper temperature.) The **Temperature** box changes from red to green when the CCD camera has reached the proper operating temperature.
3. Place the sample to be imaged in the center of the stage in the imaging chamber and close the door.
4. Select the **Fluorescent** check box.
5. Make a selection from the **Field of View** drop-down menu on the left side of the Control Panel.
6. Enter the approximate (0.5 cm) **Subject Height** (height) in the lower left entry box (or focus manually).
7. Select the **Emission Filter** and **Excitation Filter**. If the **Filter Lock** box is checked, select the excitation or emission filter of interest. Select only one, as the other filter will be selected automatically.
8. Select **High** or **Low** from the Fluor Lamp Level drop-down menu. **High** is the recommended setting
9. Set the **Exposure Time**, **Binning**, and **F/Stop**.

   Fluorescence is generally brighter than bioluminescence, so the exposure time is shorter and F/Stop higher (smaller lens opening). Typical fluorescence image camera settings might be 1 sec exposure time, Binning = Medium, and F/Stop = 2.
10. Click **Acquire**.

    After the exposure is completed, the overlay image is displayed.

    NOTE: During the fluorescent image acquisition, the Acquire button becomes a Stop button, which can be used to terminate the exposure if necessary.

11. To save the data, select **Living Image →Save Living Image Data** from the main menu bar.

    This completes the data acquisition. To obtain additional images, repeat the process, beginning with step 3.

    The image window that displays the fluorescent image includes annotations specific to fluorescence (including emission filter, excitation filter, and fluorescence level) as well as standard annotations such as exposure time, F/Stop, FOV, and acquisition date/time.

7.6 Troubleshooting

Hardware Problems

If you have difficulty during fluorescent imaging, it may be due to the lack of excitation light. Loss of excitation light can result from either a burned out quartz tungsten halogen lamb or a blown line fuse. The following procedure describes a troubleshooting process for determining the problem. Figure 7.3 on page 27 shows the fluorescence light source module.

1. Verify that the fiber optic cables are not loosened or disconnected from their proper connectors.
2. Adjust "Fluor Light Level" (excitation lamp) to avalue of "High" in the IVISAcquisition Control Panel.

3. Take a fluorescent image and check for light and fan operation by looking through the fan guard on the rear panel of the light source module. If there is neither, a blown fuseis the probable cause for light loss. See *Fuse Replacement* below.
4. Observe the operation of the system by selecting "Inspect" in the Fluorescent Lamp Level drop down list. This causes the selected filter to move into place and the lamp to turn on. Open the chamber and visually inspect to see if the excitation light is incident on the sample stage.

   If no light is detected, try a different filter. If there is still noexcitation light on the sample stage, contact PerkinElmer Technical Support (see page 2).

WARNING! DO NOT disconnect or reattach the electrical control cable that connects the fluorescence equipment (excitation filter assembly) to the light source module and IVIS Lumina Series III (electronics tray) when the power is on. See Figure 7.1 on page 26 and Figure 7.2 on page 27 for photographs of these components. Disconnecting or reconnecting the control cable when the system has electrical power will damage the system. Always turn off the rear-mounted ON/OFF switch on the IVIS Lumina Series III before making or breaking any of these cable connections.

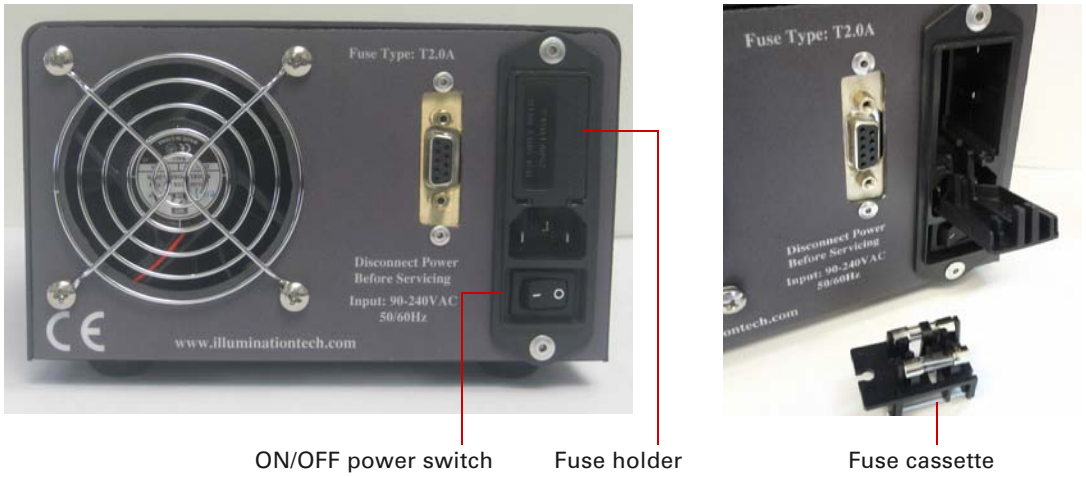
Fuse Replacement

WARNING! The following procedure can expose the user to hazardous voltages unless the electrical power to the fluorescence light source module is completely eliminated. As instructed in the procedure, turn off the lamp from the module front panel ON/OFF switch and remove the electrical power to the module by disconnecting the plug from the surge protector and the back of the module.

1. Before starting this procedure, save any important data in Living Image, and then exit the program. Next, turn off the main power at the rear of the Lumina.
2. Press the light source module ON(1)/OFF(0) switch to the OFF(0) position.
3. Remove AC line cord from the AC power receptacle on the surge protector.
4. Remove the AC line cord from the power entry module on the rear panel of the light source module.
5. Open the fuse holder door.

   The fuse holder is part of the power entry module, and is located directly below where the AC line cord plugs in (Figure 7.11). Use a thin bladed screwdriveror penknife blade if necessary. Be careful not to damage the drawer.

Figure 7.11 Fluorescent Light Source Module – Fuse Holder and Fuse Cassette



6. Examine the replacement spare fuses and verify that they are the correct rating: Fuse value: 2.0A, 5 x 20, 250V SLOW BLOW.
7. Remove fuse cassette and remove the two blown fuses (Figure 7.11). If only one is blown, it may be a good idea to replace both. Place the replacement fuses into the fuse cassette. The fuses will work in either direction.
8. Push the fuse cassette into the fuse holder and shut the fuse door. The door should click shut.
9. Reattach the AC line cord to the power entry module, then to the AC receptacle on the surge protector.
10. Return the ON/OFF switch to the ON(1) position (the red indicator will be visible on the switch).
11. Turn on the main power switch at the rear of the IVIS Lumina Series III and start the Living Image software.

NOTE: The power switch on the back of the light source module (Figure 7.11) must be in the ON position in order for Living Image software to control lamp functions.

12. Resume normal operation.

Lamp Replacement

Contact PerkinElmer Technical Service (see page 2) for lamp replacement. Replacing the lamp requires re-calibration of the IVIS Lumina SeriesIII. The procedure also requires removing the lamp module cover, which potentially exposes the user to hazardous voltages. Removing the lamp module cover will void the warranty.

7.7 Care and Maintenance of the Fluorescence Equipment

Cleaning the Fluorescence Light Source Module

If necessary, wipe the exterior surfaces of the light source module with a soft cloth.

WARNING! DO NOT use fluids to clean the exterior or interior of the IVIS Lumina Series III. Do not allow fluids of any kind to enter the light source module under any circumstances. Sprays and liquids that come into contact with the light source module or the instrument may result in damage to the system or electrocution.

If the fluorescence light source module requires more aggressive cleaning or sterilization, contact PerkinElmer Technical Support (see page 2).

Cleaning the IVIS Lumina Series III and Fluorescence Equipment

WARNING! DO NOT use fluids or moistened towels to clean the any part of the IVIS Lumina Series III where electrical or fiber optic cables make connections. Do not use fluids of any kind in the vicinity of the Excitation Filter Wheel Assembly (mounted on the rear of the imaging chamber). Turn off electrical power to the instrument before engaging in cleaning operations using fluids. The Imaging Chamber power switch is located in the rear on the electronics tray.

See *Care and Maintenance* on page 36 for instructions on cleaning the imaging chamber,

Cleaning the Optical Components and Filter Replacement

Contact PerkinElmer Technical Support for information about cleaning or sterilizing any of the optical components or the optical filter replacement (see page 2).

8 Care and Maintenance

The compounds shown in Table 8.1 do not damage the internal finish of the IVIS Lumina Series III imaging chamber and are suitable for use as cleaners, if required. Do not use any solution not included in this list. In particular, avoid strong bases, bleach, or acids that may potentially damage the unit and compromise its operation.

IMPORTANT: Do not spray cleaning solutions in the imaging chamber.

Table 8.1 Acceptable Cleaning Solutions for the IVIS Lumina Series III Imaging Chamber

Cleaning Solution	Manufacturer
Cidexplus® Solution (3.4% glutaraldehyde)	Johnson and Johnson Medical
Sporicidin® Sterilizing Solution (1.56% phenol)	Sporicidin International
Clidox-s® Disinfectant	Pharmacal Research Laboratories, Inc.
70% methyl alcohol/30% deionized water solution	
70% ethyl alcohol/30% deionized water solution	
3 - 5% bleach (in deionized water)	

NOTE: PerkinElmer makes no claims as to the sterility of the IVIS Lumina Series III imaging chamber after using the solutions in Table 8.1. Please refer to the manufacturer's literature for information as to the applicability of the compound for the organism of interest.

It is recommended to use a lint-free wipe, such as Scott Pure® wipe or a Kaydry EX-L® wipe to minimize the presence of particulate matter in the imaging chamber.

After saturating a lint-free wipe, clean the internal surfaces using a gentle circular motion. Use extra care when cleaning the radiolucent insert since it is a delicate assembly. Do not pour or spray the solution directly onto internal surfaces, especially the garage housing the scintillation sensor assembly. Rinse surfaces using a wipe saturated with sterile deionized water. Do not allow puddles of water to remain on the surfaces. To avoid any phosphorescence from the cleaner, be sure that the surfaces are dry before using the imaging chamber. Be careful not to smudge the camera lens and optical filters.

Consider dedicating an IVIS Lumina Series III for immunodeficient animals to remove the risk of cross-contamination.

9 Troubleshooting

9.1 Measured Temperature Is Not Equal to Demand Temperature

At start up, the Living Image® software programs the CCD camera to maintain the CCD at -90° C. If the camera power supply remains on (IVIS Lumina Series III box), the system maintains this temperature regardless of whether the Living Image software is open or the computer is turned on.

To check the temperature of the CCD, click the Temperature square (red or green) in the control panel in the Living Image software.

Figure 9.1 IVIS Acquisition Control Panel in the Living Image Software

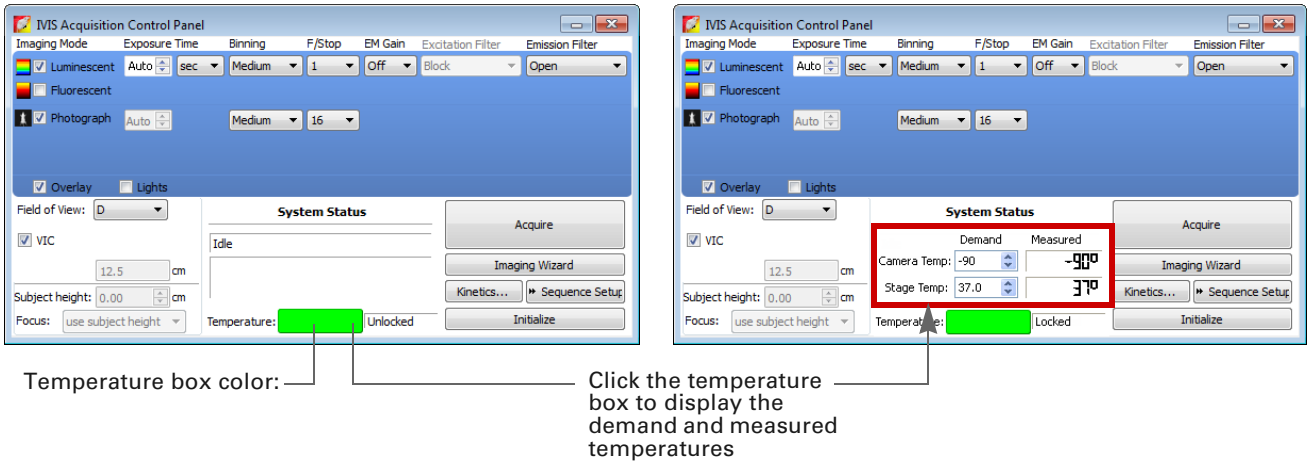


Table 9.1 Troubleshooting – Measured Temperature Does Not Equal the Demand Temperature

Problem	Possible Cause	Corrective Action
Measured temperature is warmer than the demand temperature.	Ambient temperature may be too high, camera air vents my be blocked, or system needs service. A problem may exist with the camera.	Verify that the room temperature is within operational limits. Check air vents in the camera head by removing protective cover and inspecting. Contact PerkinElmer Technical Support for assistance (see page 2).
Something is obstructing the stage.		1. Open the door to the imaging chamber and visually inspect the stage. 2. Remove anything that is physically obstructing the stage. If there is no obstruction and/or the stage still does not move, contact PerkinElmer Technical Support for assistance.

9.2 Photographic Image Is Unacceptable

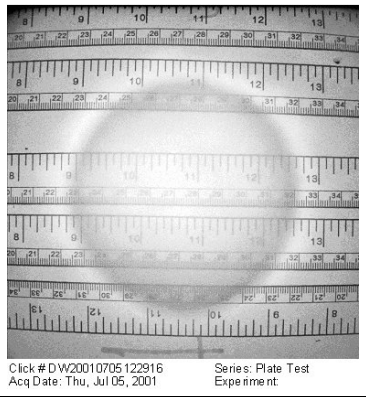
Photograph imaging parameters are automatically controlled and generally produce a good quality photo. If shiny objects are imaged, creating specular reflections, the automatic algorithm may get confused and produce an underexposed image.

Also, refer to the *Living Image Software® Manual for IVIS Lumina Series III* for further details on acquiring images.

Table 9.2 Troubleshooting – Unacceptable Photographic Image

Problem	Possible Cause	Corrective Action
Image is streaked.	Subject moved during the exposure.	Check to see if the subject may have moved. If the subject is not on the sample stage, it is probably on the floor of the imaging chamber. If the subject has moved, locate and re-anesthetize it. If gas anesthesia is being used, confirm that the anesthesia is turned on and the flow rate is appropriate.
Image is blurry.	Subject height is significantly less than or greater than 1.5 cm.	The focus is set for a sample height of 1.5 cm. Significant deviation from this height results in an out-of-focus photograph.
	Incorrect F/Stop setting.	The F/Stop for photographs should be set to F/Stop 8 or F/Stop 6. An F/Stop smaller than 8 reduces the depth of field in the photograph.
A white spot appears in the center of the field of view.	An excessively moist environment in the imaging chamber can result in condensation on the CCD window (Figure 9.2.)	Turn off the entire system and remove excess moisture in the imaging chamber. Allow the chamber to thoroughly dry. If the problem persists, contact PerkinElmer Technical Support for assistance (see page 2).

Figure 9.2 Example of Condensation



9.3 Luminescent Image Is Unacceptable

Binning, F/Stop, and exposure time affect the appearance of a luminescent image. Please refer to the *Living Image Software Manual for IVIS Lumina Series III* for instructions on setting binning, exposure time, and F/Stop values.

In order to function properly and reduce camera noise, the CCD camera must be cooled to the demand temperature before acquiring an image. If the camera is not cooled to the demand temperature, imaging may result in false positive signals.

Table 9.1 Troubleshooting – Unacceptable Luminescent Image

Problem	Corrective Action
Light contamination-Internal	Check to see that there are no extraneous light sources in the imaging chamber. Many substances phosphoresce when exposed to light. Be especially cautious of plastics and substances that contain pigment. Be sure to pre-screen any substance or material before performing actual experiments.
Light contamination-External	A two inch diameter High Reflectance Hemisphere (PerkinElmer part no. 118937) is used to help check for light leaks (Figure 9.3). Contact PerkinElmer Technical Support to purchase this accessory (see page 2). To check for light leaks using the High Reflectance Hemisphere: 3. Place the high reflectance hemisphere in the imaging chamber on the stage using a subject height of 3.5 cm at field of view D. 4. Take a luminescent image of the hemisphere using the luminescent settings: F/Stop = 1, Binning = Large (high sensitivity), and exposure time = 5 minutes. If the hemisphere can be easily seen, there is a light leak. Contact PerkinElmer Technical Support for assistance.
Camera noise	Verify that the camera is cooled to the demand temperature. Check the measured temperature in the IVIS Acquisition Control Panel Acquisition Control Panel to ensure that it is locked. If the camera temperature is locked, the camera temperature box is green. If the camera temperature box is red, click the red box to display the actual temperature. See *Measured temperature is warmer than the demand temperature.* on page 37.

Figure 9.3 High Reflectance Hemispheres

9.4 No Image Produced

If no image is produced, there may be an error in the Living Image software, a problem with the physical connections to the camera, or a hardware failure.

1. Close the Living Image® software and restart the computer.
2. Restart the Living Image software and try to acquire an image.
3. If after restarting the computer, you are still unable to produce an image, contact PerkinElmer Technical Support for assistance (see page 2).

Appendix A XWS-260 Workstation

This appendix explains how to move the IVIS Lumina Series III Series III configured with the XWS-260 workstation (Figure A.1).

CAUTION: PerkinElmer recommends that you do not move an IVIS Lumina Series III that is not located on the XWS-260 workstation. If you need to move an imaging system not on the workstation, contact PerkinElmer Technical Support for assistance (see page 2).

The procedural steps include:

- Shut down the system components and unplug them from the line power.
- Move the workstation with the components.
- Restart the system components after the power is restored.

CAUTION: The IVIS Lumina Series III has many cables and lines. It is very important to closely follow all directions to avoid damaging the system components.

Figure A.1 IVIS Lumina Series III Configured with XWS-260 Workstation

A.1 Shutting Down the Imaging System

1. Close the Living Image® software and save any information at the prompt.
2. Turn off the IVIS Lumina Series III imaging chamber.
3. Turn off the computer using the standard Windows® shut down procedure.
4. Turn off the power to the other system components and power surge protection devices.
5. Unplug the devices from the wall.

If you have any problems during the shut down procedure, please contact PerkinElmer Technical Support for assistance (see page 2).

A.2 Moving the Imaging System on the XWS-260 Workstation

1. Unlock the wheels on the XWS-260 workstation and carefully roll the workstation to the new location.

   CAUTION: When moving the imaging system on the XWS-260 workstation, be sure to grasp and push the workstation from as low as comfortably possible. PerkinElmer recommends having two people present during any major move to minimize the risk of damage to the imaging system. If the IVIS Lumina Series III is damaged during movement, PerkinElmer cannot be held responsible and any warranty will be voided.

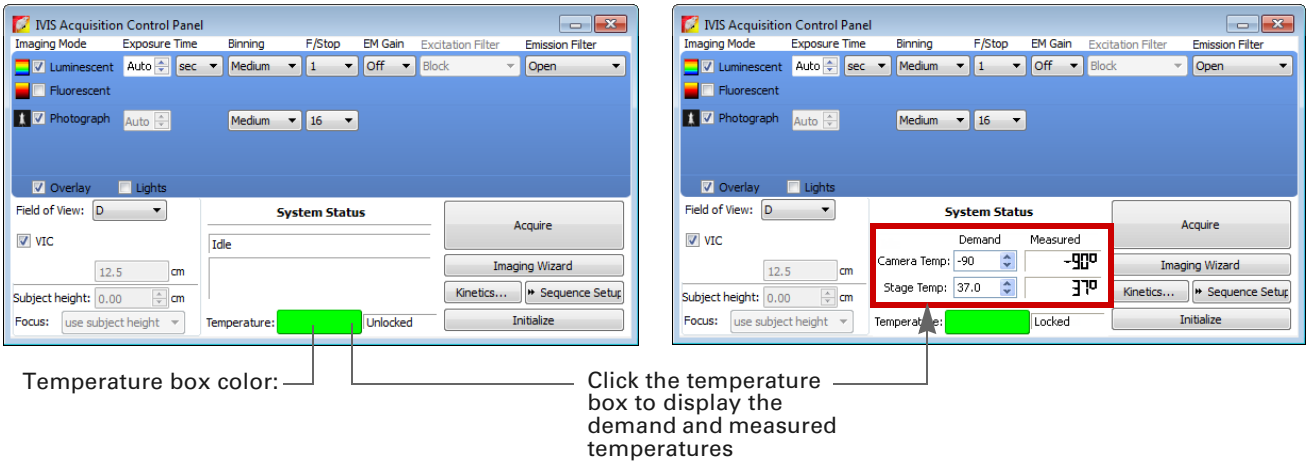
2. Lock the wheels when you are finished moving the workstation.

   Verify that the power outlets at the newlocation meet the power requirements for the system. See Chapter 5 on page 13 for equipment power requirements,.

A.3 Starting the Imaging System

1. Plug the devices into the wall sockets in the new location.
2. Turn on the power surge protection devices.
3. Turn on the computer and monitor.
4. Turn on the IVIS Lumina Series III imaging chamber (the power switch is located on the back of the unit) and verify that the other components such as the camera power supply (on the back of the unit) and fluorescence lamp are also turned to the On position.
5. Start the Living Image® software after the desktop screen is displayed.
6. Enter a User ID (up to three letters) when prompted, then click **Done**.
7. To initialize the system, click **Initialize IVIS System** in the IVIS Acquisition Control panel (Figure A.2).

Figure A.2 IVIS Acquisition Control Panel in the Living Image Software



8. Allow the system to initialize.

   You will hear the motors move. The System Status box displays the current changes.

   The temperature square in the IVIS Acquisition Control Panel is red at startup and turns green when the operating temperature is reached. The control panel displays the current temperature (Figure A.2).

   The instrument is ready for operation after the temperature is locked at -90°Ças indicated by the green light in the control panel (Figure A.2. For operating instructions, see the *Living Image® Software Manual for IVIS Lumina Series III*.)

Appendix B Options and Accessories

Table B.1 Optional Equipment and Accessories for IVIS Lumina Series III

Item	Part Number
XBP-24 Black Paper (1 tablet of 24 sheets)	117837
XLS-4 Calibrated Light Source	118897
XGI-8 Anesthesia System, 120V	118918
XGI-8 Anesthesia System, 230V	118919
XGI-8 Anesthesia System, 100V	118957
Kit, 3 Port Manifold, X-Ray Compatible, plus Accessories	125698
KH-2 Reflective Hemisphere	118937
XPP-1 Plate Positioner	118949
XPM-2 Bioluminescent Phantom Mouse	118993
XFM-2 Fluorescent Phantom Mouse	121365
XAF-8 Anesthesia System Filters	118999
XFM-1 Low Fluorescence Mat, set of 10	119000
XNC-2 Mouse Nose Cones, set of 10	119001
KS-10 Rubber Stoppers for Anesthesia Manifold	119006
XWS-260 Workbench	123325
XWS-248 Workbench	123326
XWS-272 Workbench	126138
Optical Lens Cleaner	123495
Wipe, Lint Free (50/pk)	126291
FOV Targeting Mat (each)	121313

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