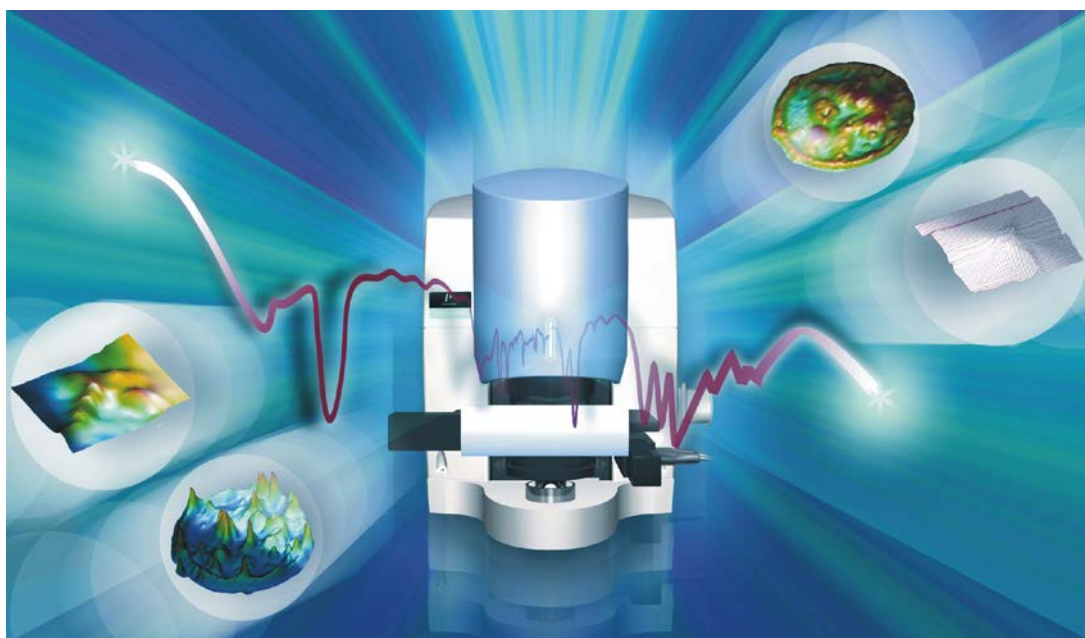


Spotlight 400 Series



User's Guide

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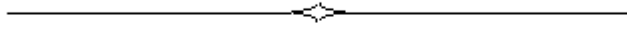
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Introduction

About This Manual

This introduction gives you information about this manual, to enable you to use it effectively when learning to operate the imaging system.

Before you start using your Spotlight imaging system we recommend that you:

- Read the rest of this Introduction and become aware of conventions and requirements;
- Read *A Guided Tour of the Spotlight Imaging System* starting on page 28;
- Read *Getting Ready to use the Imaging System* starting on page 39.

Four chapters in this User's Guide contain information on using and maintaining your imaging system:

- *Getting Ready to use the Imaging System* gives you information on how to set up your imaging system at the beginning of the day's work, to make sure that it is working properly.
- *Preparing Samples* describes techniques for preparing many types of microscopic samples. It includes descriptions of the sample preparation tools provided with, or available for, the imaging system.
- *Techniques for Collecting IR Images and Spectra* describes how you can use accessories and different techniques to collect images and spectra.
- *Maintenance* contains maintenance information. It gives you information on how to care for the imaging system and about the performance checks that we recommend that you perform routinely.

Conventions Used in this Manual

Normal text is used to provide information and instructions. *Italic* text is used to provide commentary. **Bold** text refers to text that is displayed on the screen.

UPPERCASE text, for example ENTER or ALT, refers to keys on the PC keyboard. '+' is used to show that you have to press two keys at the same time, for example, ALT+F.

All eight digit numbers are PerkinElmer part numbers unless stated otherwise.

'Spectrometer' refers to the Frontier IR System, Spectrum 100 Series, Spectrum 400 Series, Spectrum One FT-IR or Spectrum One NTS FT-NIR spectrometer used with your Spotlight imaging system.

Notes, Cautions and Warnings

Three terms, in the following standard formats, are also used to highlight special circumstances and warnings.

NOTE: A note indicates additional, significant information that is provided with some procedures.

CAUTION	<i>We use the term CAUTION to inform you about situations that could result in serious damage to the instrument or other equipment. Details about these circumstances are in a box like this one.</i>
D	Caution (Achtung) <i>Bedeutet, daß die genannte Anleitung genau befolgt werden muß, um einen Geräteschaden zu vermeiden.</i>
DK	Caution (Bemærk) <i>Dette betyder, at den nævnte vejledning skal overholdes nøje for at undgå en beskadigelse af apparatet.</i>
E	Caution (Advertencia) <i>Utilizamos el término CAUTION (ADVERTENCIA) para advertir sobre situaciones que pueden provocar averías graves en este equipo o en otros. En los recuadros como éste se proporciona información sobre este tipo de circunstancias.</i>
F	Caution (Attention) <i>Nous utilisons le terme CAUTION (ATTENTION) pour signaler les situations susceptibles de provoquer de graves détériorations de l'instrument ou d'autre matériel. Les détails sur ces circonstances figurent dans un encadré semblable à celui-ci.</i>
I	Caution (Attenzione) <i>Con il termine CAUTION (ATTENZIONE) vengono segnalate situazioni che potrebbero arrecare gravi danni allo strumento o ad altra apparecchiatura. Troverete informazioni su tali circostanze in un riquadro come questo.</i>
NL	Caution (Opgelet) <i>Betekent dat de genoemde handleiding nauwkeurig moet worden opgevolgd, om beschadiging van het instrument te voorkomen.</i>
P	Caution (Atenção) <i>Significa que a instrução referida tem de ser respeitada para evitar a danificação do aparelho.</i>



WARNING

We use the term **WARNING** to inform you about situations that could result in **personal injury** to yourself or other persons. Details about these circumstances are in a box like this one.

D

Warning (Warnung)

Bedeutet, daß es bei Nichtbeachten der genannten Anweisung zu einer **Verletzung** des Benutzers kommen kann.

DK

Warning (Advarsel)

Betyder, at brugeren kan blive **kvæstet**, hvis anvisningen ikke overholdes.

E

Warning (Peligro)

Utilizamos el término **WARNING (PELIGRO)** para informarle sobre situaciones que pueden provocar **daños personales** a usted o a otras personas. En los recuadros como éste se proporciona información sobre este tipo de circunstancias.

F

Warning (Danger)

Nous utilisons la formule **WARNING (DANGER)** pour avertir des situations pouvant occasionner des **dommages corporels** à l'utilisateur ou à d'autres personnes. Les détails sur ces circonstances sont données dans un encadré semblable à celui-ci.

I

Warning (Pericolo)

Con il termine **WARNING (PERICOLO)** vengono segnalate situazioni che potrebbero provocare **incidenti alle persone**. Troverete informazioni su tali circostanze in un riquadro come questo.

NL

Warning (Waarschuwing)

Betekent dat, wanneer de genoemde aanwijzing niet in acht wordt genomen, dit kan leiden tot **verwondingen** van de gebruiker.

P

Warning (Aviso)

Significa que a não observância da instrução referida poderá causar um **ferimento** ao usuário.

Definitions

OPERATOR: Person operating the equipment for its intended purpose.

RESPONSIBLE BODY: Individual or group responsible for the use and maintenance of the equipment and for ensuring that the **OPERATORS** are adequately trained.

Related Documents

Manuals for Spectrometers

Information on using your spectrometer can be found in the manuals that were supplied with it. The *IR & Raman Manuals CD* (L1050002) includes instrument manuals for the Frontier IR Systems, Spectrum 400 Series and Spectrum 100 Series spectrometers.

SpectrumIMAGE Help and Tutorials

In addition, the SpectrumIMAGE software has on-screen Help, which you can access by selecting **Contents** from the Help menu in each SpectrumIMAGE window or by clicking the **Help** button in a dialog.

This is a reference manual, which assumes that you are familiar with using the imaging system. Work through the on-screen tutorials, which you can access by choosing **Learning** from the Help menu on the main SpectrumIMAGE window.

Requirements for using the Imaging System

We assume that the imaging system has been properly set up and aligned. This installation must be performed by a PerkinElmer Service Engineer.

Connecting the PC to the Local Area Network

The PC provided is connected point-to-point to the imager. If you wish to connect the PC to your local area network to enable transfer of data, we recommend that you contact your local PerkinElmer Service Engineer.

Warnings and Safety
Information

The Spotlight Imaging Systems

The dual-range Spotlight 400 imaging system consists of an FT-IR imager, filter box, dual-range spectrometer (Frontier FT-IR/FT-NIR or Spectrum 400 FT-IR/FT-NIR), PC, stage controller and joystick.

NOTE: The Spotlight 400 imaging system with a Frontier FT-IR/FT-FIR or Spectrum 400 FT-IR/FT-FIR spectrometer operates in the MIR range only. The system does not include a filter box.

The Spotlight 400 imaging system consists of an FT-IR imager, an FT-IR spectrometer (Frontier FT-IR, Spectrum 100 FT-IR or Spectrum One FT-IR), PC, stage controller and joystick.

The Spotlight 400N imaging system consists of an FT-NIR imager, an FT-NIR spectrometer (Frontier FT-NIR, Spectrum 100N FT-NIR or Spectrum One NTS FT-NIR), PC, stage controller and joystick.



Figure 1 Spotlight 400 – Microscope and Frontier IR System

Summary

The PerkinElmer Spotlight 400 series imaging systems have been designed to comply with a wide variety of international standards governing the safety of laboratory equipment. In routine use, the imaging system poses virtually no risk to you. If you take some simple, common sense precautions, you can make sure that you maintain the continued safe operation of the imaging system.

- DO make sure that all parts of the Spotlight imaging system are properly connected to the electrical supply; in particular, make sure that the ground (earth) wires are securely connected.
- DO disconnect the electrical power supply before opening the main cover of the imager or the spectrometer.
- DO keep the imager dry. Avoid spilling liquid into the imager and spectrometer. Clean all external spills immediately. If anything that is spilled enters the main body of the imager or spectrometer, switch off the power and call a PerkinElmer Service Engineer.
- DO wear safety glasses and protective gloves when you are filling the MCT detector dewar in the imager with liquid nitrogen. Slowly pour the liquid nitrogen into the dewar. Stand back from the detector during filling because liquid nitrogen may be expelled from the dewar flask. Use only liquid nitrogen.
- DO NOT stare into the laser beam. Laser radiation will be present from the spectrometer. The spectrometer uses a low-power visible (red) laser and momentary exposure to the beam is not dangerous, but deliberate, direct viewing of the beam along its axis could damage your eyes.
- DO NOT use a flammable gas to purge the spectrometer or imager. The spectrometer contains a hot lamp, and fire or explosion may result. Use only clean, dry oil-free nitrogen or air to purge the instrument.
- DO read the more detailed information on safety in the following pages.

General Operating Conditions

For information about the Spectrometer, refer to the User's Guide supplied with the instrument. The *IR & Raman Manuals CD* (L1050002) includes instrument manuals for the Frontier IR Systems, Spectrum 400 Series and Spectrum 100 Series spectrometers.

Only use the imager and stage controller indoors and under the following conditions:

Temperature 15 °C to 35 °C

Relative humidity 80% maximum (non-condensing)

If possible, avoid any adjustment, maintenance or repair of the opened, operating instrument. If any adjustment, maintenance or repair of the opened instrument is necessary, this must be done by a skilled person who is aware of the hazard involved.

Whenever it is likely that the imager and stage controller are unsafe, make them inoperative. The imager and stage controller may be unsafe if they:

- Show visible damage;
- Fail to perform the intended measurement;
- Have been subjected to prolonged storage in unfavorable conditions;
- Have been subjected to severe transport stresses.

The imager and stage controller have been designed to be safe under the following conditions:

- Indoor use;
- Altitude up to 2000 m;
- Ambient temperatures of 5 °C to 40 °C;
- A maximum ambient relative humidity of 80% for temperatures up to 31 °C decreasing linearly to 50% relative humidity at 40 °C;
- Mains fluctuations not exceeding $\pm 10\%$ of the nominal voltage.



WARNING

If the equipment is used in a manner not specified herein the protection provided by the equipment may be impaired.

Electrical Safety

The imager and stage controller have been designed and tested in accordance with PerkinElmer specifications and in accordance with the safety requirements of the International Electrotechnical Commission (IEC). The imager and stage controller conform to IEC 61010-1 (Safety Requirements for electrical equipment for measurement, control, and laboratory use) as it applies to IEC Class 1 (earthed) appliances and therefore meet the requirements of EC low voltage directive 2006/95/EC.

The imager and stage controller must only be connected to equipment meeting the requirements of IEC 61010-1 (Safety requirements for electrical equipment for measurement, control and laboratory use – general requirements) or IEC 60950 (Safety of information technology equipment).

Connect the imager and stage controller to a power supply line that includes a switch or other adequate means of disconnection from the electricity supply.

Plug the imager and stage controller only into electricity supply sockets that are provided with a protective ground (earth) connection. The stage controller and the imager must be earthed.



WARNING

Any interruption of the protective ground (earth) conductor inside or outside the imager or stage controller or disconnection of the protective ground (earth) terminal can make the imager dangerous.

If fuses need replacing, use only those with the required current rating and of the specified type. Do not use makeshift fuses and do not short-circuit fuse holders.

When the imager and stage controller are connected to its electricity supply, terminals may be live. Removing covers other than those which can be removed by hand is likely to expose live parts.

NOTE: There are no user-serviceable parts in the imager or the stage controller.

Capacitors inside the imager and stage controller may still be charged even if the imager or stage controller has been disconnected from all voltage sources.

Disconnect the imager and stage controller from all voltage sources before they are opened for any adjustment, replacement, maintenance or repair. Any adjustment, replacement, maintenance or repair must be performed by a PerkinElmer Service Engineer.

Location and Ventilation

To allow for adequate cooling

The imaging system is installed by a PerkinElmer Service Engineer, who will be able to advise on the siting of the system. To allow for adequate cooling, the system should not be sited near to room heating equipment, for example, central heating radiators. There should be a minimum gap of at least 15 cm (6 inches) from the top and side surfaces of the imager and stage controller to permit adequate cooling.



WARNING

Make sure that the switches at the electrical supply inlet at the rear of the imager and of the stage controller are not obstructed.

To allow nitrogen to disperse



WARNING

Liquid nitrogen is used to cool the MCT detector.

Oxygen depletion in an enclosed space does not trigger a gasping reflex, and errors of judgment, confusion, or unconsciousness can occur in seconds and without warning.

The imager may be fitted with an MCT detector that is cooled using liquid nitrogen. A risk assessment should include personal protection protocols, and proper actions in the event of accidental spillage. 1 L (2 US pints) of liquid nitrogen evolves to 700 L (approximately 25 cubic feet) of nitrogen gas, and the cold vapor can pool at floor level. The imager should only be used in a well-ventilated area.

Warning Labels on the Instrument



Caution, risk of electric shock.



Caution, Risk of Danger

When this label is attached to an instrument it means 'Caution, risk of danger'. Refer to the manual to find out the nature of the potential hazard and any actions that have to be taken.

Imager Safety Labels

Labels are fixed to the imager in the locations shown in Figures 2–5:

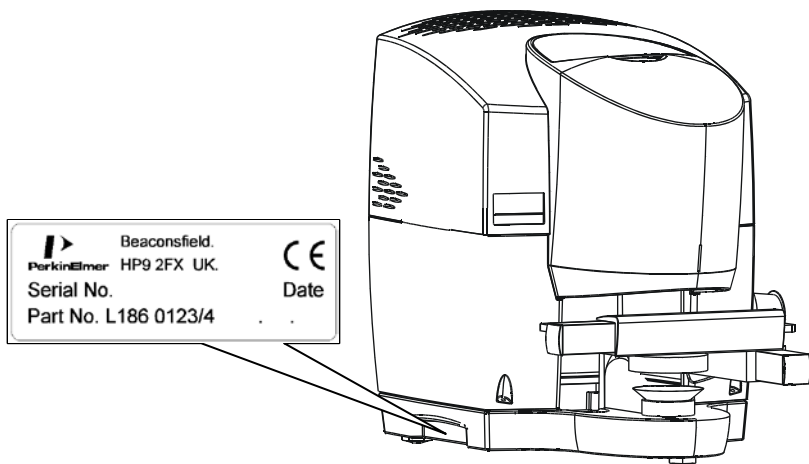


Figure 2 Label (side of imager)

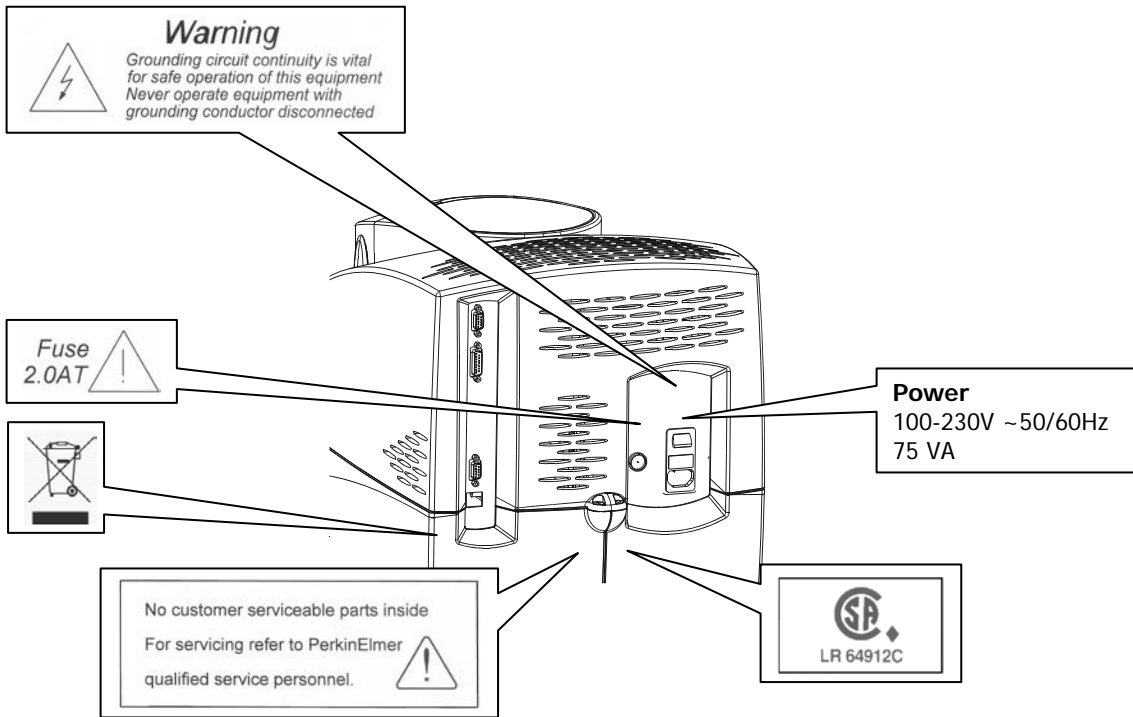


Figure 3 Labels (rear of imager)

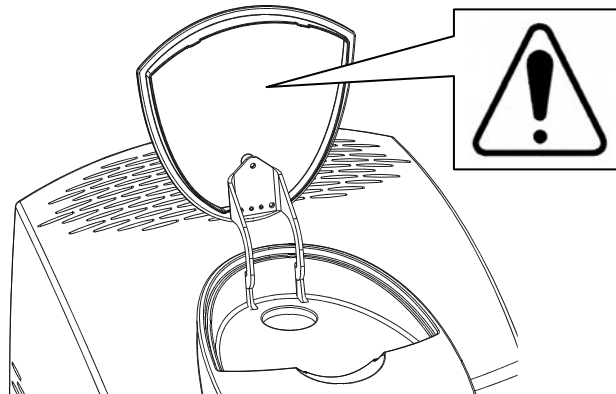


Figure 4 Label (inside dewar lid)

Refer to *Cooling the MCT Detector* on page 41.

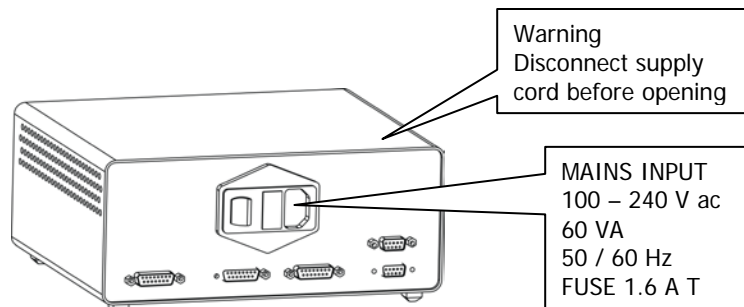


Figure 5 Label (rear of stage controller)

Mechanical Safety



WARNING

When you are using a motorized stage, do not place your fingers between the moving and fixed parts of the stage. The motors driving the stage from side to side, front to back, or up and down are powerful and do not stall easily.

Lifting the Spotlight Imaging System



The imager and spectrometer are heavy precision instruments, so two people are required for safe handling.

Consult the local codes of practice issued by safety advisors before attempting to lift these instruments.

Take care not to injure yourself or others, or to drop the instruments.

Do not move the imaging system after it has been installed without consulting your local PerkinElmer service department.

It is necessary to separate the imager from the spectrometer before lifting either instrument. If you have a dual-range imaging system, where a filter box is fitted between the imager and the spectrometer, it is necessary to remove the filter box before separating the imager from the spectrometer.

The imager weighs approximately 35 kg including the motorized stage (approximately 76 kg with packaging).

- Lift the imager only by the base.
- Do not attempt to lift the imager by the stage, cassegrain assembly or other attachments.

The spectrometer weighs approximately 34 kg unpacked (40 kg packed) and has a lifting recess on either side.

EMC Compliance

EC Directive

The Spotlight 400 series imaging systems have been designed and tested to meet the requirements of EMC Directive 2004/108/EC.

FCC Rules and Regulations

This product is classified as a digital device used exclusively as industrial, commercial, or medical test equipment. It is exempt from the technical standards specified in Part 15 of the FCC Rules and Regulations, based on Section 15.103(c).

System Requirements

Give attention to the following points before installing the imaging system.

Electrical Requirements

The power consumption of the imager does not exceed 75 VA.

The power consumption of the stage controller does not exceed 60 VA. The nominal power consumption of the spectrometer is 120 VA. The line supply must be within 10% of the nominal voltage.

If possible, do not connect any parts of the imaging system to circuits that have heavy duty equipment, such as large motors, connected.

If possible, do not use photocopiers, discharge lamps, radio transmitters, and other equipment with large or frequent transient loads, on the same supply circuit.

Imager

The imager can operate on electricity supplies of 50 or 60 Hz and on a voltage range of 100 to 240 V. The primary fuse (2 A T, 250 V, part number 04970839) for the imager is in the drawer on the mains inlet connector.

NOTE: No voltage selection is required.

Stage Controller

The stage controller can operate on electricity supplies of 50 or 60 Hz and on a voltage range of 100 to 240 V. No voltage selection is required. The primary fuse (1.6 A T, 250 V, part number 09991641) is located in a drawer on the mains inlet.

Site Requirements

Minimum bench dimensions of 170 x 75 cm (68 x 30 inches) are needed to accommodate the imager, filter box (if fitted), spectrometer, PC and ancillaries.

To get the best performance from your imaging system:

- Place the imaging system in an environment that is relatively dust-free.
- Make sure that the bench top is free from vibrations or mechanical shocks, and is flat and level.
- Do not place the imaging system near to room heating equipment, for example, central heating radiators.

Leave at least 15 cm (6 inches) from any vertical obstacle to the sides of the imager and stage controller, to permit an adequate flow of cooling air.

NOTE: Do not stand the stage controller on its side as this will cover some of the cooling holes.

Safety Specifications

Imager

Power supply	100–230 V \pm 10%, 50/60 Hz, \pm 10%
Primary fuse	2.0 A, time-lag
Weight	32 kg without the motorized stage

Stage Controller

Power supply	100–240 V \pm 10%, 50/60 Hz
Primary fuse	1.6 A T, (time-lag) The stage controller is not designed for use with all three motors simultaneously over extended periods.
Maximum speed	6 mm/s
Maximum motor voltage	24 V
Stepping resolution	Maximum 6400 steps/rev, 156 nm/step
Baud	9600
Travel	75 mm x 50 mm or 160 mm x 160 mm
Weight	Stage 2.7 kg Stage controller 2.8 kg



Overview of the Spotlight
Imaging System

A Guided Tour of the Spotlight Imaging System

The Spotlight 400 series imaging systems enable you to collect images from extremely small samples. The PerkinElmer cassegrain collection optics give high-performance infrared microspectroscopy. The imager includes a camera and viewing system that magnifies the visible-light image of the sample so that you can see, position, and isolate a point of interest. The SpectrumIMAGE software enables you to control the operation of your imaging system and to collect an image of the sample, which is displayed in the Monitor Visible window.

A range of detectors is available for the Spotlight 400 imaging system. The standard MCT (Mercury Cadmium Telluride) detector collects images over a spectral range of 7800 cm^{-1} to 710 cm^{-1} . The Spotlight with Long Wavelength option collects images over an extended range of 7800 cm^{-1} to 580 cm^{-1} . For work in the near-infrared region, the InGaAs (Indium Gallium Arsenide) detector offers improved performance between 7800 cm^{-1} and 3900 cm^{-1} and, unlike the MCT detectors, does not require cooling with liquid nitrogen.

The imager includes the following features:

- A high-performance cassegrain mirror system; this has a wide collection angle (high numerical aperture) and is highly efficient in collecting infrared radiation for microspectroscopy.
- Images or spectra can be collected using reflectance, diffuse reflectance or transmittance sampling modes; these have different detector gain settings to help optimize the quality of data from a variety of samples.
- Array and single point detectors for collecting either IR images or spectra from a single point.
- Internal coaxial LED illumination, with variable intensity.
- An automatic infrared aperture that closes to the size and rotation selected when you choose a scan or monitor command in Point mode.
- A motorized stage, controlled by a joystick and the SpectrumIMAGE software, that enables you to find points on your sample and focus the imager.
- SpectrumIMAGE software to control the operation of the imager; for example, selecting Image mode or Point mode, focus, illumination, stage position, adjusting the correction, and changing sampling modes.
- Auto-correction to optimize the position of the lower cassegrain for maximum energy throughput in Image mode.
- Dual-resolution IR imaging ($6.25\text{ }\mu\text{m}$ and $25\text{ }\mu\text{m}$).
- An optional micro-ATR objective for data collection from optically thick and non-reflective samples. (This is mainly applicable to mid-infrared applications.)
- Data collection, post-run processing and multi-dimensional graphic display facilities for IR and visible images.
- The same cassegrain is used to send both infrared radiation and visible light to the remote aperture. The imager continuously views and monitors infrared concurrently; there is no beampath switching between visible and infrared.
- The lower cassegrain can be removed. This provides space for thick samples to be studied in reflectance or diffuse reflectance mode.

Connections

Figures 6–9 show the input and output connections on the imager and stage controller.

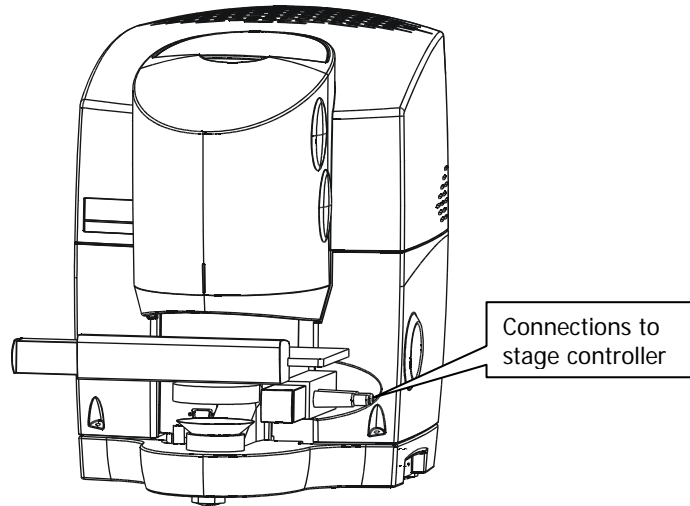


Figure 6 Connections to Stage Controller

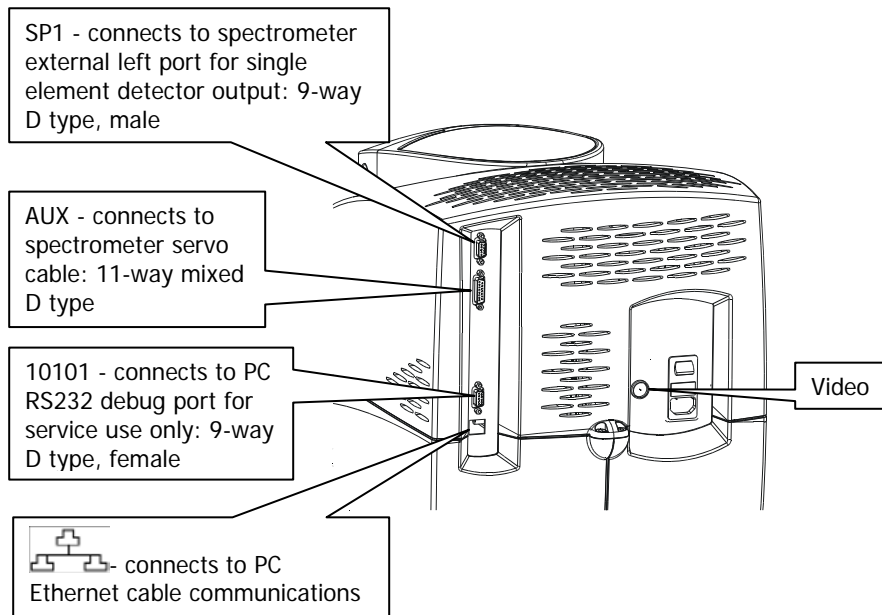


Figure 7 Electrical connections (top rear of imager)

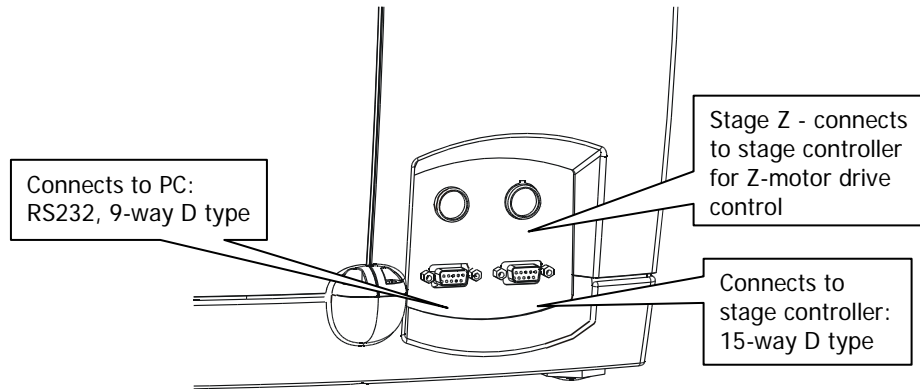


Figure 8 Electrical connections (bottom rear of imager)

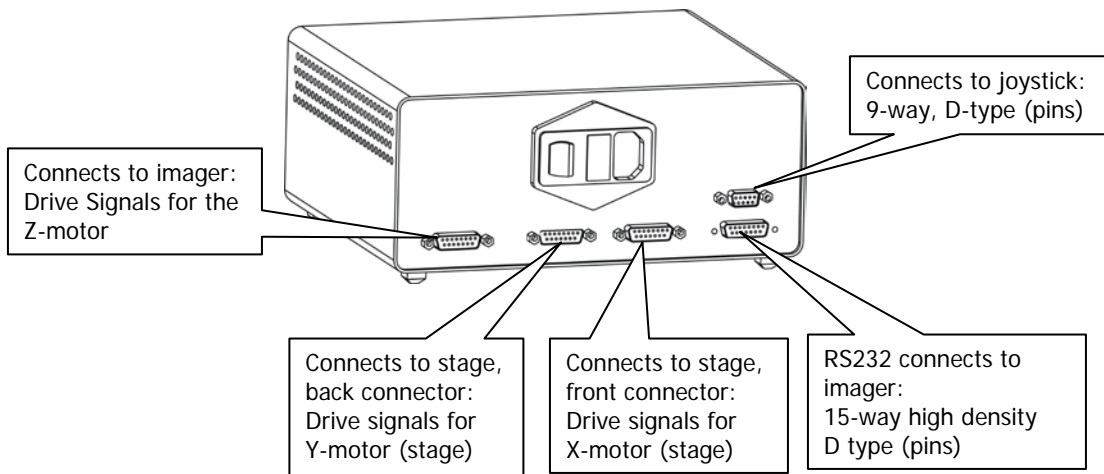


Figure 9 Stage Controller electrical connections

NOTE: If your system includes a filter box, see *Imager Connections* on page 105 for more information on the connections.

Modes of Operation

The imager has three main modes of operation, namely Image mode, Point mode, and ATR Imaging mode.

In Image mode you can quickly produce an IR image from a particular area of the sample. Simply indicate the area you want to scan by drawing a box around it, and then click a button to collect the IR image. From the image, individual spectra can be extracted for any point on the sample.

SpectrumIMAGE software also enables you to mark points of interest on the sample. In Point mode, you can collect individual spectra (either at the current aperture position or at positions you have marked) with or without an ATR objective. You can also collect spectra at regularly spaced intervals along a line (line-scanning) or within a marked area (mapping).

NOTE: Point mode is not available if you are using the Spotlight with Long Wavelength option.

The ATR Imaging mode is only available to users of the *Spotlight ATR Imaging Accessory* (part number L1860320). It allows you to collect data from an area of the sample surface with a spatial resolution up to four times better than conventional reflection or transmittance IR imaging techniques.

Mode		Samples
Image mode	Transmittance	Thin solids, fibers, films
	Reflectance	Polished or smooth surfaces
	Diffuse Reflectance	Rough or poorly reflecting surfaces, powders
Point mode	Transmittance	Single areas between 10 μm and 100 μm of thin solids, fibers and films
	Reflectance	Single areas between 10 μm and 100 μm of polished or smooth surfaces
	Diffuse Reflectance	Single areas between 10 μm and 100 μm of rough or poorly reflecting surfaces and powders
	With ATR objective	Non-reflecting sample surfaces (use with care on hard surfaces)
ATR Imaging mode	With ATR Imaging Accessory	Single rectangular areas (sides typically between 25 μm and 500 μm) of a rough or poorly reflecting sample surface

NOTE: Diffuse reflectance mode increases the gain of the detector to compensate for the loss of signal from poorly reflecting surfaces. This may cause saturation of the detector in some circumstances, in which case you should use the reflectance mode.

For further information on each of these modes, see the SpectrumIMAGE software on-screen Help.

The Optical System

The imager has optics for infrared microspectroscopy and visible light. A video camera enables you to view the sample. The two systems intersect at the aperture. When a sample on the sample stage is in focus, its conjugate image is focused at the remote aperture.

You can choose between transmittance, reflectance or diffuse reflectance operation. Reflectance and diffuse reflectance modes use the same optical system. This section describes what happens within the optical system when the imaging system changes from visible light to infrared mode in transmittance and reflectance/diffuse reflectance operation.

Dichroic mirrors form part of the optical system. The dichroic mirrors used in the imager reflect infrared and transmit visible light.

Viewing

When you view a sample with the video camera, you are really looking at the conjugate image of the sample, located at the remote aperture (Figure 10).

The Z-control on the joystick enables you to focus the optical image. This control moves the sample position up and down until the conjugate image is focused at the remote aperture.

The joystick also enables you to move the position of the sample so that the area of interest is in the center of the field of view. The automatic infrared aperture then enables you to isolate an area of interest in Point mode. In Image mode the apertures are not needed.

Viewing in Transmittance

When the imager is in viewing mode in transmittance (Figure 10):

- The lower fold mirror, beneath the cassegrains, receives light from the LED source and directs it up through the lower dichroic mirror onto the lower cassegrain.
- The lower cassegrain condenses the beam to an appropriate size for a microscopic sample and focuses it at the sample position.
- The upper cassegrain collects light from the sample and sends it upward through the aperture and the upper dichroic mirror.

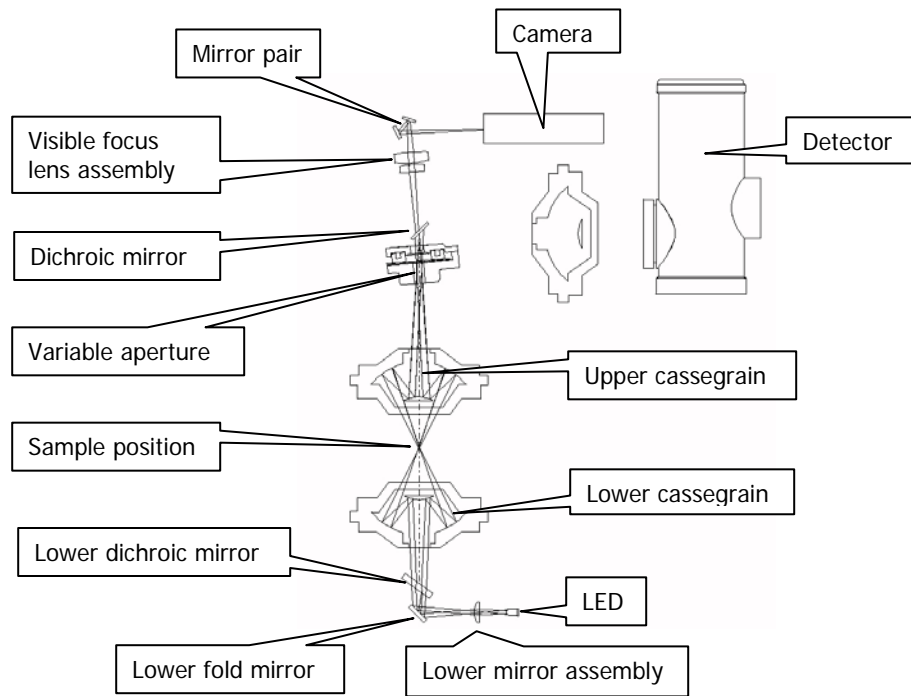


Figure 10 Path of the visible beam for viewing a sample in transmittance

Viewing in Reflectance/Diffuse Reflectance

When the imager is in viewing mode in reflectance or diffuse reflectance (Figure 11):

- The reflectance illuminator assembly directs light from the upper LED down through one side of the cassegrain onto the sample.
- The upper cassegrain collects the reflected light from the sample and sends it upward through the aperture.

The lower 'transmission' illuminator is also active in reflectance and diffuse reflectance modes, giving simultaneous illumination from above and below.

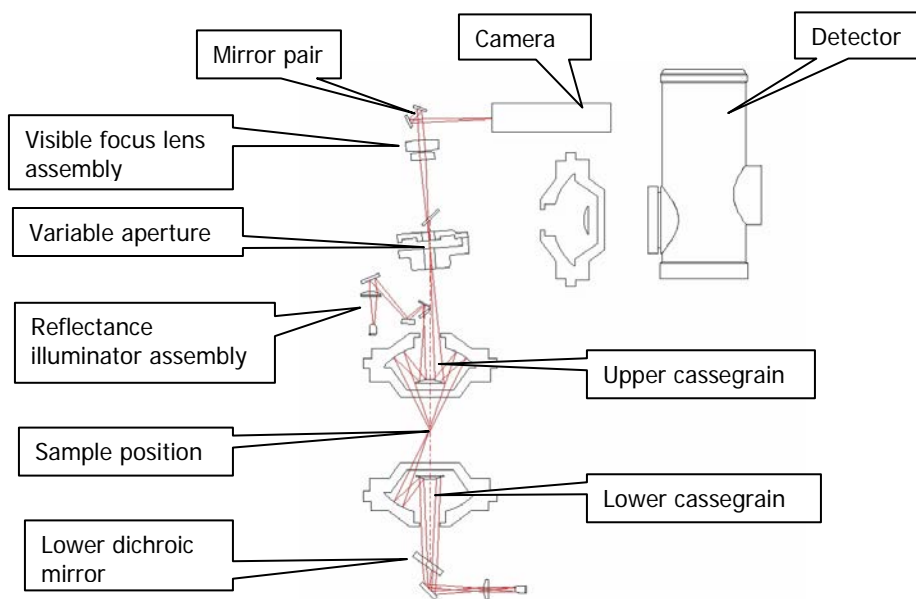


Figure 11 Path of the visible beam for viewing a sample in reflectance/diffuse reflectance

Collecting an Image

The upper cassegrain is used for both visible light and infrared radiation. For this reason, when you adjust the sample position so that the visible image of the sample is in focus, the sample is also correctly positioned for collecting the infrared image. Similarly, when you adjust the aperture in Point mode so that the required area of the sample is isolated visually, you have also isolated the area of the sample from which the image is to be collected. In Image mode the apertures are not used to isolate the sampling area, and the visible image is used to determine the dimensions of the IR image.

Collecting an Image in Transmittance

Collecting an image in transmittance differs from viewing in transmittance as follows (Figure 12):

- Instead of receiving light from the illuminator, light from the spectrometer is reflected off the toroid onto the lower dichroic mirror which sends it through the lower cassegrain.
- The upper dichroic mirror reflects the beam onto the detector cassegrain.
- The detector cassegrain focuses the beam on to the detector.

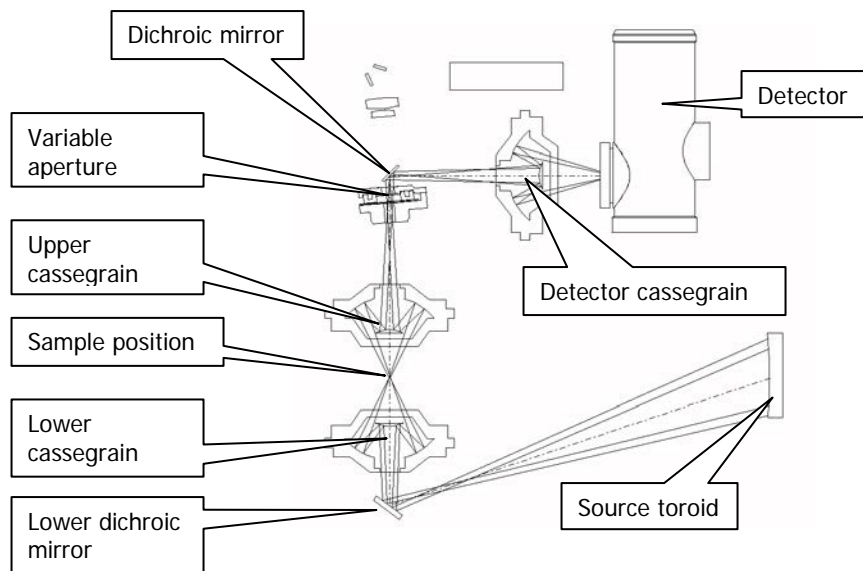


Figure 12 Path of the infrared beam for collecting an image in transmittance

Collecting an Image in Reflectance/Diffuse Reflectance

Collecting an image in reflectance or diffuse reflectance differs from viewing as follows (Figure 13):

- The toroid moves to send the beam to the Reflectance illuminator assembly dichroic mirror, which sends it through the upper cassegrain.
- The beam is reflected off the sample and back through the other side of the cassegrain, toward the remote aperture.
- The detector cassegrain focuses the beam on to the detector.

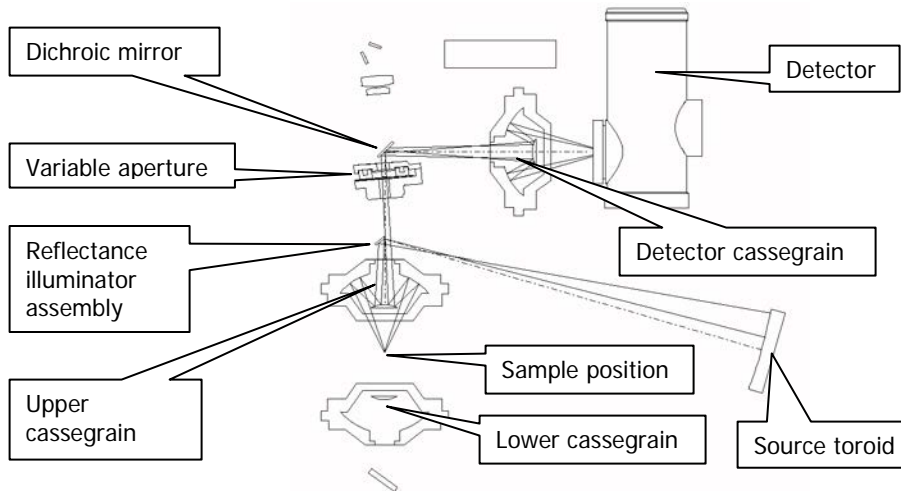


Figure 13 Path of the infrared beam for collecting an image in reflectance/diffuse reflectance

Changing the IR Magnification

Between the upper cassegrain and the variable aperture there is a mechanism for changing the IR magnification. This is not shown in Figure 10 to Figure 13 for simplicity, as for visible viewing and for low IR magnification, it is not used. When changing from low IR magnification, which gives a pixel size of $25\ \mu\text{m}$, to high IR magnification, which gives a pixel size of $6.25\ \mu\text{m}$, the mechanism rotates to place a series of magnifying mirrors in the IR beam.

Spotlight Requirements

A Spotlight 400 series imaging system requires driver software and may require an additional card to be installed in the PC. If you encounter a problem with the PC, contact a PerkinElmer Service Engineer. Do not attempt to remove the card from the PC or install the software on another PC.

The SpectrumIMAGE software is installed in conjunction with Spectrum software.

Hardware Requirements

The PC you install the software on must meet the following specification:

- Intel® Pentium 4, 2 GHz processor (or greater) – dual-core or hyper-threaded preferable
- 40 GB Hard disk with at least 1 GB free space as an NTFS drive

NOTE: We have locked the system into using an NTFS drive because the alternative FAT32 file system doesn't provide enough protection at a folder and file level to ensure that users and groups of users cannot delete or amend data files, while at the same time being able to create new data files.

- At least 3 GB of Random Access Memory (RAM)
- A graphics card with an ATI chip-set with the capability of displaying 32-bit color at a resolution of 1280 x 1024, with a refresh rate of 75 Hz
- DVD drive
- A keyboard and PS/2®-style mouse
- Serial (RS232) port – for stage control box (systems with motorized stages only)
- 1 free PCI slot
- 2 network ports
- USB port

NOTE: If you should need to change the PC we recommend that you contact a PerkinElmer Service Engineer about PC configurations.

Software Requirements

All Spotlight 400 imaging systems are supported by SpectrumIMAGE version 1.6 or later, which requires that a 32-bit operating system is installed on the PC, either Windows® XP Professional Service Pack 3, or later, or Windows® 7 Professional Service Pack 1, or later.

Internet Explorer

Internet Explorer 6 or later is used for displaying the HTML help pages.

QuickTime

QuickTime is used to run the animations in the tutorials and on-screen Help. QuickTime was installed on your PC during installation of SpectrumIMAGE software.

Spectrum

Spectrum software must be installed before SpectrumIMAGE.

Getting Ready to use the
Imaging System

Before Using the Imaging System

Before you use the Spotlight 400 series imaging system you must:

- Make sure the spectrometer is switched on;
- Switch the stage controller and imager on;
- Cool the detector (if using an MCT detector; the InGaAs detector does not require cooling);
- Set up the imager;
- Focus the imager.

We recommend that you use these procedures at the beginning of the day's work, or any time the imaging system has not been in use or has been used by others.

Cooling the MCT Detector

When an MCT (Mercury Cadmium Telluride) detector is fitted in the imager, it must be cooled to 77 K before you collect spectra. It is mounted in a dewar that can be filled with liquid nitrogen. As you fill the dewar, the temperature of the detector drops, and the preamp supplies power to the detector. Use the following procedure to cool the MCT detector.



WARNING

Do not site the instrument in a poorly ventilated area.

Oxygen depletion in an enclosed space does not trigger a gasping reflex, and errors of judgment, confusion, or unconsciousness can occur in seconds and without warning.

See Location and Ventilation on page 18.



WARNING

The extremely low temperature of liquid nitrogen can burn skin and eyes. Avoid exposure by wearing heavy gloves and safety goggles whenever you work with it.



WARNING

When liquid nitrogen warms to room temperature, nitrogen gas vaporizes so rapidly that resulting pressures can send a funnel or detector cap suddenly and forcefully shooting upward from the top of the imager.



WARNING

Be sure to wait the specified time when filling the funnel and before replacing the detector cap. This enables the bubbling nitrogen to settle down and the pressure to dissipate. In addition to wearing safety goggles at all times, stand back from the imager after each time you fill the funnel.

Open the flap covering the dewar.

1. Remove the dewar cap.
2. Place the small funnel supplied with the imager in the opening in the detector dewar (Figure 14).

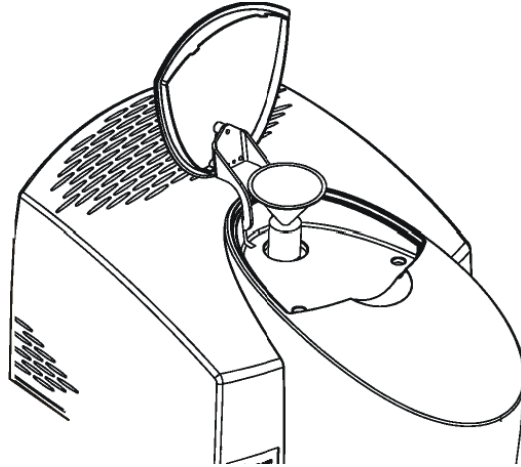


Figure 14 The dewar opening with the funnel inserted

CAUTION

Stand where you can see the inside of the funnel as you pour the nitrogen in. Pour slowly, so that neither the funnel nor the dewar overflows. If liquid nitrogen runs down the outside of the dewar, it can damage the optics of the imager.

3. Carefully fill the funnel with liquid nitrogen. Stand back and let the funnel empty completely.
The liquid nitrogen bubbles rapidly as it drains into the dewar. This first amount of liquid nitrogen vaporizes completely as it cools the dewar.
4. Add another one and a half funnels of liquid nitrogen. Stand back and wait two minutes.
This nitrogen also vaporizes as the dewar continues to cool. The two minute wait enables the bubbling to settle down and the pressure of the vaporizing nitrogen to dissipate.
5. Continue to pour liquid nitrogen into the funnel, adding a little more each time the funnel empties.
The funnel takes longer to empty as the dewar fills. This happens after two to three more funnels of liquid nitrogen. Because the dewar has now cooled, the liquid nitrogen does not vaporize, but instead fills the dewar.
6. Remove the funnel and wait two minutes.
The liquid nitrogen settles down and bubbling slows.
7. When the nitrogen stops bubbling, refit the detector cap.
The filled dewar cools the MCT to the correct operating temperature for several hours. After that, the dewar begins to return to room temperature, and the preamp switches off power to the MCT.

Setting up the Imaging System

CAUTION

For information about setting up your Spotlight 400 series imaging system for use in ATR Imaging mode, refer to the Spotlight ATR Imaging Accessory User's Guide (part number L1050048) on the Spectrum Manuals CD (part number L1050002).

To avoid damaging the instrument, it is essential that you remove any accessories, such as single-point ATR objective, and the lower cassegrain (as described in Collecting the Image of a Thick Sample on page 66) BEFORE you select ATR Imaging mode.

To set up your Spotlight 400 series imaging system for use in Image mode or Point mode:

1. Make sure the spectrometer is switched on.
It can take the spectrometer up to two hours to equilibrate after being switched off overnight.
2. Switch on the imager at the rear switch.
The blue LED light at the front comes on.
3. Switch on the stage controller.
A light at the front comes on.
4. At your PC, click **Start** and then select **SpectrumIMAGE** in the **SpectrumIMAGE** group in the **PerkinElmer Applications** group under **Programs**.
The SpectrumIMAGE software starts. When the start-up animation finishes a dialog is displayed, which may require login details.
5. Enter your **Name**.
6. Select the **Sampling Mode** from the drop-down list.

OR


Select **Work offline** if you just want to work with previously collected data.
7. Make sure that the lower cassegrain is fitted, that if the ATR objective is fitted it is retracted, and that there is no sample on the stage, then click **OK**.
The imager initializes.

Focusing the Imager

The focus of the imager is changed by moving the sample stage up or down. The Z-control on the joystick, or the Focus control on the Control dialog, enables you to focus the image in the Monitor Visible window.



The default position for the lower cassegrain gives optimal illumination for thin samples. For optimal performance it may be necessary to refocus the lower cassegrain to compensate for the sample's refractive index.

Focusing the Visible Image

1. Click  in the Stage Control window to move the sample stage to the Load position.
2. Place your sample on the sample stage.
3. If you want to examine the sample in reflectance mode, click **Reflectance** on the Control dialog.
4. From the GoTo menu in the Stage Control window, select Center of Stage.
The sample stage moves so that the center of the sample stage is illuminated.
5. Use the Z-control on the joystick to focus on the sample.
You may need to change the illumination of the sample, or the correction.

OR

In the Focus section of the Control dialog, click **Auto**.
The image of the sample is focused.

6. If you need fine focus, use the **Focus** control on the Control dialog.
7. If you are examining the sample in transmission mode, you may need to adjust the position of the lower cassegrain so that the field of view is evenly illuminated:
Adjust the **Correction** control by clicking on the up () or down () buttons.

OR

Select **Auto** in the Correction section of the Control dialog.
This is especially useful if the sample is in a compression cell or held between windows of high refractive index.

NOTE: If your Spotlight 400 is supplied with an InGaAs detector and you are working in transmittance at low magnification (large pixels), the SpectrumIMAGE software may report an overload error when monitoring an IR function or collecting data. To resolve this, fit a 32%T attenuator to the external beam window of the spectrometer. See *Fitting an Attenuator to the Spectrometer* on page 45 for details.

This attenuator should be kept in place during data acquisition.

8. Adjust the **Illumination** on the Control dialog to give good illumination.

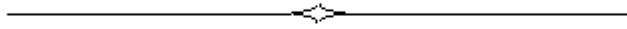
Fitting an Attenuator to the Spectrometer

If your Spotlight 400 system is supplied with an InGaAs detector, you will be provided with an Attenuator kit (part number L1160560) containing 1%T, 4%T, 6%T, 14%T and 32%T attenuators. The attenuators attach magnetically to the external beam window of the spectrometer.

Use of the 32%T attenuator is recommended if an overload error message is displayed when using a large aperture. This attenuator should be kept in place during data acquisition.



Figure 15 KBr Window with 14%T Attenuator



Preparing Samples

Sample Preparation

The Spotlight imaging system enables you to examine the sample in the Monitor Visible window and to select the area where you want to collect images or spectra. To make sure that you collect good quality spectra, it is important that you prepare samples properly. Sample preparation is needed when the sample is too thick for transmission work, or the area of interest is on the inside (for example in a laminated sample).

- If you are going to collect transmission spectra, the sample should ideally be thin enough (approximately 5 to 20 μm) to give good detail and undistorted absorption bands.
- The area of the sample must be large enough to give an adequate signal or the scan time must be increased.
- Preparing a sample, therefore, often involves flattening it; this both thins it and increases its area. The sample can be flattened by rolling, squeezing, or pressing.

This chapter tells you how to prepare samples for spectroscopy with the Spotlight imaging system. It includes:

- A list of useful tools;
- A list of window materials commonly used for mounting samples;
- Descriptions of special techniques used to prepare particular types of samples.

If you are not familiar with the techniques described here we recommend that you work through the SpectrumIMAGE on-screen tutorials, which you can access by selecting **Learning** from the Help menu on the main SpectrumIMAGE window.

Tools for Sample Preparation

This section lists the tools you need for preparing samples:

- Tools provided with the imaging system;
- Tools in the microsampling toolkit;
- Materials to have available;
- Specialized accessories you may want to purchase.

Tools Provided with the Imaging System

The following items for use in sampling are provided in the Sampling Accessories Kit that is supplied:

Item	Use
Holder for 13 mm disks	Supporting 13 mm disks on the sample stage.
Slides, glass (box)	Supporting samples for sample preparation.
Rotating 13 mm disk holder	Supporting samples and allows rotation of the sample.
Support for large samples	Clips on to the sample stage. Supporting bulky samples so that the stage clip does not interfere with them.
Gold mirror slide	Background sample for measurements in reflectance mode.
Gold mirror and white reflector slide	Background sample for measurements in reflectance and diffuse reflectance modes (supplied with InGaAs systems).
KBr windows (2)	Supporting samples.

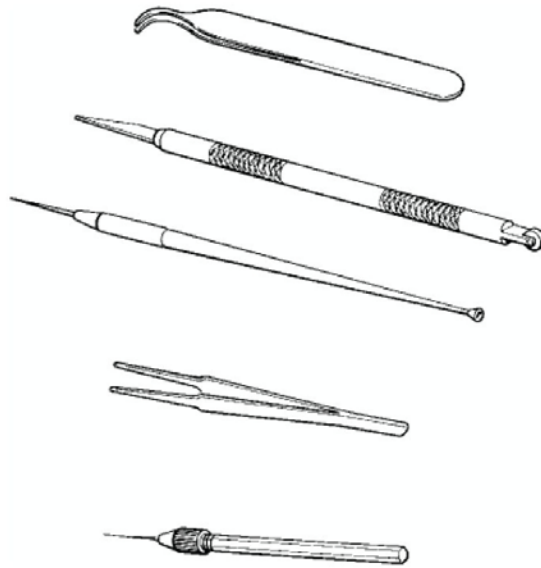


Figure 16 Some of the tools in the microsampling toolkit

The following tools are provided in the Microsampling toolkit:

Tool	Use
Steel tweezers	Picking up extremely small objects
Roller knife	Cutting (knife end) and flattening (roller end)
Steel probe	Pulling samples apart, separating fibers
Forceps, 4½ inch, Cd plated	Picking up small objects
Tungsten alloy needle	Transferring particles
Pin vise	Holds needles for sharpening or for flattening samples
Interchangeable handle for micro tools	Handle for tungsten needle or steel probe

Other Useful Tools

Depending on the type of samples that you usually work with, it may be helpful to have some of the following tools:

Tool	Part Number
Microprobe with right angle bend	N9302606
Forceps, round tips	N9302607
Forceps, narrow needle points	N9302608
Windows (all 13 mm diameter):	
BaF ₂ (1 mm thick)	N9302611
BaF ₂ (2 mm thick)	N9302612
ZnSe (1 mm thick)	N9302613
NaCl (2 mm thick)	N9302614
KBr (2 mm thick)	N9302615
Wide-tipped forceps, hooked	09908138
Wide-tipped forceps, flat	09908400
1.5 mm microdisk. Fits in 13 mm disk holder to support very small samples.	01861043

These items are available from your local PerkinElmer sales office or agent.

Specialized Accessories

The following accessories are extremely useful in preparing certain types of samples (as described in *Techniques for Preparing Samples*, starting on page 54):

- Miniature Diamond Anvil Cell (part number N9302618);
- Fiber Optic Illuminator (part number N9302602);
- Microtome.

Items to Have Available

In addition to the items provided with the Spotlight imaging system, we recommend that you have the following available:

- Tape with adhesive on both sides (“double-sided tape”) for holding long or large samples on the sample stage;
- Single-edge razor blades for cutting samples.

Common Window Materials

For work in the mid-infrared using the Spotlight 400 imaging system, both liquid and solid samples are often mounted on salt windows. Very thin windows, 1 to 2 mm thick, give the best spectra. The following materials are commonly used in windows:

- KBr: Potassium bromide is inexpensive, and it transmits infrared radiation to below 400 cm^{-1} . The major disadvantage of this material is that it is hygroscopic, so that the windows fog easily.
- BaF₂: Barium fluoride is not hygroscopic. Its transmittance cut-off is 750 cm^{-1} . It can break or crack easily.
- NaCl: Sodium chloride transmits infrared down to 600 cm^{-1} . Otherwise, its properties are similar to KBr.
- ZnSe: Zinc selenide is not hygroscopic. Its transmittance cut-off is 650 cm^{-1} . ZnSe is more durable than the other windows but is yellow, so that the field of the Monitor visible window appears yellow.

The measuring range of the Spotlight 400 system in Image mode is 7800 cm^{-1} to 710 cm^{-1} . With the Long Wavelength option, this range becomes 7800 cm^{-1} to 580 cm^{-1} .

The Spotlight 400N FT-NIR imaging system has a range of $7800\text{--}2400\text{ cm}^{-1}$ (with an MCT detector) or $7800\text{--}3900\text{ cm}^{-1}$ (with an InGaAs detector). For most near-infrared work in the region $7800\text{--}4000\text{ cm}^{-1}$, samples can be mounted on standard quartz microscope slides.

Techniques for Preparing Samples

This section describes some useful techniques for preparing various types of samples.

Flattening Solids

Flattening samples by pressing or squeezing often enables you to make thick samples thin enough to give good infrared spectra. Because the samples are usually quite small, only moderate force is necessary.

Rolling with the roller knife

The roller end of the roller knife provided in the microsampling tool is one of the simplest and most effective devices for flattening samples. It is especially useful for flattening fibers or particles.

You can treat different types of sample in different ways:

- If the sample is soft, you can roll it on a small salt window.
- If the sample is hard, you can roll it on a hard surface, such as glass or metal. A flat, black cap from a jar makes a good surface for rolling a light-colored sample.
- If you roll the sample on a small, flat piece of metal you can view it and collect spectra in reflectance or diffuse reflectance mode. Samples rolled on windows transparent to infrared can be examined in transmittance.
- If you flatten fibers on a glass microscope slide, they can then be peeled off and mounted either on a window or over the aperture for the microscope slide.

Squeezing with a pellet press

You can squeeze samples between the polished anvils of a KBr pellet press without KBr. To collect the spectra use one of the following methods:

- Peel the flattened sample off the anvil with a probe or knife and place it on a sample mount. Collect the transmittance spectrum.
- Leave the sample on the polished anvil and collect the reflection spectrum. Use a clean area of the anvil as the reference.

Using a diamond anvil cell

See the procedure in *Polymers* (page 56) for information on flattening samples with a miniature diamond anvil cell.

Compressing between infrared transmitting windows

Pressing two windows together, with the sample between them, compresses the sample. This also provides optical contact between the windows and the sample, reducing surface scattering.

Windows made of NaCl or KBr are relatively soft. If your sample is hard, or if it is wet, use BaF₂ or ZnSe.

Pressing with the heel of a probe

Press on small samples with the flat end of the probe handle. Even moderate pressure usually produces considerable thinning.

Pressing with a needle

Pressing with the point of a needle or probe applies a high force per unit area, because the area of contact is small.

Rolling a hard sample with the side of a sewing needle held in a pin vise presses it into a flake.

Slicing Samples from Solids

Cutting a wedge of sample

Cutting a wedge-shaped piece from its edge enables you to produce a thin sample while destroying very little of the original. This technique can be used with laminates, plastics, films, paint chips and paper.

To cut a wedge-shaped piece from a relatively thick sample:

- Hold the sample in tweezers as you slice a thin wedge from it with a razor blade. Taper the wedge to as thin a slice as possible.

To cut a wedge-shaped piece from a relatively thin sample:

1. Place the sample between two offset glass slides. Allow a triangular portion of the sample to protrude as shown on the left in Figure 17.

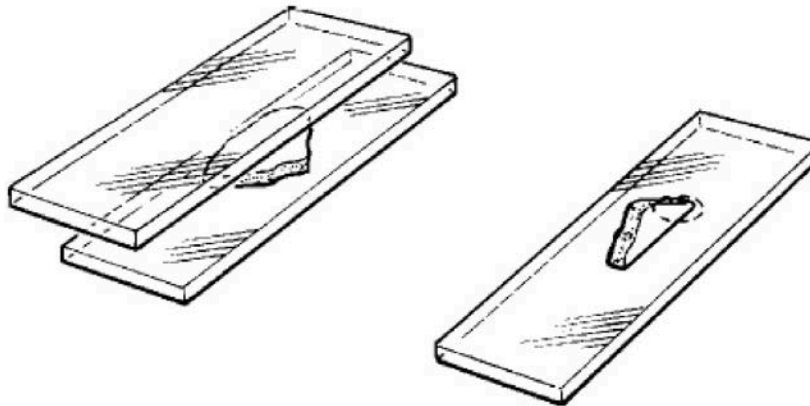


Figure 17 Cutting a wedge-shaped sample

2. Run a razor blade or the roller knife along the edge of the upper slide. The triangular piece of the sample is sliced off, giving a wedge-shaped sample.
3. To mount the sample, rotate it so that it is positioned as shown on the right in Figure 17.
4. Position it under the imager so that the infrared beam goes through the thin end of the wedge.

Microtoming

A microtome enables you to slice a sample into thin cross-sections, 0.5 to 20 μm thick. It is commonly used to prepare samples for light microscopy; the same range of thicknesses is also appropriate for infrared microspectroscopy.

If you are trying to identify the individual components of a laminate, microtomed samples give the best results.

Samples are often embedded in a supporting medium before they are microtomed. If you must use an embedding material, choose it carefully, so that it does not alter the sample by reacting with it, dissolving it, or contaminating it. Some commonly used materials are:

- **paraffin wax:** This is the preferred medium for infrared spectroscopy. It produces few spectral interferences, and it can usually be easily removed from the sample with warm xylene.
- **β -pinene wax:** This material is similar to paraffin.
- **plastic embedding materials:** These can be used depending on the size and porosity of the sample.
- **acrylic and epoxy resins:** Although these are commonly used in light microscopy, they are not recommended for infrared, because they are hard to remove and can cause spectral interferences.

Polymers

Pressing or squeezing enables you to reduce the pathlength of polymer samples such as paint chips, thick films, elastomers, or fibers.

Diamond anvil cell

The miniature diamond anvil cell, shown in Figure 18, enables you to press polymers (or other compressible samples). It enables you to both thin the sample and collect its spectrum in the same device; this is an advantage when you have limited material available. It is small enough to be easy to manipulate, and fits in the recessed retainer in the support for large samples. By collecting a background spectrum of an empty area of the cell, you can completely compensate for the absorption bands of the diamonds.

To thin a sample in the miniature diamond anvil cell:

1. Loosen and remove the three screws that hold the cell together.
2. Lift off the top half of the cell and set it aside.
3. Place the sample on the bottom half of the cell. (The sample must be small.)

- Put the top half back on the cell, lining up the red dots on the top and bottom halves. Do not tighten the screws yet; applying uneven shear forces may damage the diamonds.

One diamond can damage another.

CAUTION

- Press down on the cell with your thumb to thin the sample. Replace the three screws and tighten them finger tight.

NOTE: If the spectrum collected with the diamond anvil cell shows interference fringes, place some KBr in the cell and collect a background spectrum through it.

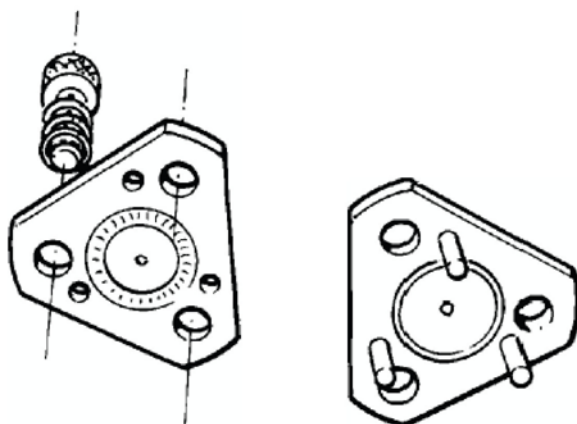


Figure 18 The miniature diamond anvil cell

Pressing elastomers between windows

If your sample is elastic and you are compressing it between windows, you must apply pressure continuously. Use the following procedure:

- Press on the windows with a probe, flattening the sample.
- While maintaining the pressure, apply small amounts of quick-setting nitrocellulose cement to the edges of the salt plates.

When the cement is dry, the sample remains compressed.

The compression cell (see *Using the Compression Cell* on page 65) enables you to compress this kind of sample.

Filled polymers

When a polymer contains a high concentration of fillers, and you want to analyze the polymer, you have to prepare a sample for analysis that is free of filler.

Often you can obtain a suitable sample by cutting a thin wedge of the material with a sharp blade. If the filler is not uniformly dispersed, you can find clear regions of polymer for analysis.

You can use pyrolysis to remove the fillers. As you heat the sample, the polymer volatilizes, and the fillers are reduced to ash. The sample can be pyrolyzed in the following ways:

Using a disposable pipette:

1. Place the polymer in a disposable pipette and seal the large end.
2. When this end cools, tap the polymer into it, then heat the sample gently.
The pyrolyzed polymer condenses on the walls of the pipette. The filler is left behind as ash.
3. Score and break the pipette between the ash and the pyrolysate.
4. Add a drop of solvent to the pyrolysate to wash it on to a salt plate.

If the amount of sample is small, use a capillary tube instead of a disposable pipette.

Using a microbrush to pyrolyze micro amounts:

1. Seal the end that is away from the brush fibers and tap the sample particle into this end then heat it gently with a microtorch.
2. After pyrolysis, break off and discard the end of the tube that contains the ash.
3. While holding the fibers of the brush against the salt plate, add a drop of solvent to the broken end.
4. Allow the solvent containing the pyrolysate to flow into the fibers.
When it evaporates, the pyrolysate remains on the salt plate for analysis.

Particles

Crushing

Crushing enables you to thin samples such as large particles that cannot be sliced. This can be done in various ways:

- If the sample is small, crush it with the roller end of the roller knife.
- If the sample is larger, use a pestle and mortar.

Separating by aperturing

Powders and other particulate solids may contain several different components. Instead of separating them, use the infrared aperture to isolate the component you want to sample:

1. Spread the sample out with a probe so that you can visually distinguish the components.
2. Looking at the Monitor Visible window, find a particle of the component you want to sample.
3. Center this particle in the field of view.
4. Adjust the infrared aperture until only the particle that is of interest is visible.

You can easily pick up extremely small particles and transfer them with a very fine-pointed tungsten needle. Scoring the surface of the salt plate with the needle makes a simple map to help you positively identify the particles under the imager.

Transferring with a tungsten needle

When necessary, sharpen the tungsten needle.

Nujol or fluorolube mulls

Suspending fine particles of a solid sample in nujol or fluorolube reduces or eliminates the surface reflections that can distort absorption measurements. These oils also reduce the amount of radiation lost to reflection or scattering.

If the film is thin enough, you can correct the spectrum for the presence of the oil by subtracting a spectrum of the pure liquid. It is difficult, however, to obtain the correct thickness for a good subtraction.

Fibers

You can roll fibers to flatten them (see *Flattening Solids* on page 54), or they can be pressed in a diamond anvil cell (see *Polymers* on page 56). The following is another approach.

Fibrous Solids

If a fibrous sample, such as paper, is too thick, tear it and examine the torn edges. The edges contain single fibers and thin clumps of fibers.

Coatings on Substrates

If the sample is coated on a substrate, the method for collecting its spectrum depends on the nature of the substrate:

- If the substrate is reflective, you can analyze the sample in reflectance. If the sample is only weakly reflective or scatters the signal strongly, use the diffuse reflectance sampling mode.
- If the substrate is opaque, scrape off a sample of the coating; use the roller knife to scrape a small piece on to a KBr or BaF₂ disk.

Liquids

Solutions of samples

Although liquids are seldom analyzed with the imaging system, sometimes the sample of interest is in solution.

1. Transfer the solution on to a salt plate.
2. Allow the solvent to evaporate, leaving the sample on the plate.

Micropipettes

You can use a micropipette to apply liquid to the surface of a salt plate, or to the edge of the junction between two salt plates. In the latter case, the liquid flows between the plates by capillary action.

Preventing liquids from spreading

If the amount of liquid being transferred to the salt plate is very small, restrict it to a small area of the plate. There are several ways to do this:

- Use a microbrush to transfer solutions. The bristles of the microbrush hold the liquid in a small region of the salt plate until the solvent evaporates.
- Repeatedly jab a small area of the salt plate with a tungsten probe. Leave the resulting small salt particles in the well that is produced.

The capillary spaces between the salt particles retain the liquid and minimize spreading.

- Place the salt plate on a small metal washer that is being gently heated.

Because there is more heat at the outside of the salt plate than near the center (over the hole in the washer), the droplet of liquid is forced toward the center.





Techniques for Collecting
IR Images and Spectra


Techniques for Collecting IR Images and Spectra


This chapter describes how accessories and collection techniques enable you to collect images and spectra from different types of sample.

The main steps required to scan an image, from loading the sample to selecting the area you wish to scan are outlined below. For further information see the on-screen Help.

NOTE: Image mode must be selected in the Control window in the SpectrumIMAGE software before you can scan an image.

1. In the Stage Control window, click  , or select **Load Position** from the GoTo menu to drive the sample stage to the loading position.
2. Position the sample in the center of the sample stage.
3. Click  , or display the GoTo menu and select **Center of Stage** to drive the sample stage so that the sample is in the center.
4. To focus the visible image displayed in the Monitor Visible window, use the Z-control on the joystick or the **Focus** control on the Control window.
5. The Monitor Visible window displays only the field of view of the imaging system, which is a small area. Select a default survey size from the View menu in the Stage Control window and then start the survey by selecting **Visible Image Survey** from the Survey menu in the Stage Control window.
A visible image is collected and displayed in the Stage Control window.

NOTE: To get an overall picture of your sample, click  in the Stage Control window.

6. Using the joystick, move to a clear area that you wish to use for the background.
7. In the Stage Control window, click .
8. Check the settings in the Scan Image Background dialog and then click **Start** to begin scanning.
9. Using the joystick, move back to the region you want to scan.
10. In either the Monitor Visible window or the Stage Control window, draw a grow box around the area you want to scan.

11. In the Stage Control window, click . The Start Image dialog is displayed.
12. Enter a filename for the new image and then click **Start Image**. Data collection begins and the new image is displayed in a new window.

Using the Compression Cell

The optional compression cell (part number N1870185, Figure 19) enables you to flatten soft materials. It also enables you to hold specimens flat and in optical contact with salt windows. The cell consists of an aluminum block, machined to accept salt windows, with window retainers and a special wrench to apply pressure across the windows. The sample is held between the two windows. The compression cell fits into the sample slide holder on the stage of the imager. 1 mm and 2 mm thick windows of 13 mm outer diameter can be used with the cell; two KBr windows (2 mm thickness) are included. The cell can apply pressure without rotating the windows, and therefore avoids scratching them.

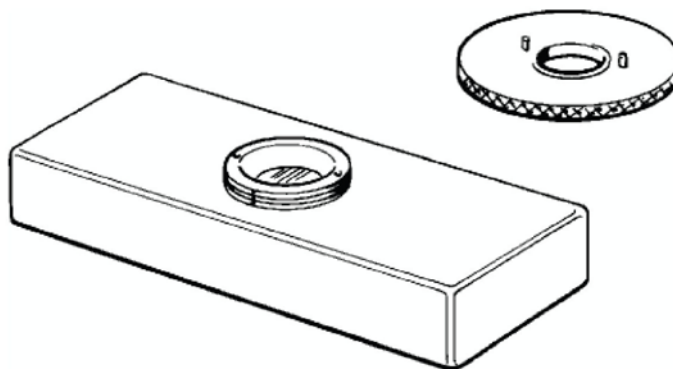


Figure 19 The compression cell

Although thinning can be performed with the compression cell, it does not replace the miniature diamond anvil cell as a sample-thinning device. The primary application of the compression cell is for keeping specimens flat over the entire visual field of view.

Using the Hot Stage

The optional hot stage (part number N1870184, Figure 20) enables you to study temperature-dependent phenomena in microsamples. The hot stage consists of a temperature controller and a heating block that accepts infrared windows. The heating block contains an integral thermocouple, and the temperature is digitally displayed in degrees Celsius on the controller. The hot stage can heat samples up to 250 °C in 1 degree increments. A target temperature can be selected and maintained. The hot stage is held in the slide clip on the sample stage of the imager. The imager requires no modifications to accept the hot stage.

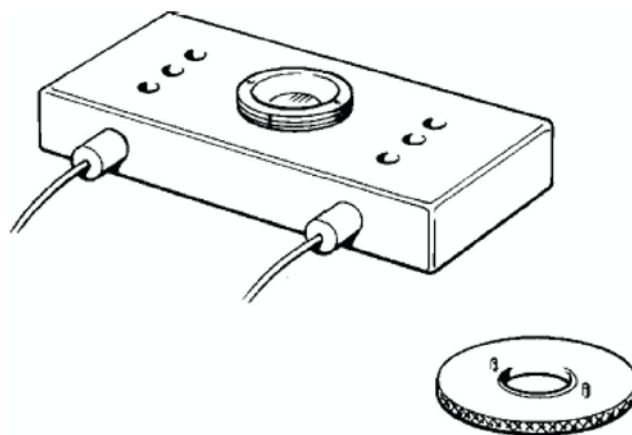


Figure 20 The hot stage

The hot stage enables you to study phase transitions and temperature-dependent chemical reactions. Infrared microscopy can provide detailed molecular structural information for systems undergoing phase transitions; this information is not available from thermal data only. Polymers, pharmaceuticals and liquid crystals are examples of materials where investigations of phase transition are important.

Collecting the Image of a Thick Sample

Lowering the stage using the Z-control on the joystick enables you to focus on a thick sample. For very thick samples you may have to remove the lower cassegrain assembly; then only the reflectance or diffuse reflectance sampling modes can be used to view the sample and collect its image.

To collect the image of a thick sample

1. Click **Zero** on the Control dialog.
The lower cassegrain moves to the position where the infrared beam is focused if no sample is on the stage.
2. Move the sample stage to its highest possible position.

3. Release the locking lever, at the back of the lower cassegrain assembly on the right (Figure 21).

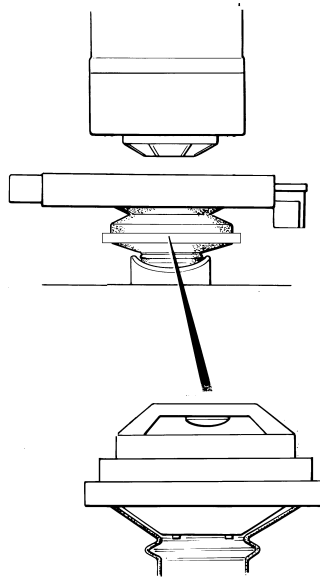


Figure 21 The lower cassegrain

4. Gently slide the cassegrain assembly forward and out of the dovetail connector.

NOTE: To make withdrawal easier, pull the locking lever gently.

5. Click **Park** on the Control dialog.
The lower cassegrain bracket moves down.
6. Click **Park** again.
The lower cassegrain bracket moves to its lowest position.
7. Lower the stage using the Z-control on the joystick.

CAUTION

Once the lower cassegrain is removed, lowering the stage when at the limit of its travel backwards will cause it to collide with the dovetail connector. This will cause permanent damage to the stage motor gear box. If necessary, remove the dovetail connector to avoid damaging the instrument.

To remove the dovetail connector:

- Loosen the two fixing screws at the front of the connector using a 3 mm hex key, and pull the connector away from the microscope (see Figure 22).



Figure 22 Location of the dovetail connector behind the stage

To refit the lower cassegrain after use

1. If necessary, refit the dovetail connector using the fixing screws.
2. Raise the stage using the Z-control on the joystick to its highest position.
3. Click **Zero** on the Control dialog.
4. Slide the cassegrain assembly back into the dovetail connector, as far as it goes.

NOTE: To make replacement easier, pull the locking lever gently.

5. Tighten the locking lever.
6. Make sure that the cassegrain is correctly seated.
7. Place the gaiter over the cassegrain if the purge facility is required.

Collecting an Image in an Inert Atmosphere

The optional purge system enables you to purge the spaces around the sample and the infrared beam (typically with nitrogen or dry air), to provide a dry, controlled atmosphere.



WARNING

Do not use flammable gases for purging.

Parts of the purging system

Some parts of the purging system are shown in Figure 23.

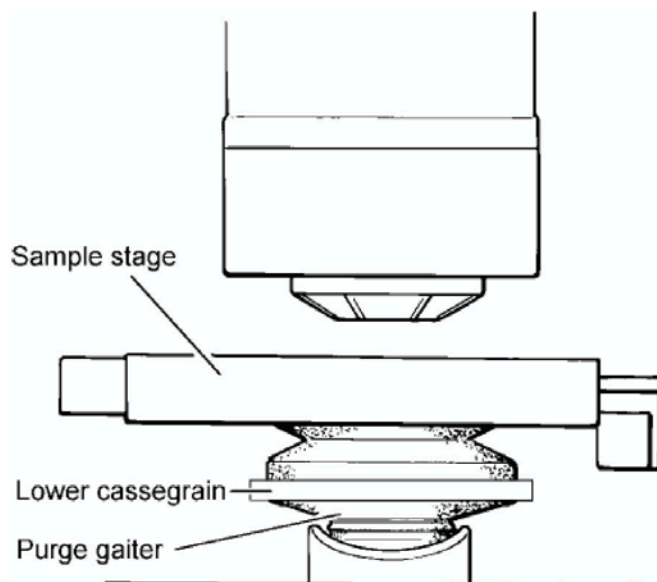


Figure 23 Parts of the purge system

The purge system consists of:

- The gas inlet connector on the metal plate at the rear of the imager and the connector at the rear of the spectrometer;
- The purge coupling molding connecting the spectrometer to the imager;
- The lower purge gaiter under the lower cassegrain.

When all of these parts are in place, the gas entering through the inlet displaces air from the path of the infrared beam.

Purging the system

1. Make sure that all parts of the purge system, as listed above, are in place.
2. Set up the imager.
See *Getting Ready to use the Imaging System* starting on page 39.
3. Place the sample in position.
4. Purge the imager and spectrometer for 15 to 20 minutes at a rate of 10 L min⁻¹.
5. Collect the background spectrum and the image of the sample.
See the on-screen Help for further information about collecting images.

Changing the sample

If you have changed a sample, any air that has entered the system must be flushed out.

Viewing a Sample with the Visible Polarizer

Polarized visible light can enable you to identify areas or structures that differ chemically and to solve problems commonly found in infrared microspectroscopy applications.

The Theory of Light Polarization

Ordinary light and infrared radiation consists of waves vibrating in all possible planes perpendicular to the direction of propagation. This is represented in the left side of Figure 24.

Conventionally the plane of the light is taken to be the plane of the continuously varying electric vector.

If the light passes through a polarizer, the polarizer allows the passage of only those waves that have their plane of vibration in one particular direction. The light that emerges is said to be polarized, and is represented on the right side of Figure 24.

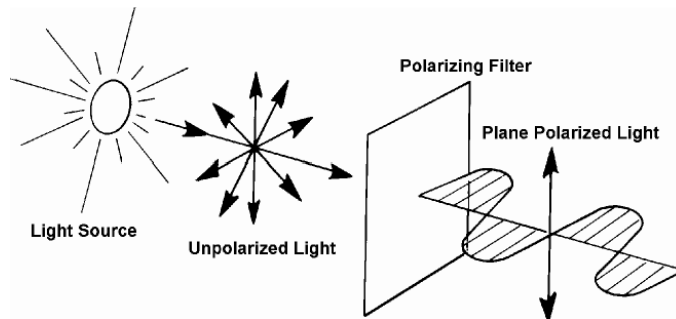


Figure 24 Representation of unpolarized light (left) and polarized light (right)

Because all components of the wave in the plane of polarization are transmitted, the ideal polarizer allows 50% of the light through.

If a second polarizer is placed in the path of the polarized light two things may result:

- If the second polarizer is placed in the same direction as the first (as at the top of Figure 25), the polarized light can pass straight through.
- However, if the second is placed at a right angle to the first, a situation which is referred to as crossed polarizers, the passage of the polarized light is blocked, that is, extinction occurs (as at the bottom of Figure 25).

The second situation occurs because the light transmitted by the first polarizer oscillates in exactly the plane that is blocked by the second polarizer.

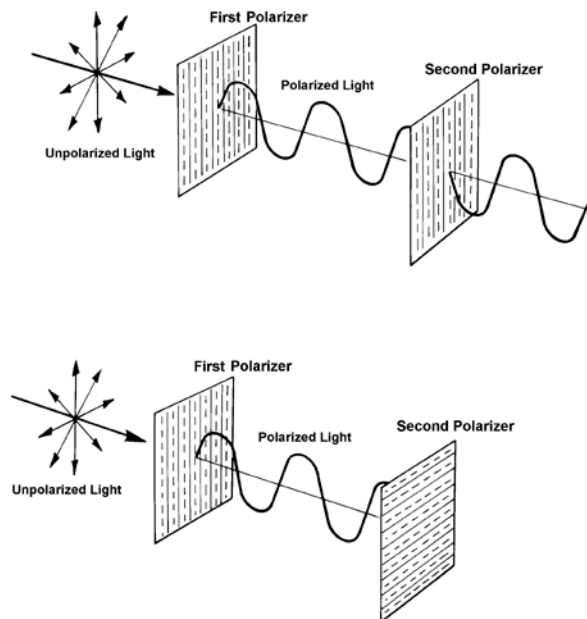


Figure 25 Polarizers parallel (top) and polarizers crossed (bottom)

Some materials are *anisotropic* (or *birefringent*): their refractive index depends on their orientation. These materials can alter the polarization of light passing through them; this is dependent on the wavelength of the transmitted light.

When you look at an anisotropic sample using polarized light, the change in polarization caused by the sample means that some light leaks through the second polarizer. Because the change in polarization is dependent on the wavelength, the color of the light emerging changes with the distance traveled through the sample and the amount of birefringence encountered.

Applications

Differences in the birefringence of an object or area may be an indication of chemical disparity. This can be useful in visibly separating or identifying an object or area of interest before collecting an infrared spectrum. Some examples are given below:

Laminates

Many polymer structures consist of different layers of material and adhesives of varying thicknesses bonded together in order to meet physical requirements. If you view a cross section of the structure using polarized light, you can identify the individual layers and set the apertures to collect separate infrared spectra from each layer. This is useful for identifying the materials used to create the structure.

Polymer films

Although they may appear identical when using normal light, the film and inclusion present in a polymer film may exhibit different degrees of birefringence when viewed using polarized light. If this is so, you can visibly identify the inclusion and collect an infrared spectrum to determine its composition.

Rocks minerals and crystals

Most crystals are characteristically birefringent and thus are ideally suited to this technique. Viewing a mixture consisting of crystals, such as an artificial sweetener, pharmaceutical powder, or an illicit substance, enables you to visibly separate the components by their relative size, shape and birefringence.

Fibers

Polarization may enable you to identify and separate fibers in a clump or to view a bicomponent fiber. Most fibers in their natural state are optically thick and their cylindrical shape can cause lensing effects. For these reasons, fibers are usually flattened in preparation for infrared microspectroscopy. This flattening affects the birefringence of the structure and may degrade the usefulness of this technique.

Biological substances

Birefringence can occur in some biological substances. You can collect infrared spectra of thin sections of these materials. In some cases, polarized light can reveal structures or chemical disparities in these structures, and infrared spectra can be collected from the different regions of interest.

Equipment

The equipment for visible polarization studies consists of two parts: the polarizer and the analyzer (Figure 26).

The polarizer polarizes the incoming beam from the illuminator and the analyzer contains a polarizing element that you can rotate to any orientation. It is placed in front of the camera.

The polarizers for both transmittance and reflectance are built into the imager, and are automatically switched into the beam when the analyzer is inserted.



Figure 26 The analyzer

Operation

Insert the analyzer into the slot in the right-hand side of the front cover (Figure 27); push the analyzer in with the wheel facing towards you. It has two positions:

- The first position allows the full beam to pass through.
- When the analyzer is inserted fully, the polarizing element is in the beam.

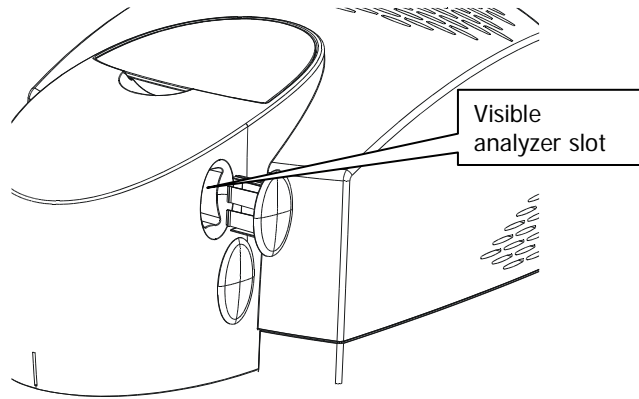


Figure 27 Analyzer position

When the polarizing element is in the beam, rotate the wheel while viewing the Monitor Visible window.

Collecting Images Using the Infrared Polarizer Accessory

An absorption band in the infrared range occurs when a vibration is accompanied by a change in dipole moment. The electric vector of the incident radiation must have a component in the direction of the dipole moment change.

In polarization spectroscopy, the absorption bands of greatest interest are those in which the direction of dipole moment change is related to a bond direction, for example, the nitrile stretching vibration. If, in a particular sample, all the bonds of a particular type are aligned in a specific direction, the strength of the absorption depends on the polarization of the incident radiation, that is, whether the electric vector is parallel to or perpendicular to the bond direction.

For example, stretching an acrylic fiber aligns the molecules with the general direction of the polymer chains parallel to the fiber axis, and the nitrile groups tend to be oriented perpendicular to the axis. If the spectrum is collected with the infrared radiation polarized perpendicular to the axis, the nitrile absorption peak is much stronger than if the spectrum is collected with radiation polarized parallel to the axis. The ratio of the two intensities (called the *dichroic ratio*) is a measure of the extent of alignment of the nitrile groups and thus of the polymer chains.

The Spotlight imaging system enables you to collect polarization spectra of very small samples. These include:

- Single filaments (typically $14 \times 70 \mu\text{m}$);
- Films;
- Single crystals;
- Liquid crystals.

Equipment

The optional infrared polarizer has a rotatable silver bromide element in an aluminum mount (Figure 28).

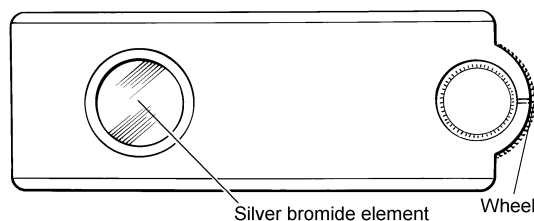


Figure 28 The infrared polarizer

Using the Polarizer

CAUTION

The polarizer element is extremely fragile. Do not touch it with anything. It cannot be washed, dusted, or blown upon by air. If damaged, it cannot be repaired. When it is not in use, protect it in the case supplied.

1. Remove the snap in cover that masks the aperture for the infrared polarizer (Figure 29).

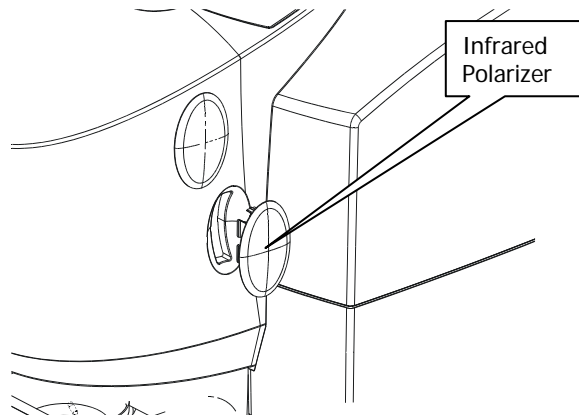


Figure 29 Infrared polarizer position

2. Slide the analyzer into the vertical slot on the sample holder that can be seen through this aperture.
The flat side of the analyzer must be towards the rear of the imager, and the wheel facing outwards.
3. Push the analyzer into the slot until it stops.
4. Turn the wheel to orient the polarizing element.

NOTE: Both the scribed line and the uneven coloration are usual, and do not affect the performance of the element.

Collecting Spectra with the ATR Objective

NOTE: The ATR objective is optional and mainly applicable to mid-infrared applications.

The ATR (Attenuated Total Reflectance) technique enables the collection of spectra from materials that are too opaque for transmission measurements, and too strongly absorbing for good reflectance measurements. Spectra can be collected with little sample preparation.

SpectrumIMAGE software enables you to automatically image a sample using the optional ATR objective. Spectra are collected by touching the ATR objective on the sample and collecting the spectrum generated from the surface layer of the sample. See the on-screen Help for further information.

NOTE: You must be in Point mode in the SpectrumIMAGE software when using the ATR objective, as the ATR objective cannot be used in Image mode. Point mode is not available if you are using the Spotlight with Long Wavelength option.

The ATR objective uses a crystal made from a material that transmits infrared radiation, and has a high refractive index. An infrared beam enters the crystal and is internally reflected within the crystal, creating an evanescent wave. At each reflection inside the crystal, the wave continues beyond the crystal surface into a sample that is held in close contact. The penetration depth depends on the refractive indices of the crystal and the sample. For a germanium crystal, the penetration depth for most samples is less than 1 μm . The penetration depth also varies with the wavenumber of the infrared radiation:

$$d_p = \frac{\lambda}{2\pi n_1 (\sin^2 \theta - n_{21}^2)^{1/2}}$$

Where:

n_1 is the refractive index of the ATR crystal (4.0 for germanium)

λ is the wavelength of the radiation

θ is the angle on incidence of the beam

n_{21} is the ratio of the refractive indices of the sample and the ATR crystal.

ATR objective

The ATR objective enables the microscopic examination of samples in order to locate the exact area of interest. The ATR crystal is moved vertically out of the beam path to enable you to view the sample, and place the area of interest in the center of the field of view. The crystal is then placed onto the sample under positive pressure to collect a spectrum.

The crystal has a small contact area formed by the flattened point of a cone; this ensures a 100 μm diameter contact area.

Optical path

Infrared radiation is directed into the crystal from the front half of the upper cassegrain, and is focused at the sample position. It is reflected once within the crystal, then the totally internally reflected beam is collected by the rear portion of the upper cassegrain, which focuses it on the remote aperture. The radiation is directed onto the detector in the imager.

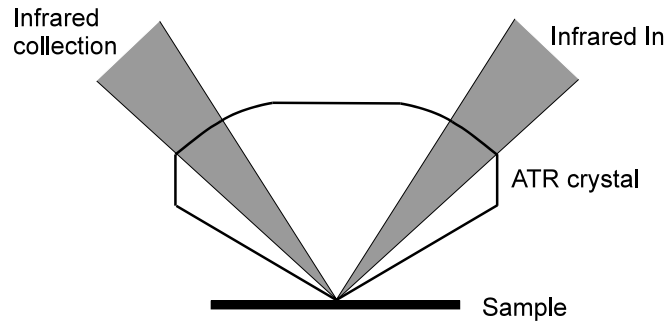


Figure 30 ATR optical path

ATR Objective Specification

The ATR cassegrain is fitted to the imager and the ATR crystal holder is supplied separately.

ATR crystal materials

Germanium or Silicon

Range of measurement

Ge: 5500 cm^{-1} to 600 cm^{-1}

Si: 7800 cm^{-1} to 800 cm^{-1}

Area of contact with sample

Nominally 100 μm diameter flat surface. Single internal reflection from surface.

Maintenance

The ATR assembly may be removed (see *Removing the ATR Crystal Holder from the Imager* on page 85), cleaned (see *Cleaning the ATR Objective Crystal* on page 87), or replaced and aligned by the user (see *Fitting the ATR Crystal Holder to the Imager* on page 80).

A replacement crystal is provided with each objective.

Fitting the ATR Crystal Holder to the Imager

1. Switch off the imager at the electricity supply.
2. Stop any laser radiation from entering the imager by switching the internal beam of the spectrometer to the internal sample compartment.
For further information on how to do this, see the Spectrum software on-screen Help.
3. Hold the crystal assembly under the upper cassegrain.
Be careful not to change the alignment of the two adjusting levers.
4. Fit the two adjustment thumb-nuts (Figure 31).
The assembly is aligned if the adjusting levers have not been moved.
5. Direct the infrared beam to your imager.
6. Switch on the imager.

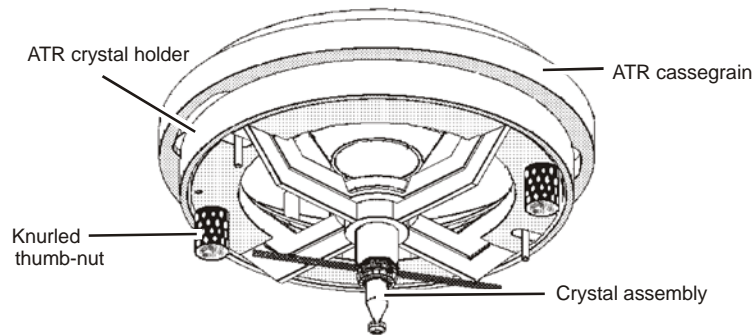


Figure 31 The ATR crystal holder and ATR cassegrain

Measuring the Energy in Reflectance Mode

1. Make sure that your spectrometer is switched on, and the source has warmed up.
2. Make sure that the imager, PC, and stage controller are switched on and that SpectrumIMAGE software is running on your PC.
3. Place the slide holding the reference mirror (supplied with the imager) on the sample stage.
4. Make sure **Point mode** is selected on the Control dialog.
5. Make sure that the imager is in reflectance by choosing **Reflectance** on the Control dialog.
6. Look at the image in the Monitor Visible window and focus the beam on the surface of the mirror using the Z-control on the joystick.
There are usually dust particles or scratches on the surface that you can use to focus on.
7. Click **Aperture** on the Control dialog.
The IR Aperture dialog is displayed.
8. Enter 100 in the **Width** and **Height** text boxes.
9. Click **OK**.
The IR Aperture dialog closes and the aperture size is set.
10. In the Stage Control window, display the Scan menu and select **Monitor**.
The Monitor Visible window is used to monitor the energy reaching the detector.
11. Record the level of energy measured.
12. Click **Exit**.
The Monitor Visible window returns to monitoring the visible image.

NOTE: The energy level can also be measured in diffuse reflectance mode using this procedure, using the white reflector slide supplied with InGaAs systems.

Adjusting the Height of the Crystal

NOTE: The crystal is mounted on a bayonet assembly similar to that on some light bulbs. You must always support both ends of the toggle bar when lowering or raising the crystal.

CAUTION

To prevent serious damage to the crystal, do not move the sample stage while the ATR crystal is in the lower, working position and touching or near to the sample.

1. Lower the crystal onto the reference mirror by turning the bar clockwise and slowly lowering the toggle bar.
2. Using the knurled height-adjustment nut, adjust the height of the crystal so that it just rests on the mirror under positive pressure (Figure 32).

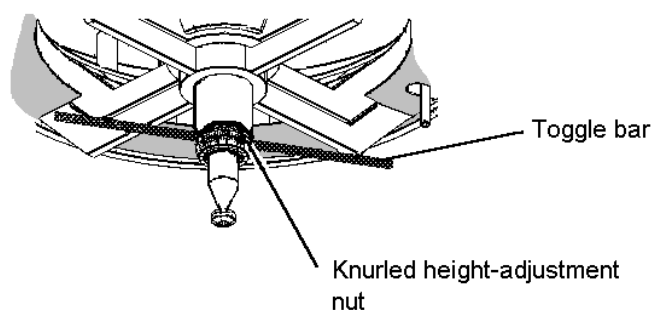


Figure 32 Adjusting the ATR crystal height

Centering the Crystal

1. Retract the crystal using the toggle bar, by lifting the bar then twisting counterclockwise.
The crystal is retracted.
2. Place a piece of black PVC electrical tape on a microscope slide, and put the slide on the microscope stage.
3. Move the stage so that the tape is directly below the crystal, and focus the beam on the tape.
4. Lower the crystal onto the tape.
The crystal makes an impression in the soft tape.

5. Retract the crystal using the toggle bar.

When the crystal is retracted, the tape returns to its previous shape. The impression disappears after a few seconds so you must perform the next three steps quickly.

To increase the life of the impression, raise the sample stage slightly using the arrow buttons in the Focus section of the Control dialog.

6. Focus the visible illumination on the tape.
7. In the Monitor Visible window look at the impression of the crystal on the tape. Decide whether the impression is in the center of the field of view.
If the impression is in the center of the field of view, the assembly is aligned.

If the impression is not in the center of the field of view:

1. Loosen the two knurled thumb-nuts under the ATR crystal holder half a turn.

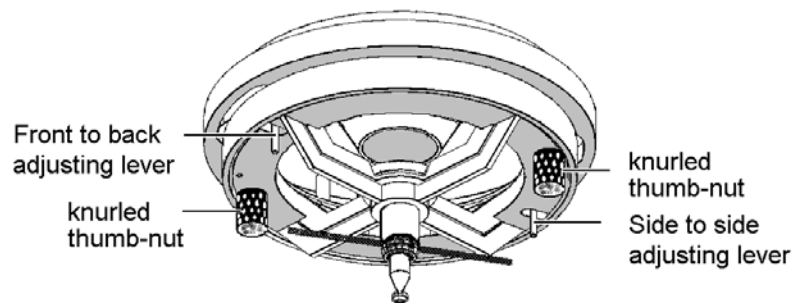


Figure 33 Centering the ATR crystal

2. Move the ATR crystal holder using the adjusting levers until it is centered.
One lever moves the assembly backwards and forwards and the other from side to side.
3. Tighten the knurled thumb-nuts.
4. Move the microscope slide to a fresh area of tape.
5. Lower the crystal onto the tape.
6. Retract the crystal using the toggle bar.
7. Look through the imager at the impression of the crystal on the tape, and decide whether the impression is in the center of the field of view.
8. If necessary, repeat steps 1 to 7 until the ATR objective is aligned.

Checking the Infrared Alignment

1. Place the slide holding the reference mirror (supplied with the imager) on the sample stage.
2. Look at the image in the Monitor Visible window and focus the imager on the surface of the mirror with the Z-control on the joystick.
There are usually dust particles or scratches on the surface that you can use to focus on.
3. Lower the crystal onto the reference mirror.
4. Select **Aperture** on the Control dialog.
The IR Aperture dialog is displayed.
5. Enter 100 in the **Width** and **Height** text boxes.
6. Click **OK**.
The IR Aperture dialog closes and the aperture size is set.
7. To monitor the energy reaching the detector, in the Stage Control window display the Scan menu and select **Monitor**.
The Monitor Visible window is used to monitor the energy reaching the detector.
The energy level measured should be 20–30% of that measured before.
8. Click **Exit**.
The Monitor Visible window returns to monitoring the visible image.

Removing the ATR Crystal Holder from the Imager

To use the imager for conventional microspectroscopy or imaging, you need only retract the ATR crystal from its working position. If, however you need a very large working distance, for example if the sample is recessed, the ATR crystal holder can be removed.

1. Switch off the imager at the electricity supply.
2. Stop any laser radiation from entering the imager by switching the internal beam of the spectrometer to the internal sample compartment.
See the Spectrum on-screen Help for further information.
3. Make sure that the ATR crystal is in the raised position.
4. Screw the plastic protective cover in place, over the crystal.
5. While holding the crystal assembly, unscrew the two adjustment thumbnuts completely. Allow the assembly to drop vertically from the cassegrain. Be careful not to disturb the two adjusting levers. Any movement of the adjusting levers will change the alignment of the assembly when it is refitted.
6. Store the assembly carefully for future use.
When refitted, the assembly will still be aligned if the adjusting levers have not been moved.
7. Direct the infrared beam back to the imager.

Fitting a Crystal Assembly to the ATR Objective

1. Remove the ATR crystal holder, as described on page 85.
2. Place the crystal holder on a bench, with the crystal upwards.
3. While holding the crystal assembly in place, unscrew the knurled height adjusting nut. The crystal assembly is under spring pressure. If you do not hold the assembly in place, the spring may be lost.
4. Remove the crystal assembly.
Leave the spring in the ATR crystal holder.
5. Place the new crystal assembly into the ATR crystal holder and refit the height-adjusting nut.
Take care not to damage the crystal.
6. Refit the ATR crystal holder to the imager as described on page 80.
7. Align and adjust the ATR crystal holder.

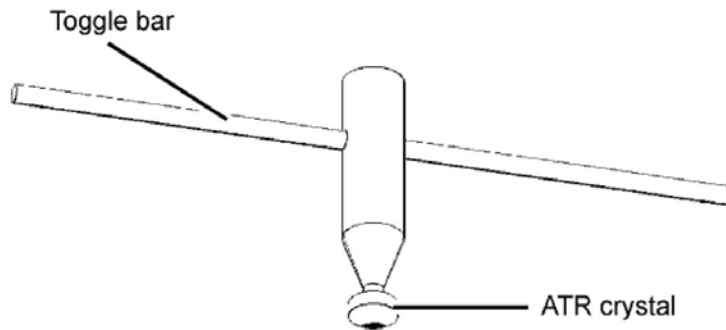


Figure 34 The ATR crystal assembly

Cleaning the ATR Objective Crystal

The ATR objective crystal is in contact with the sample under test, so it may become dirty during use. A dirty crystal may give spectra of the contaminant rather than the sample under test.

Great care must be taken when cleaning the crystal. It should be cleaned using the minimum mechanical pressure, with a soft brush or lens tissue. Isopropanol or n-hexane may be used to clean the crystal.

NOTE: Do not use acetone or xylene to clean the crystal.

1. Pour a small volume of a solvent into a shallow dish.
2. Using the Z-control on the joystick, lower the sample stage to allow the dish to be placed on the stage under the crystal.
3. Using the Z-control on the joystick, raise the stage so that the tip of the crystal is just immersed in the solvent.

NOTE: Do not immerse the whole of the ATR crystal in the solvent.

4. Leave the crystal in the solvent for 5 minutes.
We recommend that you do not leave the crystal in the solvent for longer periods.
5. If any material remains on the crystal, rub the crystal gently with a soft brush or lens tissue.

Collecting Spectra Using the ATR Crystal

Collecting a Background Spectrum

CAUTION

To prevent serious damage to the crystal, do not move the sample stage while the ATR crystal is in the lower, working position and touching or near to the sample.

1. Place the slide holding the reference mirror (supplied with the imaging system) on the microscope slide.
2. Make sure that **Point mode** is selected on the Control dialog.
3. Make sure that the imager is in reflectance mode by choosing **Reflectance** on the Control dialog.

NOTE: Diffuse reflectance mode can also be used with the ATR crystal. A white reflector is a suitable background reference material (a slide is supplied with InGaAs systems).

4. Look at the image in the Monitor Visible window and focus on the surface of the mirror using the Z-control on the joystick.
There are usually dust particles or scratches on the surface that you can use to focus on.
5. Lower the ATR crystal to the working position.
6. Set the infrared aperture to a suitable size and rotation.
Refer to *Setting the Infrared Aperture* in the on-screen Help.
The contact area of the ATR objective is approximately 100 μm in diameter. We therefore recommend that you use a maximum aperture size of 100 \times 100 μm .
7. Display the Options menu on the Stage Control window, and select **Set Background Position**.
The background position is set. Do not move the stage before you collect the background spectrum. If you move the stage, you may cause serious damage to the ATR crystal.
8. Display the Scan menu in the Stage Control window and select **Background**.
The Scan Background dialog is displayed.
9. Select the Scan Parameters and Display Mode you want.

10. Click **OK**.

CAUTION

*If you have moved the stage since setting the background position, a dialog is displayed. If you click **Yes**, the stage will move and may cause serious damage to the ATR crystal. If you click **No**, the current stage position becomes the background position.*

11. The spectrometer starts to scan and spectra are displayed as they are collected. When scanning has finished, the results are displayed.
12. Retract the ATR crystal.

Collecting a Sample Spectrum

The SpectrumIMAGE software enables you to collect line scans and maps and to collect spectra at marked points using the ATR objective in Point mode. For information on how to do this, refer to *Mapping* and *Line Scanning* in the on-screen Help.

1. Retract the ATR crystal.
2. Place the sample on the slide holding the reference mirror, or remove the reference mirror slide from the stage and put a new slide with the sample on it on the stage.
3. Make sure that **Point mode** is selected on the Control dialog.
4. Make sure that the imager is in reflectance mode by choosing **Reflectance** on the Control dialog.

NOTE: Diffuse reflectance mode can also be used with the ATR crystal.

5. Move the sample to the center of the field.
Even though the imager may not be focused on the sample, you can see a change in the light intensity as the sample moves into the center of the field of view.
6. Look at the image in the Monitor Visible window and focus on the sample using the Z-control on the joystick, then move the area of interest into the center of the field of view.
7. Lower the ATR crystal to the working position.

Monitoring

Before you collect the sample spectrum, you should monitor the spectrum to make sure that there is good contact between the crystal and the sample.


1. In the Stage Control window, display the Scan menu and select **Monitor**.
The Monitor dialog is displayed.
2. Click **Ratio**.
3. Enter the **Scan Parameters** that you require.

4. Click **OK**.

The Monitor window becomes the Monitor IR Ratio window. The spectrum at the current aperture position is displayed.

5. If your sample is soft, you may need to increase the contact of the crystal with the sample.

To increase the contact, raise the stage slightly using the arrows in the **Focus** section of the Control dialog.

6. When you are happy with the displayed spectrum, click .

7. Display the Scan menu in the Stage Control window and then select **Sample Ratio**. The Scan Sample Ratio dialog is displayed.

8. Select the Scan Parameters and Display Mode you want.

9. Click **OK**.

The spectrometer starts to scan and spectra are displayed as they are collected.

10. If you want to view the sample, retract the ATR crystal.

Reflectance FT-IR Microspectroscopy

The Spotlight imaging system enables you to collect images and spectra from samples that display any of the three types of reflectance: diffuse reflectance, specular reflectance, or reflection-absorption.

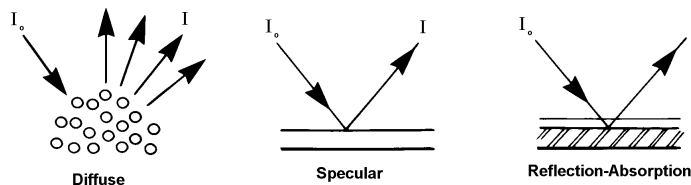


Figure 35 Three types of reflectance

Figure 35 shows how the incident radiation (I_0) is reflected in each type of reflectance. It is not uncommon for two or more of these processes to occur simultaneously, depending on the structure of the sample.

Diffuse Reflectance

In diffuse reflectance, the incident radiation is reflected in all directions from the surface of the sample. This type of reflectance is seen in samples with matt surfaces, such as paper. The broad collection angle of the imager enables it to capture a large proportion of the diffusely reflected radiation and send it to the detector. In addition, the diffuse reflectance sampling mode compensates for any loss of signal by increasing the gain of the detector.

A problem often encountered when using the diffuse reflectance technique is that there is a large specular component in the reflected radiation. Figure 35 shows three diffuse reflectance spectra of polymethyl methacrylate (PMMA) shavings. In the top spectrum, the shavings were neat. In the next two spectra, the PMMA was diluted with successively larger amounts of KBr.

In the neat sample, the presence of interfering specular reflectance introduces non-linearities to the spectral data. For example, the relative intensities of the strong C=O and C–O stretching absorptions are not as expected (arrows on the top spectrum).

As the sample is diluted in a non-absorbing matrix (KBr), specular reflectance is minimized. The bottom spectrum more closely matches an absorption spectrum.

NOTE: Sample cups for obtaining diffuse reflectance spectra of powders are available from PerkinElmer: regular cup (part number 01862760) and micro cup (01862761).

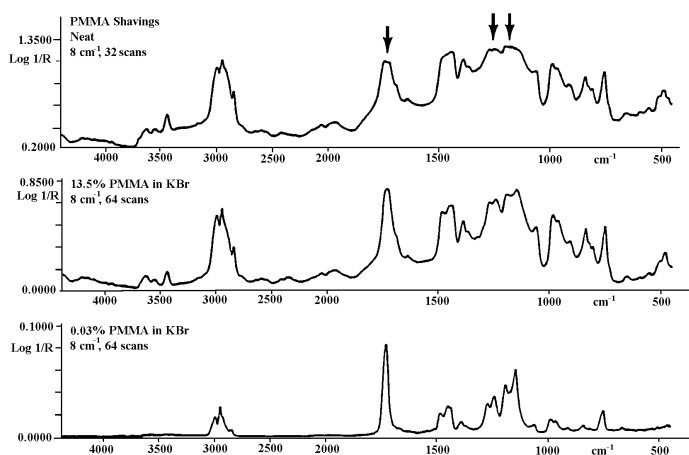


Figure 36 Diffuse reflectance spectra of PMMA

Diffuse reflectance spectra of powders and pharmaceutical tablets can be collected in the region $7800\text{--}4000\text{ cm}^{-1}$ and to lower wavenumbers if required, using the Spotlight 400N imaging system. These spectra generally do not suffer the non-linearity problems to the same extent as the corresponding mid-infrared diffuse reflectance spectra in the $2000\text{--}1000\text{ cm}^{-1}$ region.

Specular Reflectance

NOTE: Specular reflectance is mainly applicable to mid-infrared applications.

Specular reflectance is reflection in one direction (Figure 35). This is the type of reflection that occurs from a smooth, polished surface.

Absorption information cannot be obtained directly from a specular reflectance spectrum of a dielectric material, because the reflectance spectrum is governed by dispersion in the refractive index. However, you can use the Kramers-Kronig integration to calculate the absorbance spectrum from the specular reflectance spectrum. This integration is performed by the Kramers-Kronig process in Spectrum.

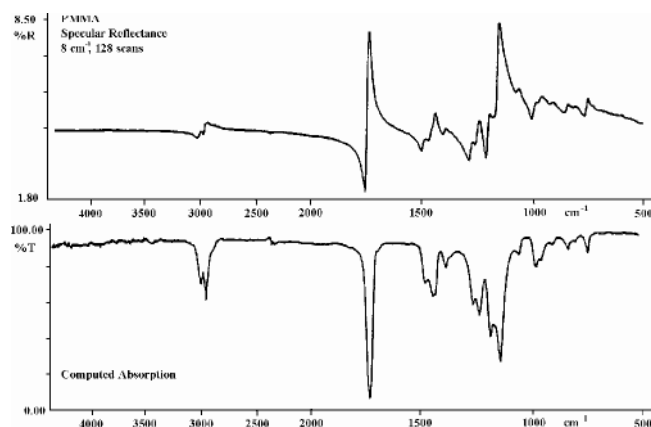


Figure 37 Specular reflectance spectrum of a PMMA plate and the absorption spectrum calculated from it with the Kramers-Kronig command

The greatest limitation to this method is the presence of an interfering diffuse reflection signal. This occurs when the sample surface is not perfectly smooth. The Kramers-Kronig integration is not appropriate for spectra from such samples.

Reflection-Absorption

NOTE: Reflection-absorption is mainly applicable to mid-infrared applications.

Reflection-absorption occurs when the incident radiation passes through a thin, absorbent film that is on a reflective surface (typically a metal) and is then reflected back through the film (Figure 35). The absorbance spectrum of the film can be collected directly. Figure 38 shows the spectrum of residual oil on an electronic contact. The sample diameter was 100 μm .

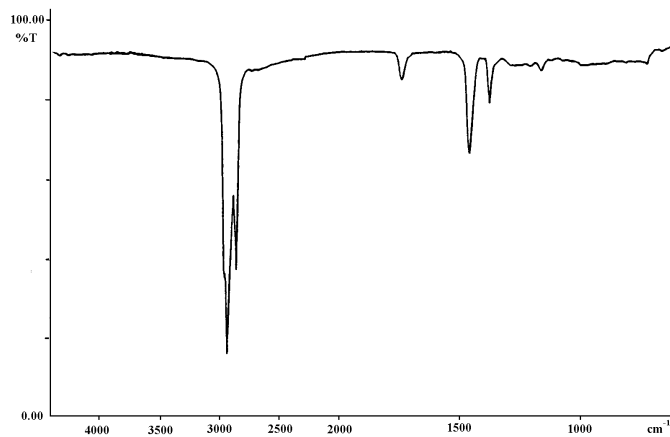
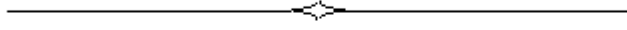


Figure 38 Reflection-absorption spectrum of residual oil on an electronic contact



Maintenance

Maintenance



Before cleaning the imager, switch off the mains voltage and remove the mains cord.

A Responsible Body must perform routine maintenance on your imager to make sure that it is safe and performing well. This involves:

- Inspecting the imager;
- Protecting the imager;
- Cleaning the optics;
- Cleaning the imager cover;
- Renewing the fuse;
- Testing the stage controller;
- Renewing the stage controller fuse;
- Service.

This chapter contains a list of the available spare parts and optional equipment for the imager.

If you need to replace a part, use only PerkinElmer approved spare parts.

CAUTION

The covers of the imager must only be removed by a PerkinElmer Service Engineer.

Inspecting the Imager

At least once a year, or whenever the imager has been subjected to adverse environmental conditions, visually inspect the housing to make sure that no covers are loose or distorted.

At the same time, make sure that all required labels are firmly in place (see Figures 2–5 on pages 19–20).

Protecting the Imager

The most important rule in caring for the imager is to keep it as free from dust and dirt as possible. Dust, fingerprints, and smears on the optics reduce the quality of the images it produces.

Whenever the imager is not in use, cover it with its plastic cover.

CAUTION *Ensure the power is switched off when the imager is covered.*

Cleaning the Optics

The imager was aligned, cleaned, and sealed at the factory. Do not attempt to take it apart.

CAUTION *Avoid the excessive use of solvents. Flowing solvents dissolve the cement on cemented optics; dissolved cement can damage mirror surfaces.*

Cleaning the Cover

CAUTION *Ensure the power is switched off and the supply lead is disconnected before cleaning the cover.*

You can clean the outside of the imager or stage controller using a damp cloth. Mild detergent may be used, if necessary. Always perform a patch test on an inconspicuous area before you clean the entire instrument.

Avoid spilling liquid into the imager or stage controller. Clean all external spills immediately. If anything that is spilled enters the main body of any part of the system, switch off the power and call a PerkinElmer Service Engineer.

Replacing the Imager Fuse

1. Switch off the imager and disconnect it from the power supply.
2. Insert a screwdriver into the slot at the side of the fuse drawer; pull out and flip to one side over the mains inlet.
The fuse may now be removed.
3. Fit the replacement fuse into the fuse drawer.
You require a 2.0 A time-delay fuse (part number 04970839).
4. Refit the fuse drawer.

Testing the Stage Controller

The motor that drives the stage has a resolution of up to 1 μm . Although the stage controller has such high precision, its motor can still move the stage at a speed of up to 6 mm per second.



WARNING

When you are using a motorized stage, do not place your fingers between the moving and fixed parts of the stage. The motors driving the stage are powerful and do not stall easily.

The joystick operates in the following manner:

- Left / Right joystick movement gives X movement of the stage.
- Top / Bottom joystick movement gives Y movement of the stage.
- Twisting the joystick gives Z movement of the stage:
Left twist (counterclockwise) gives upward stage movement;
Right twist (clockwise) gives downward stage movement.

To test the stage controller:

1. Start the SpectrumIMAGE software.
SpectrumIMAGE initializes the sample stage.
2. Make sure that you can move the stage in every direction, using both the mouse and the joystick.

Replacing the Stage Controller Fuse

1. Switch off the stage controller, disconnect it from the power supply and remove the mains cord.
2. Insert a screwdriver into the slot at the side of the fuse drawer, and pull out and flip to one side over the mains inlet.
The fuse may now be removed.
3. Fit the replacement fuse into the fuse drawer.
Make sure that you fit the fuse in the top slot. You require a 1.6 A time-lag fuse (part number 09991641).
4. Refit the fuse drawer.

Service

All optical and mechanical equipment requires periodic servicing to keep it performing properly and to compensate for wear. We recommend that the Spotlight imaging system is cleaned, examined, and adjusted periodically by a PerkinElmer Service Engineer.

NOTE: If you experience unexpected problems with the imager, contact your PerkinElmer office or representative immediately.

Accessories

Description	Part Number
Spare fuse for imager (2 A time-lag, 250 V)	04970839
Spare fuse for stage controller (1.6 A time-lag, 250 V)	09991641
Visible Polarizer Kit	L1860294
Sampling Accessory Kit	L1860250
MCT Detector Accessory Kit	L1860554
IR Polarizer Assembly	L1860408
Holder for 13 mm Sampling Disks	L1860409
Hot Stage Accessory (110 V)	N1870184
Compression Cell	N1870185
Hot Stage Accessory (220 V)	N1870188
Rotatable Sample Disk Holder	N1873039
Holder for Reflection-Transmission Measurements	N1873124
BaF ₂ Window (1 mm thick)	N9302611
BaF ₂ Window (2 mm thick)	N9302612
ZnSe Window	N9302613
NaCl Window	N9302614
KBr Window	N9302615
Miniature Diamond Anvil Cell	N9302618
Spare Germanium crystal	L1860268
Spare Silicon crystal	L1860269
Gold Mirror and white reflector Slide (InGaAs systems only)	L1860438

Sample Preparation Tools

These are described in *Tools for Sample Preparation* on page 49.

Electrical Connections

Fitting the Plug

The power cable for the electrical supply plugs into the back of the imager. It has a molded connector at one end. If it is necessary to fit a plug on the power cable, use the wire color code below:

Plug Pin	Wire Color (100/110/120 V)	Wire Color (220/230/240 V)
Ground (Earth)	Green or Green/Yellow	Green/Yellow
Live	Black	Brown
Neutral	White	Blue



WARNING

To ensure safe and satisfactory operation of the instrument, it is essential that the green or green/yellow ground (earth) wire of the power cord is connected to a ground that complies with the regulations of the local electricity supply authority (or equivalent body); ground circuit continuity is essential for safe operation of the equipment.

Connecting the Imager to the Electrical Supply

The imager operates on an electrical supply with a frequency of 50 or 60 Hz and at voltages in the range 100 to 240 V without adjustment.

The stage controller will operate at all voltages in the range 100–240 V and frequencies of 50 or 60 Hz without adjustment.

Fit the molded connector of the power cable into the inlet at the rear of the imager.

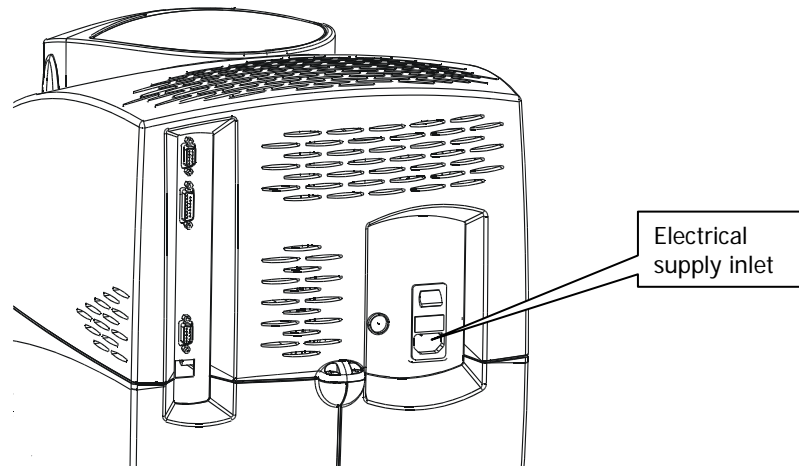



Figure 39 Location of the electrical supply inlet

Imager Connections

- The cable from the imager to the spectrometer comes from the preamp inside the imager, to a connector marked  on the rear of the spectrometer.
- The connection labeled MOTOR X on the stage controller plugs into the connector next to the red dot on the motorized stage.

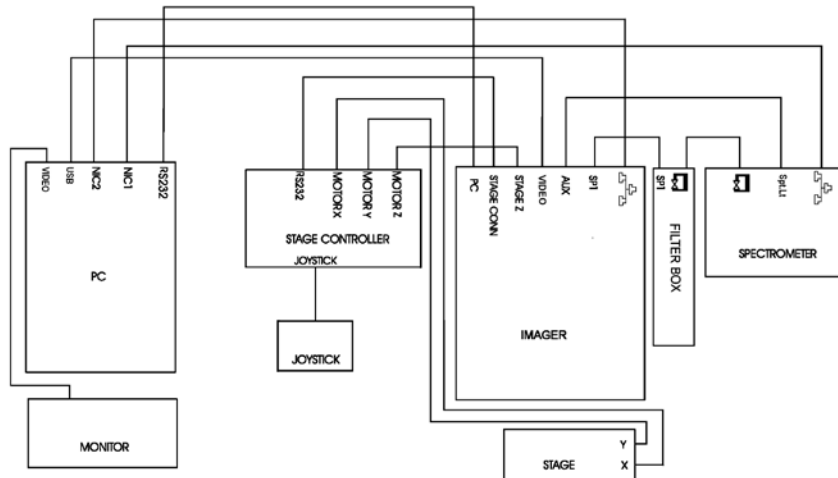
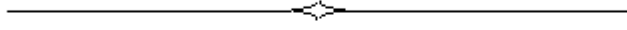


Figure 40 Electrical connections for the imager with USB camera, dual-range spectrometer with filter box, stage controller, & PC

NOTE: The Filter Box connectors are not marked. The gender of the connectors ensures that they are connected correctly.



Appendices

Appendix 1: Installing SpectrumIMAGE Software

NOTE: Spectrum software must be installed BEFORE you start to install SpectrumIMAGE.

CAUTION

If your Spotlight system appears to be faulty, consult a PerkinElmer Service Engineer.

Do not attempt to remedy the problem by re-installing the SpectrumIMAGE software. Important setup or diagnostic information could be lost.

You may need to install a later version of the SpectrumIMAGE software if, for example, you have purchased an accessory that is not supported by the software installed on your PC.

CAUTION

Check the documentation supplied with the accessory to make sure that it is compatible with your Spotlight imaging system.

If you believe a hardware or firmware upgrade may be required, consult a PerkinElmer Service Engineer.

Before installing the SpectrumIMAGE software, you must uninstall any existing SpectrumIMAGE software.

Uninstalling an earlier version of SpectrumIMAGE software

CAUTION

Before you begin, copy the C:\WINDOWS\pelimage.ini file to a safe location on your PC hard drive and to a memory stick.

These backups ensure that, if necessary, the initialization settings for your Spotlight system can be recovered by a PerkinElmer Service Engineer.

NOTE: You must be logged in at Administrator level in Windows before uninstalling or installing software.

1. If you have not done so already, copy the **C:\Windows\pelimage.ini** file to a safe location on your hard drive and to a memory stick.
2. Display the Control Panel, and then select **Add/Remove Programs** (Windows XP) or **Programs and Features** (Windows 7).

3. Select **PerkinElmer SpectrumIMAGE** in the list of programs.
4. Click **Remove** (Windows XP) or **Uninstall** (Windows 7).
The SpectrumIMAGE software is uninstalled, and you are prompted to restart your system.

NOTE: The C:\Windows\pelimage.ini file is not removed. This allows a new SpectrumIMAGE installation to preserve settings from the old.

Installing the SpectrumIMAGE software

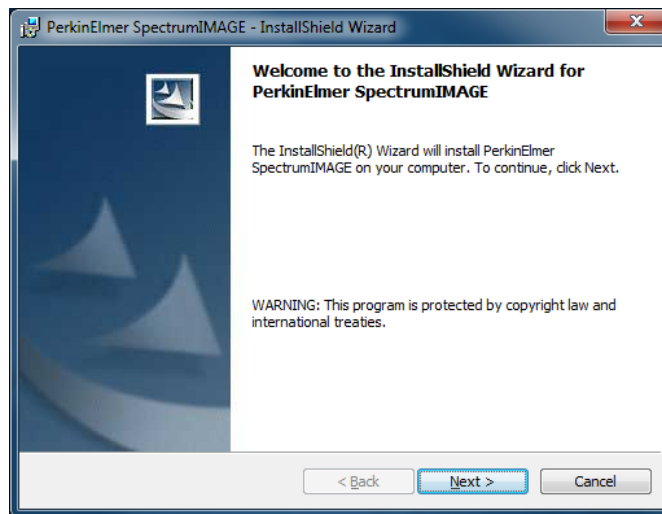
NOTE: Before installing SpectrumIMAGE, read and print the release notes which can be found as a .rtf file at the root of the *SpectrumIMAGE Software DVD*. These notes contain important information that may not be in this User's Guide or the on-screen Help.

After uninstalling any existing SpectrumIMAGE software:

1. Close all open or minimized software windows.
2. Insert the SpectrumIMAGE DVD.

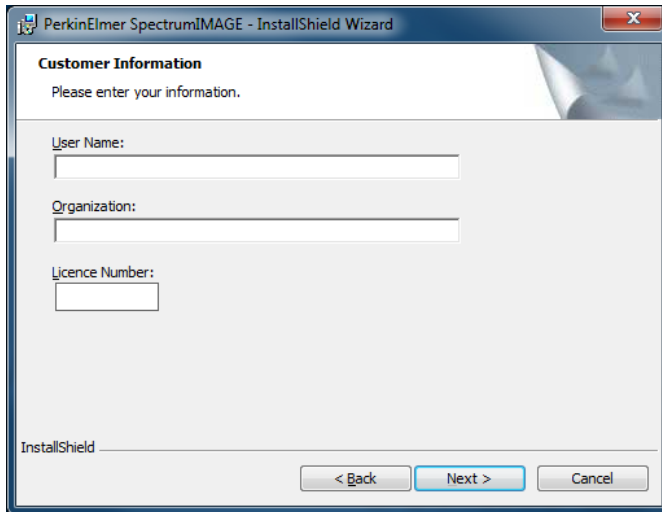
If the DVD does not run automatically, click **Start** and then **Run**. Click **Browse**, select the **SpectrumIMAGE** DVD, and then **setup.exe**. Click **Open** and then **OK**.

After the installation files are prepared, the InstallShield Wizard is displayed.

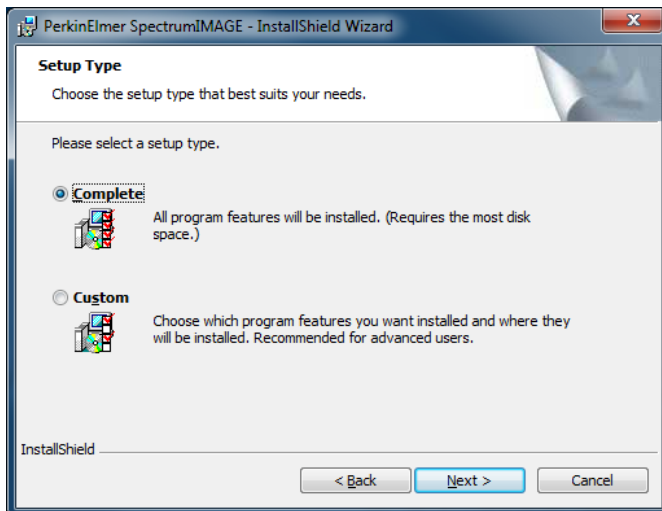


3. Click **Next**.
The License Agreement page is displayed.

4. Select **I accept the terms of the license agreement** and then click **Next**.
The Customer Information page is displayed.

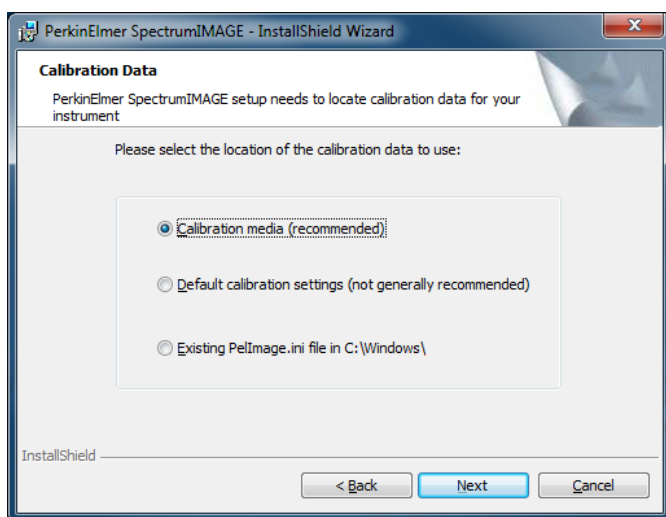


5. Complete the **User Name**, **Organization**, and **Licence Number** fields, and then click **Next**.
The Setup Type page is displayed.



6. Click **Next**.

The Calibration Data page is displayed.



NOTE: For a new SpectrumIMAGE installation, the **Existing PellImage.ini file in C:\Windows** option is not present.

7. For a new SpectrumIMAGE installation, select **Calibration media (recommended)**.

OR

If you are upgrading from an earlier version of SpectrumIMAGE, select **Existing PellImage.ini file in C:\Windows**.

CAUTION

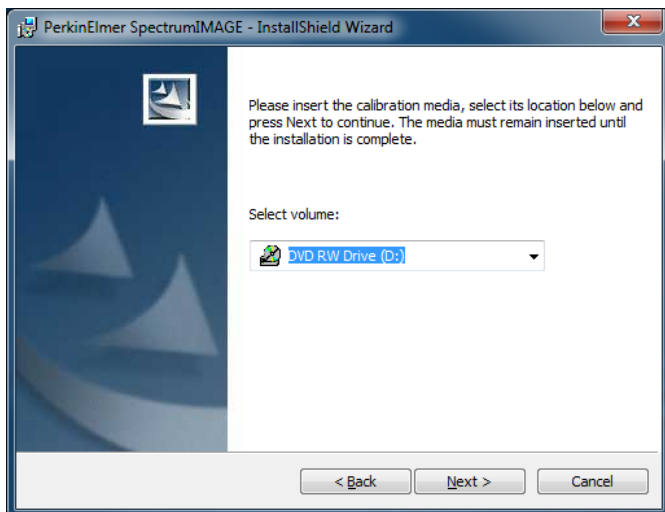
*Do not select **Default calibration settings (not generally recommended)**. This option is only for PerkinElmer Service Engineers.*

For a new installation, the default calibration settings do not include the factory settings for your Spotlight system that are supplied on its Calibration data disk.

If you are upgrading from an earlier version of SpectrumIMAGE, the default calibration settings include neither the factory settings for your Spotlight system, nor any settings that have been customized since the system was installed.

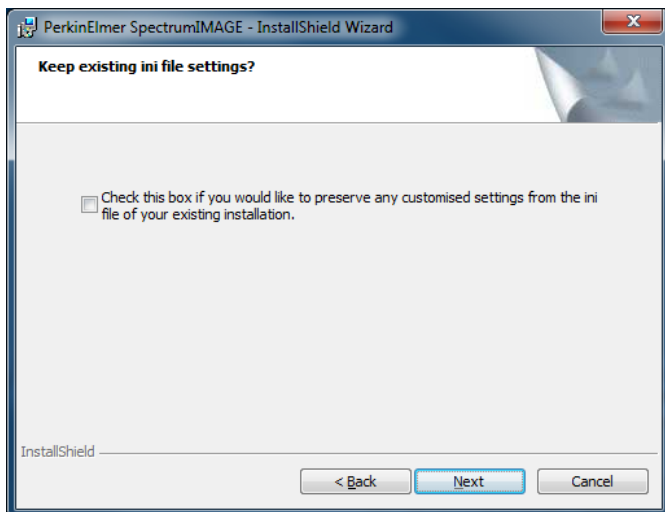
8. Click **Next**.

For a new SpectrumIMAGE installation, you are reminded that the calibration media must remain inserted until the installation is complete.



OR

If you are upgrading an earlier SpectrumIMAGE installation, the Keep existing ini file settings? page is displayed.



This option allows you to keep any customized settings for your system as well as the existing calibration settings.

- For a new SpectrumIMAGE installation, select the location of the calibration media from the drop-down list.

OR

If you are upgrading from an earlier version of SpectrumIMAGE, select **Check this box if you would like to preserve any customized settings from the ini file of your existing installation.**

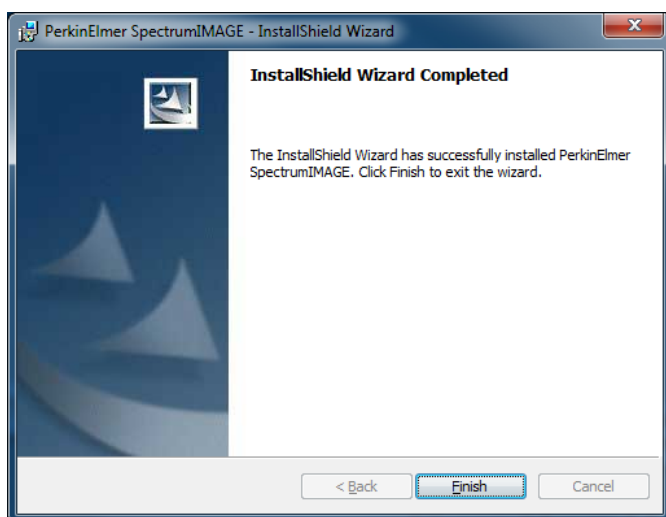
NOTE: If you do not select **Check this box if you would like to preserve any customized settings from the ini file of your existing installation**, your customized settings revert to their factory defaults.

- Click **Next**.

During installation, C:\Windows\pelimage.ini is created. If a previous installation of SpectrumIMAGE has already created this file on your PC, any new settings (such as those required for the ATR Imaging Accessory) are merged with the old settings. The InstallShield Wizard is now ready to install SpectrumIMAGE.

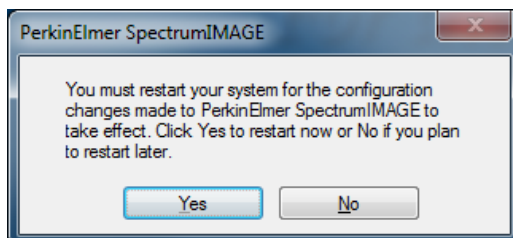
- Click **Install**.

A progress bar is displayed until the InstallShield Wizard has completed the installation.



- Click **Finish**.

You are prompted to restart your PC.



- Remove the calibration media (if applicable), and then click **Yes**.

The PC restarts.

You may need to modify the display properties and power save options for your PC. Refer to *Appendix 2: Amending the Power Options Display Settings* on page 114.

Appendix 2: Amending the Power Options Display Settings

For optimum performance it may be necessary to amend the power options display settings for your PC.

Power Options

1. Display the Power options in the Control Panel.
2. For Windows XP, ensure that the following options on the Power Schemes tab for the currently selected power scheme are set to **Never: Turn off monitor, Turn off hard disks, System standby and System hibernates.**

OR

For Windows 7, select **Change plan settings** for the currently selected power scheme, then **Change advanced power settings** and ensure that the following options are set to **Never (0 minutes): Turn off display after, Turn off hard disk after, Sleep after and Hibernate after.**

Display Options

Windows 7

1. Place the mouse pointer in a clear area of the desktop, click the right mouse button and then select **Screen Resolution.**
2. Set the screen **Resolution** to **1280 by 1024** (or better).
This is the recommended screen resolution for SpectrumIMAGE.
3. Click **Advanced Settings.**
The settings dialog for the graphics card is displayed.
4. Select the **Monitor** tab, and then set the **Screen refresh rate** to **75 Hertz.**
5. Click **Apply**, and then click **OK.**
6. Close the Control Panel.
7. Reboot the PC.

Windows XP

1. Place the mouse pointer in a clear area of the desktop, click the right mouse button and then select **Properties.**
The Display Properties dialog is displayed.
2. Select the **Settings** tab and set the **Screen resolution** to **1280 by 1024** (or better).
This is the recommended screen resolution for SpectrumIMAGE.

3. Click **Advanced**.
The settings dialog for the graphics card is displayed.
4. Select the **Monitor** tab, and then set the **Screen refresh rate** to **75 Hertz**.
5. Click **Apply**, and then click **OK**.
6. Close the settings dialog for the graphics card and then close the **Display Settings** dialog.
7. Reboot the PC.

Appendix 3: Decontamination

Before using any cleaning or decontamination methods except those specified by PerkinElmer, users should check with PerkinElmer that the proposed method will not damage the equipment.

Customers wishing to return instrumentation and/or associated materials to PerkinElmer for repair, maintenance, warranty or trade-in purposes are advised that all returned goods must be certified as clean and free from contamination.

The customer's responsible body is required to follow the "Equipment Decontamination Procedure" and complete the "Certificate of Decontamination". These documents are available on the PerkinElmer public website:

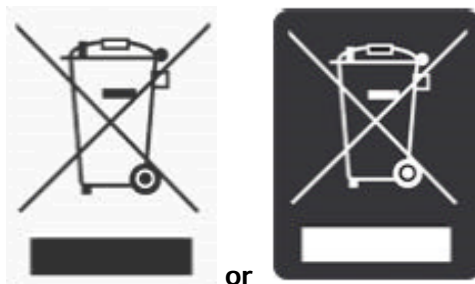
<http://las.perkinelmer.com/OneSource/decontamination.htm>

Alternatively, if you do not have access to the internet contact Customer Care:

Customer Care USA:	1-800-762-4000	(inside the USA)
(8:30 a.m. – 7 p.m. EST)	(+1) 203-925-4602	(outside the USA)
Customer Care Canada:	800-561-4646	
Customer Care EU:	0800 40 858	(Brussels)
	0800 90 66 42	(Monza)

If you are located outside of these regions, please call your local PerkinElmer sales office for more information.

Appendix 4: WEEE Instructions for PerkinElmer Products



A label with a crossed-out wheeled bin symbol and a rectangular bar indicates that the product is covered by the Waste Electrical and Electronic Equipment (WEEE) Directive and is not to be disposed of as unsorted municipal waste. Any products marked with this symbol must be collected separately, and in accordance with the regulatory guidelines in your area.

The objectives of this program are to preserve, protect and improve the quality of the environment, protect human health, and utilize natural resources prudently and rationally. Specific treatment of WEEE is indispensable in order to avoid the dispersion of pollutants into the recycled material or waste stream. Such treatment is the most effective means of protecting the customer's environment.

The requirements for waste collection, reuse, recycling, and recovery programs are set by the regulatory authority in your location. Contact your local responsible person (such as your laboratory manager) or authorized representative for information regarding applicable disposal regulations.

See the PerkinElmer web address below for information specific to PerkinElmer products, and contact details for the Customer care department in your region.

<http://las.perkinelmer.com/OneSource/Environmental-directives.htm>

Products from other manufacturers may also form a part of your PerkinElmer system. These other manufacturers are directly responsible for the collection and processing of their own waste products under the terms of the WEEE Directive. Please contact these manufacturers directly before discarding any of their products. Consult the PerkinElmer web address (above) for manufacturer's names and web sites.

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