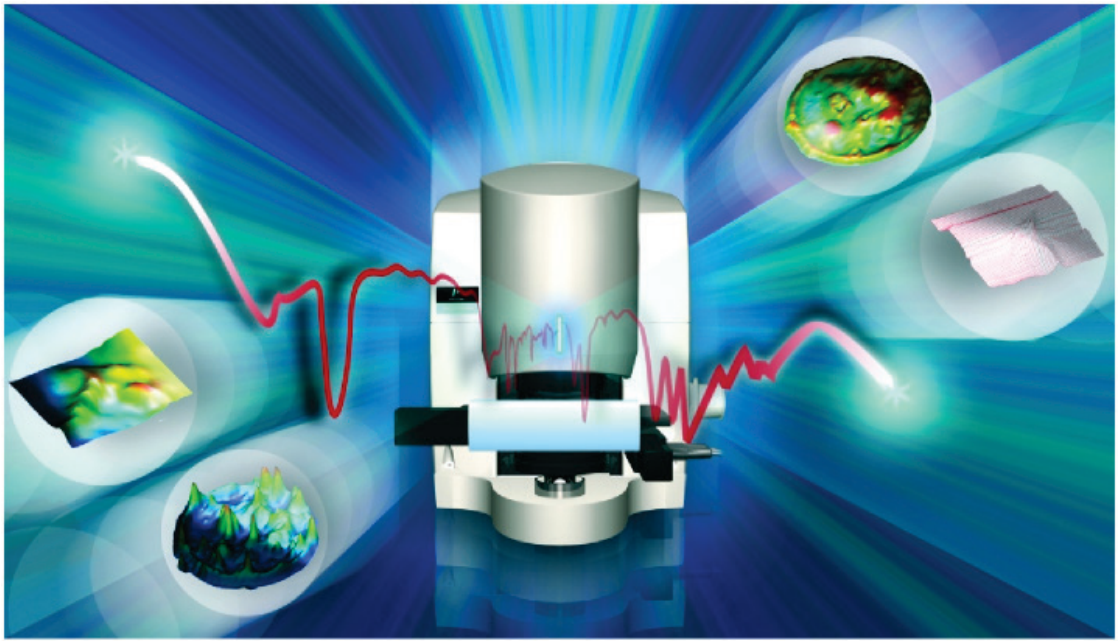
MOLECULAR SPECTROSCOPY

Spotlight 200



User's Guide

PerkinElmer®

Release History

Part Number	Release	Publication Date
L1050036	H	October 2015

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Contents

Introduction

About this Manual

This introduction gives you information about this manual, to enable you to use it effectively when learning to operate the Spotlight 200.

Before you start using your Spotlight 200 we recommend that you:

- Read the *Warnings and Safety Information* starting on page 13;
- Read the rest of this *Introduction* and become aware of conventions and requirements;
- Read *Overview of the Spotlight 200* starting on page 29;
- Read *Getting Ready to Use the Spotlight 200* starting on page 41.

Four chapters in this User's Guide contain information on using and maintaining your Spotlight 200:

- *Getting Ready to Use the Spotlight 200* gives you information on how to set up your Spotlight 200 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 Spotlight 200.
- *Techniques for Collecting Spectra* describes how you can use accessories and different techniques to collect spectra.
- *Maintenance* contains maintenance information. It gives you information on how to care for the Spotlight 200 and about the performance checks that we recommend that you perform routinely.

Conventions Used

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 or Spectrum One spectrometer supplied with your Spotlight 200.

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	*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.*
D	***Caution (Achtung)*** *Bedeutet, daß die genannte Anleitung genau befolgt werden muß, um einen **Geräteschaden** zu vermeiden.*
DK	***Caution (Bemærk)*** *Dette betyder, at den nævnte vejledning skal overholdes nøje for at undgå en **beskadigelse af apparatet**.*
E	***Caution (Advertencia)*** *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.*
F	***Caution (Attention)*** *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	***Caution (Attenzione)*** *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.*
NL	***Caution (Opgelet)*** *Betekent dat de genoemde handleiding nauwkeurig moet worden opgevolgd, om **beschadiging van het instrument** te voorkomen.*
P	***Caution (Atenção)*** *Significa que a instrução referida tem de ser respeitada para evitar a **danificação do aparelho**.*

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 Spectrometer

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. The *Spectrum Two Manuals CD* (L1050242) contains the instrument manual for the Spectrum Two spectrometer.

Spectrum Software

Spectrum software has on-screen Help, which you can access by choosing the **Contents** command from the Help menu, by pressing the F1 key, or by clicking the **Help** button in a dialog. The Help information assumes that you are familiar with the hardware components of the Spotlight 200 microscope contained in this guide.

Requirements for using the Spotlight 200

We assume that the Spotlight 200 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 Spotlight 200. 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 200

The Spotlight 200 consists of a microscope, spectrometer, PC, stage controller and joystick.

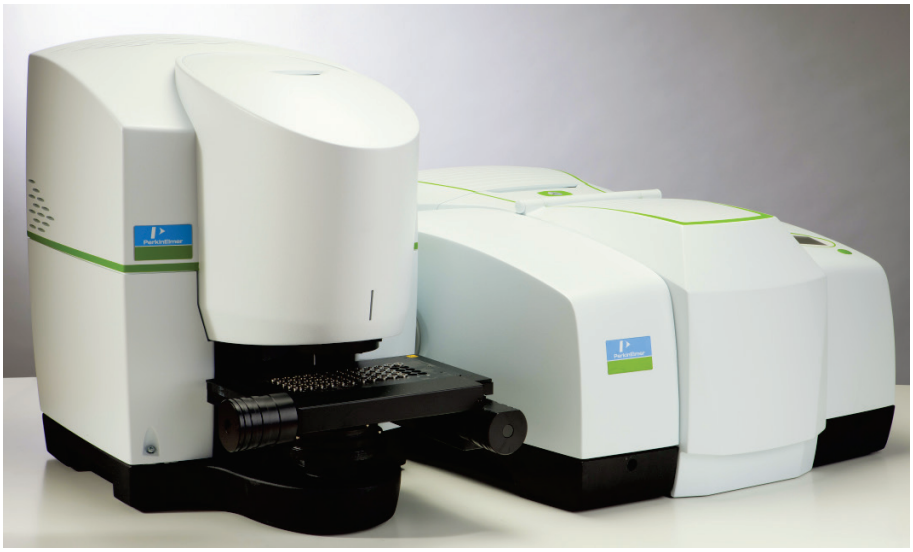


Figure 1 Spotlight 200 – microscope and Frontier IR System

Figure 2 Spotlight 200 – microscope and Spectrum Two System

Summary

The PerkinElmer Spotlight 200 has been designed to comply with a wide variety of international standards governing the safety of laboratory equipment. In routine use, the Spotlight 200 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 Spotlight 200.

- DO make sure that all parts of the Spotlight 200 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 microscope and spectrometer.

- DO keep the microscope dry. Avoid spilling liquid into the microscope and spectrometer. Clean all external spills immediately. If anything that is spilled enters the main body of the microscope or spectrometer, switch off the power and call a PerkinElmer Service Engineer.

- If your Spotlight 200 is fitted with an MCT (mercury cadmium telluride) detector, DO wear safety glasses and protective gloves when you are filling the detector dewar in the microscope 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 use a flammable gas to purge the spectrometer or microscope. 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.

- If your Spotlight 200 is fitted with an automated ATR objective, DO NOT look directly at either the stage lighting LEDs or the window at the back of the weighbridge, particularly if you are using an optical magnifier that could focus the visible or infrared beams from these sources.

- 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.

The microscope 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 microscope and stage controller conform to IEC publication 61010−1 ("Safety requirements for electrical equipment for measurement, control, and laboratory use") as it applies to IEC Class 1 (earthed) appliances and therefore meets the requirements of EC low voltage directive 2006/95/EC.

Only use the microscope 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 and repair of the opened, operating instrument. If any adjustment, maintenance and 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 microscope and stage controller are unsafe, make them inoperative. The microscope 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 microscope 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 ±10% of the nominal voltage. For example, 230 V ± 10%.

WARNING

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

Electrical Safety

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

Only plug the microscope and stage controller into electricity-supply sockets that are provided with a protective ground (earth) connection. The stage control box and the microscope must be earthed.

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 microscope 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 microscope or the stage controller.

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

Disconnect the microscope 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.

The microscope 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).

WARNING

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

Location and Ventilation

The Spotlight 200 is installed by a PerkinElmer Service Engineer, who will be able to advise on the positioning 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 microscope and stage control box to permit adequate cooling.

WARNING

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

Warning Labels

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 which have to be taken.

Microscope Safety Labels

The following safety labels are fixed to the microscope.

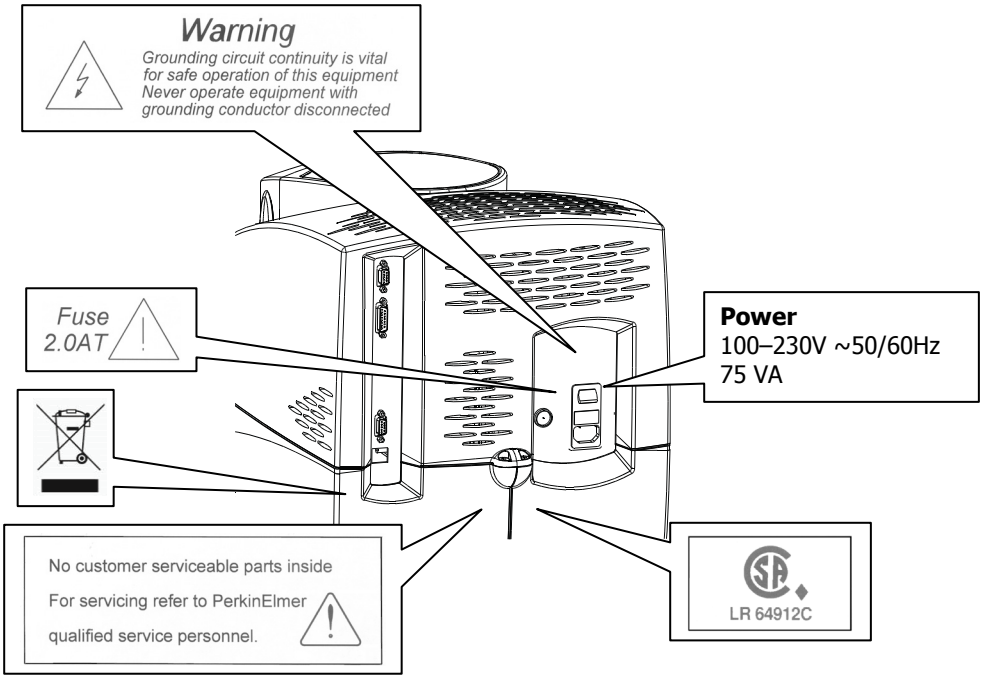


Figure 3 Rear of Microscope

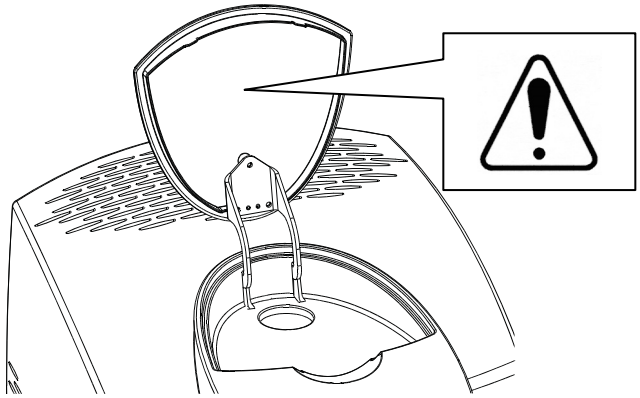


Figure 4 Inside Dewar lid – MCT detector versions only

NOTE: See *Cooling the MCT Detector* on page 43 for information on how to fill the Dewar.

Stage Controller Safety Labels

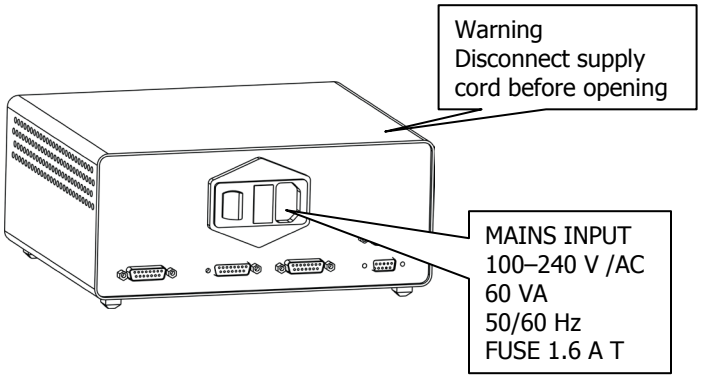


Figure 5 Rear of Stage Controller

Warning Signs on the Microscope

Caution, risk of electric shock.

Caution, risk of danger.

Refer to accompanying documents to find out the nature of the potential hazard and any actions which have to be taken.

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 200

WARNING

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.

The microscope weighs approximately 35 kg, including the motorized stage.

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

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

Do not move the Spotlight 200 after it has been installed without consulting your local PerkinElmer service department.

EMC Compliance

EC directive

The Spotlight 200 has been designed and tested to meet the requirements of the EMC Directive 2004/108/EC.

The Spotlight 200 complies with the EMC standard EN61326, (EMC standard for electrical equipment for measurement, control and laboratory use) and EN55011 (ISM) class A (rf emissions).

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 Spotlight 200.

Electrical Requirements

The power consumption of the microscope 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 (65 VA for the Spectrum Two).

The line supply must be within 10% of the nominal voltage. For example, 230 V ± 10%.

If possible, do not connect any parts of the Spotlight 200 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.

Microscope

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

NOTE: No voltage selection is required.

Site Requirements

A minimum bench space of 170 x 75 cm (68 x 30 inches) is required to accommodate the microscope, spectrometer, PC and ancillaries.

To get the best performance from your Spotlight 200:

- Place the Spotlight 200 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 Spotlight 200 near to room heating equipment, for example central heating radiators.

Leave a gap of at least 15 cm (6 inches) to the sides of the microscope and stage controller box to permit an adequate flow of cooling air.

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

Safety Specifications

Microscope

Power supply	100–230 V ± 10%, 50/60 Hz ± 10%
Primary fuse	2.0 A T (time-lag), 250 V
Weight	32 kg without the motorized stage

Stage Controller

Power supply	100–240 V ± 10% , 50/60 Hz ± 10%
Primary fuse	1.6 A T (time-lag), 250 V The control box is not designed for use with all three motors simultaneously over extended periods
Maximum speed	6 mm/s
Maximum motor voltage	24 V dc
Stepping resolution	Maximum 6400 steps/rev, 156 nm/step
Baud rate	9600
Travel	75 mm x 50 mm or 215 mm x 100 mm
Weight	Stage 2.7 kg Control box 2.8 kg

Overview of the Spotlight 200

A Guided Tour of the Spotlight 200

The Spotlight 200 enables you to collect IR spectra from extremely small samples. The PerkinElmer cassegrain collection optics give high-performance infrared microspectroscopy. The microscope 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 image of your sample is displayed in the Monitor Visible window on your PC monitor. Spectrum software enables you to control the operation of your Spotlight 200, and collect IR spectra from the sample.

The microscope 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.
- Spectra can be collected in either reflectance or transmittance modes.
- 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.
- A motorized stage, controlled by a joystick and the Spectrum software, enables you to find points on your sample and focus the microscope. Two sizes of stage are available:
  - Sample sizes up to 75 × 50 mm (part number L1860160)
  - Sample sizes up to 160 × 69 mm (part number L1860161)

  Some versions of the microscope feature LED lighting of the stage area.
- Spectrum software, which controls the operation of the microscope, for example: focus, illumination, stage position, adjusting the correction, and changing between reflectance and transmittance sampling modes.
- Auto-correction to optimize the position of the lower cassegrain for maximum energy throughput.
- A micro-ATR objective for data collection from optically thick and non-reflective samples. Manual and automatic ATR objectives are available; the automatic ATR option also includes a weighbridge to measure the pressure applied to the sample during analysis.
- Automatic image analysis, data collection and post-run processing of collected spectra.
- A single cassegrain to send both infrared radiation and visible light to the remote aperture. The microscope continuously views and monitors infrared concurrently; there is no beampath switching between visible and infrared.
- A removeable lower cassegrain to provide space for thick samples to be studied in reflectance mode.
- An InGaAs (indium gallium arsenide), DTGS (deuterated triglycine sulfate), or an MCT (mercury cadmium telluride) MIR detector.

Connections

Figure 6 to Figure 9 show the input and output connections on the microscope and stage controller.

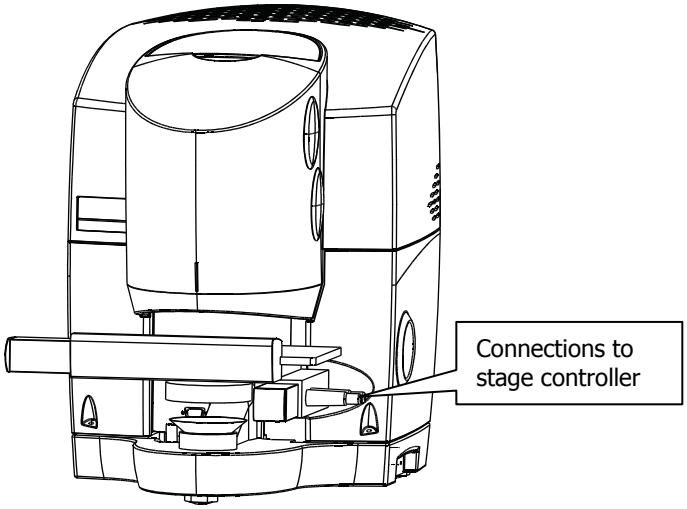


Figure 6 Connections to Stage Controller

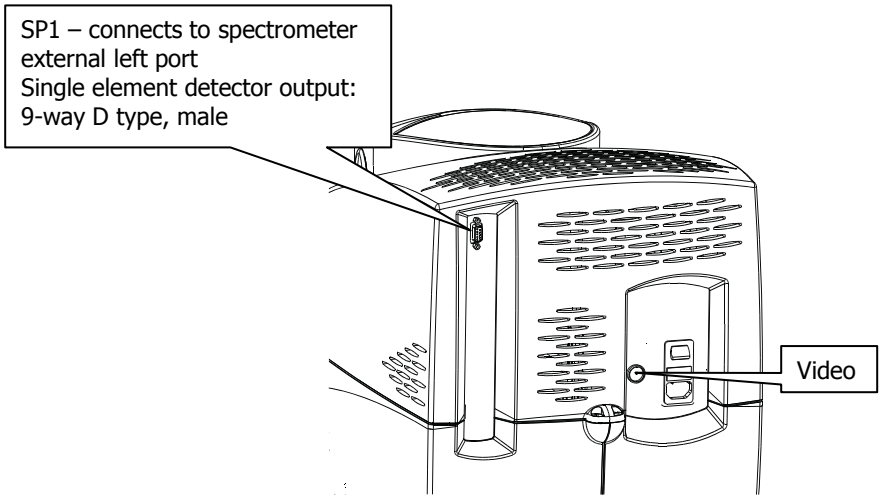


Figure 7 Electrical Connections (top rear of imager)

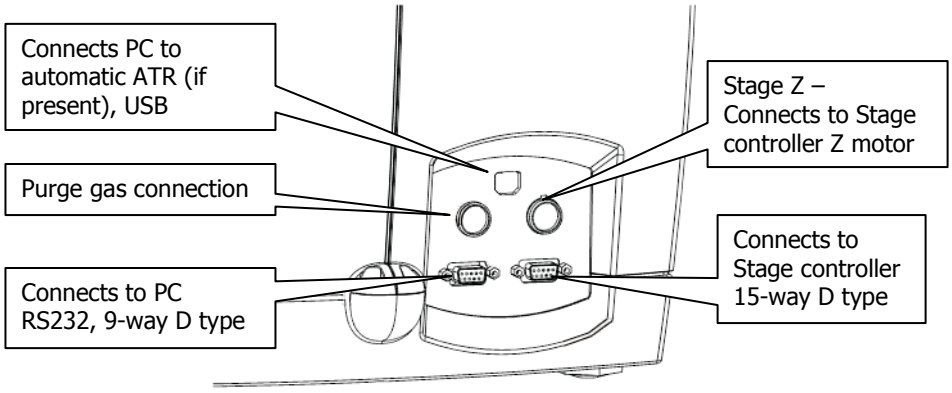


Figure 8 Electrical Connections (bottom rear of imager)

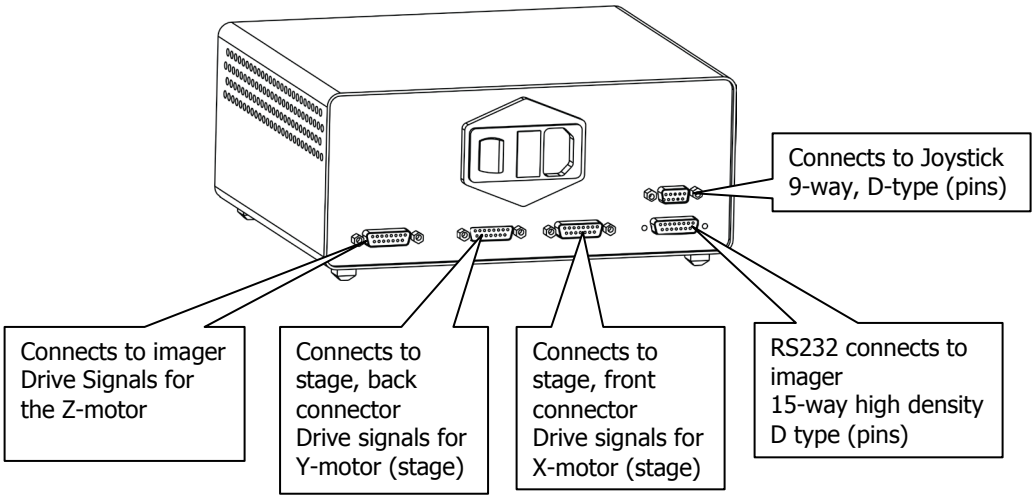


Figure 9 Stage Controller Electrical Connections

Operation

Spectrum software enables you to mark points of interest on the sample. 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 or within a marked area.

For further information, see the Spectrum on-screen help.

The table below lists the modes of operation that should be used for particular samples.

Transmittance	Single areas of thin solids, fibers and films. The optimal aperture range depends upon the detector type, as follows: **MCT detector:** Between 10 μm and 100 μm. **DTGS detector:** Between 50 μm and 200 μm. **InGaAs detector:** Between 10 μm and 200 μm.
Reflectance	Single areas of coatings and thick solids. The optimal aperture range depends upon the detector type, as follows: **MCT detector:** Between 10 μm and 100 μm. **DTGS detector:** Between 50 μm and 200 μm. **InGaAs detector:** Between 10 μm and 200 μm.
With ATR objective	Thick, non-reflecting samples.

NOTE: In general, the larger the aperture setting, the better the spectral quality. Sample sizes may be larger than the maximum aperture sizes given in the table above.

The Optical System

The microscope 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.

Spotlight 200 enables you to select between transmittance and reflectance operation. This section describes what happens within the optical system when the system changes from visible light to infrared mode in transmittance and reflectance operation.

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

Visible Light Optics

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.

Visible light optics in transmittance

When the microscope 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.

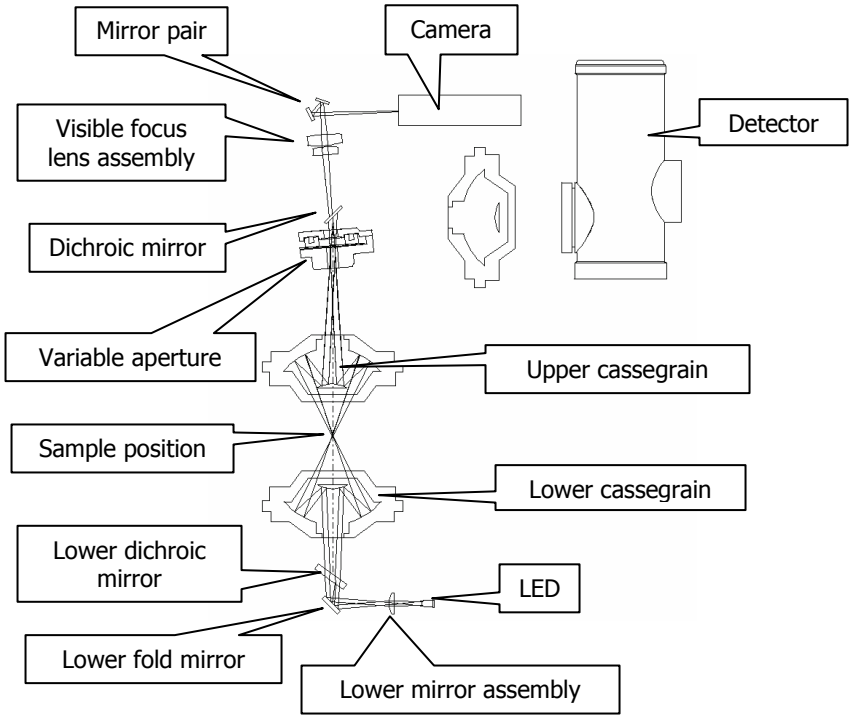


Figure 10 Path of the Visible Beam for Viewing a Sample in Transmittance

NOTE: Figure 10, above, illustrates a Spotlight 200 with an MCT detector. Other detector types may look different, but the optical arrangement is the same in all cases.

Visible light optics in reflectance

When the microscope is in viewing mode in 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 mode, giving simultaneous illumination from above and below.

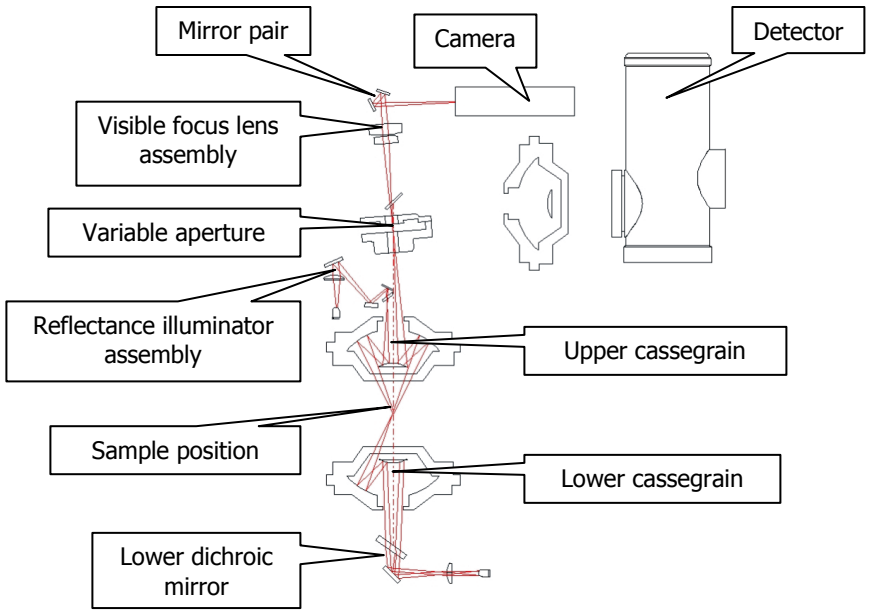


Figure 11 Path of the Visible Beam for Viewing a Sample in Reflectance

NOTE: Figure 11, above, illustrates a Spotlight 200 with an MCT detector. Other detector types may look different, but the optical arrangement is the same in all cases.

Infrared Optics

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 infrared spectra. Similarly, when you adjust the aperture so that the required area of the sample is isolated visually, you have also isolated the area of the sample from which the IR spectrum is to be collected.

IR optics in transmittance

IR optics in transmittance differs from when viewing a sample 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.

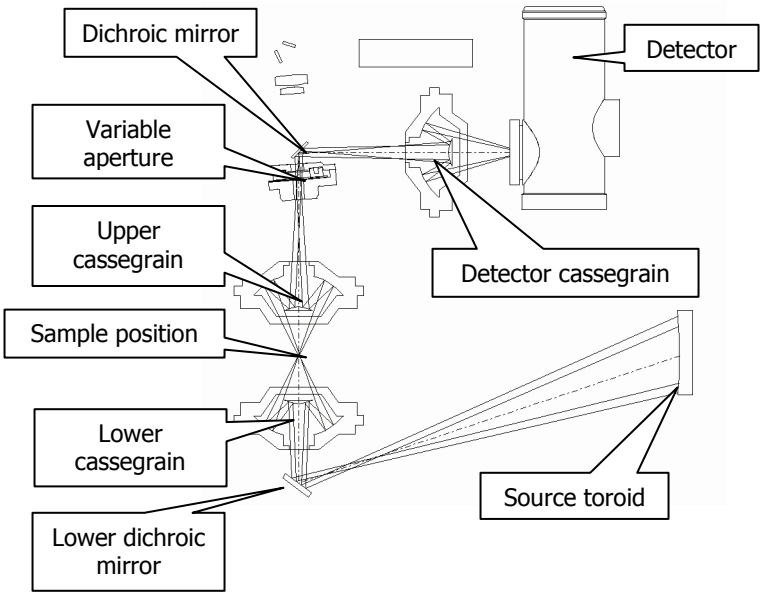


Figure 12 Path of the Infrared Beam for Collecting an Image in Transmittance

NOTE: Figure 12, above, illustrates a Spotlight 200 with an MCT detector. Other detector types may look different, but the optical arrangement is the same in all cases.

IR optics in reflectance

IR optics in reflectance differs from viewing in reflectance 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.

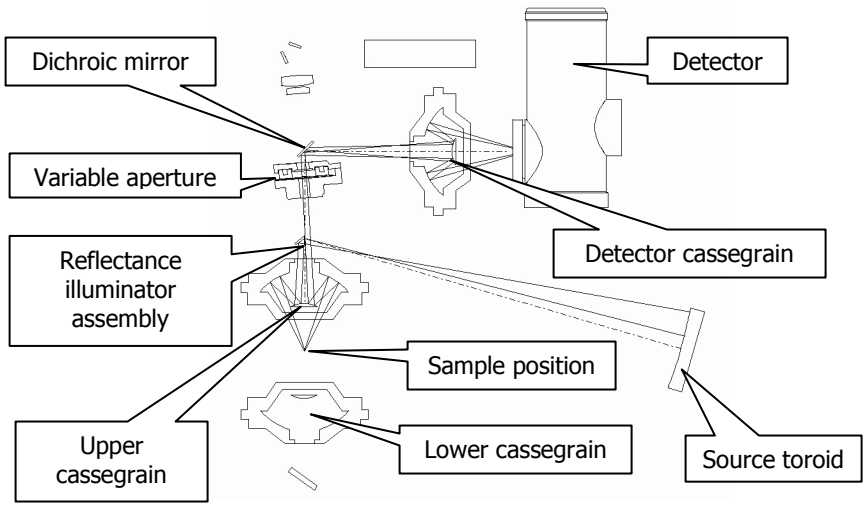


Figure 13 Path of the Infrared Beam for Collecting IR Spectra in Reflectance

NOTE: Figure 13, above, illustrates a Spotlight 200 with an MCT detector. Other detector types may look different, but the optical arrangement is the same in all cases.

Spotlight System Requirements

Spotlight 200 requires additional cards 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 cards from the PC or install the software on another PC.

Hardware Requirements

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

- Intel® Pentium 4, 2 GHz processor (or greater) – dual-core or hyper-threaded preferable
- At least 3 GB of Random Access Memory (RAM)
- 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.

- 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
- USB port
- 1 free PCI slot
- Network port.

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

Software Requirements

This software requires that either Windows® XP Professional Service Pack 3 (or greater), or Windows® 7 Professional (32-bit or 64-bit), or Windows® 8.x Pro (32-bit or 64-bit) operating system is installed on the PC before you install Spectrum.

Microsoft Service Packs and Updates can be downloaded from www.microsoft.com/downloads.

Getting Ready to Use the Spotlight 200

Before Using the Spotlight 200

Before you use the Spotlight 200 you must:

- Make sure the spectrometer is switched on;
- Switch the stage controller and microscope on;
- Cool the detector (MCT detector versions only);
- Set up the microscope;
- Focus the microscope.

We recommend that you use these procedures at the beginning of the day's work, or any time the Spotlight 200 has not been in use or has been used by others.

Cooling the MCT Detector

If the microscope is fitted with an MCT (mercury cadmium telluride) detector, the detector must be cooled to −196 °C 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.

NOTE: If your Spotlight 200 is fitted with a DTGS or InGaAs detector, cooling with liquid nitrogen is not required.

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 microscope.

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 microscope after each time you fill the funnel.

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.

1. Open the flap covering the dewar.

2. Remove the dewar cap.

3. Place the small funnel supplied with the microscope in the opening in the detector dewar (Figure 14).

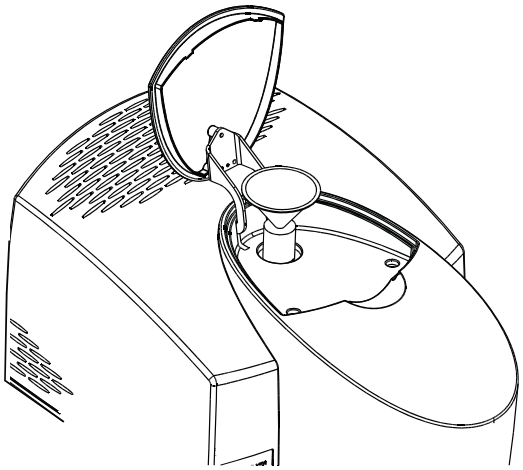


Figure 14 The Dewar Opening with the Funnel Inserted

CAUTION	*Stand where you can see the inside of the funnel as you pour the nitrogen in, but without positioning your head over the funnel itself. 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 microscope.*

4. 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.

5. 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.

6. 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.

   Now the dewar has cooled, the liquid nitrogen does not vaporize, but instead fills the dewar.

7. Remove the funnel and wait two minutes.

   The liquid nitrogen settles down and bubbling slows.

8. 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 Spotlight 200

To set up your Spotlight 200:

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 microscope at the rear switch.
   The blue LED light at the front comes on.

3. Switch on the stage controller.
   The green LED light at the front comes on.

4. At your PC, click **Start** and then select **Spectrum** in the **PerkinElmer Applications** group under **All Programs**.
   Spectrum software starts.

5. Enter your **User name** and, if required, your **Password**.

6. Select the spectrometer connected to your Spotlight 200.

7. Select **Instrument** from the **Setup** menu, and click the **Setup Instrument BeamPath** tab.

8. Click the microscope detector  to direct the beam to the microscope.
   The beampath diagram and accessory toolbar are updated to show that the microscope is connected.

9. Click the microscope  button on the accessory toolbar.

10. Make sure that the lower cassegrain is fitted, that the ATR objective crystal (if fitted) is retracted, and that there is no sample on the stage, and then click **Start**.
    The microscope initializes.

NOTE: If you want to avoid initializing the microscope at the start of a session, click **Skip** to apply the settings from the previous session. This option is only available if the microscope has previously been initialized.

Focusing the Microscope

The focus of the microscope is changed by moving the sample stage up or down. The Z-control on the joystick, or the **Auto-Focus** button on the Setup Microscope Basic tab, enables you to focus the image in the Camera View.

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 image

1. Select **Microscope** > **Stage Move** > **To Load Position** to move the sample stage to a position where a sample can be loaded easily.

2. Place your sample on the sample stage.

3. Select the **Sampling Mode** in the Setup Microscope Basic tab.
   You can also switch between Transmittance and Reflectance modes using the button on the accessory toolbar.

4. Select **Microscope** > **Stage Move** > **To Center 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

   Click **Auto-Focus** in the Setup Microscope Basic tab.

   OR

   Click the  button in the Camera View toolbar.
   The image of the sample is focused.

> **NOTE:** If your Spotlight 200 system is supplied with a Frontier or Spectrum 400 FT-IR/FT-NIR dual-range spectrometer and this has been setup to work in the near infrared, you may find that there is a red tint to the image when viewed in the visible range. To remove this, fit a 1%T attenuator to the external beam window of the spectrometer. See *Fitting an Attenuator to the Spectrometer* on page 48 for details.
>
> This attenuator should be removed during data acquisition.

6. If you need fine focus, use the **Adjust Up**  or **Adjust Down**  buttons on the accessory toolbar to move the stage a small distance along the z-axis.
   You can select the size of the Z-Axis Adjustment in the Setup Microscope Basic tab.

7. If you are examining the sample in transmittance mode, you may need to adjust the position of the lower cassegrain so that the field of view is evenly illuminated (this is especially useful if the sample is in a compression cell or held between windows of high refractive index):
   Adjust the **Correction** control in the Setup Microscope Advanced tab by clicking the up  or down  buttons.

   OR

   Click **Maximize Energy** in the Correction section of the Setup Microscope Advanced tab.

   OR

   Click the  button in the Camera View toolbar.

NOTE: If your Spotlight 200 system is supplied with a Frontier or Spectrum 400 FT-IR/FT-NIR dual-range spectrometer and you are using a large aperture in the near infrared, the 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 48, for details.

This attenuator should be kept in place during data acquisition.

8. Adjust the **Illumination** control in the Setup Microscope Basic tab to give the required level of illumination.

   OR

   Click **Auto** in the Illumination section in the Setup Microscope Basic tab.

   OR

   Click the  button in the Camera View toolbar.

Fitting an Attenuator to the Spectrometer

If your Spotlight 200 system is supplied with a Frontier or Spectrum 400 FT-IR/FT-NIR dual-range spectrometer, 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 to the external beam window of the spectrometer magnetically.

When examining samples in the visible range on systems fitted with an MCT detector, use of the 1%T attenuator is recommended if the image appears with a red tint. However, the attenuator should be removed during data acquisition.

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

Setting Scan Parameters

1. Select **Instrument** from the **Setup** menu.
2. Click the **Setup Instrument Basic** tab.
3. Click the **Resolution** setting to display the drop-down list.

   For mid-infrared measurements, it is recommended that the spectral resolution is set to 8 cm^{-1}; for near infrared, this value should be set to either 8 or 16 cm^{-1}.

4. Select the **Start** and **End** values for the scan.

   For mid-infrared analysis, it is normal to set the upper wavenumber limit to 4000 cm^{-1} and the lower limit to the low wavenumber specification of the detector, as shown in the table below.

Detector type	**Low wavenumber limit (cm^{-1})**
MCT mid-band	580
MCT wide-band	450
DTGS	380

   The Service engineer can advise you of the lower limit to the low wavenumber, at the time of installation, or it will be recorded on the Service Installation test spectra.

   For near infrared measurements, on a system fitted with an InGaAs detector, the upper wavenumber value is typically set to between 15800 and 8000 cm^{-1} and the lower limit to 4000 cm^{-1}.

NOTE: To work in the near infrared range of the spectrum, the Spotlight 200 must be attached to a dedicated NIR spectrometer or a dual-range spectrometer which has been set up to operate in the near infrared range.

The **Setup Instrument Basic** tab also provides an option for selecting the number of accumulations. There are no firm rules about these selections since the number of accumulations needed to generate acceptable spectra will depend on the nature of the sample, the size of the area being analyzed and the requirements of the application.

For single point analyses where the analysis is relatively quick, it is probably better to "over-scan"; 50 accumulations taken at 8 cm^{-1} resolution using an MCT or InGaAs detector (20 accumulations for a DTGS detector) can be collected reasonably quickly.

For mapping or imaging experiments, the total time for the analysis is normally very important and most analysts set the number of accumulations to be the very minimum required to get a sufficiently useable spectrum. In mapping, this may be 1 to 5 accumulations per point and for imaging it may be 15 or 30 accumulations per pixel for the background image and 2 or 4 accumulations per pixel for the sample image.

Optimizing Transmittance measurements

In most cases, samples for transmittance measurements are placed flat on top of an IR-transmitting window such as NaCl, KBr, BaF_2, ZnSe or diamond. In some cases, such as flattened fibers, the sample is simply secured across an open aperture mounted in the standard sample holder.

These samples should be prepared such that they are thin, ideally 10 to 25 microns, but certainly thinner than 50 microns (see section on sample preparation) and also they should sit as flat on the window as possible.

In transmittance measurements, the infrared beam passes through the window supporting the sample and this will alter the beam characteristics (focus, etc.). This variation will be different for different window materials depending on their refractive indices. To optimize the infrared energy through the window (and sample), the lower cassegrain is moved up or down using the **Correction** facility on the **Setup Microscope Advanced** tab to compensate for this refractive index variation.

1. Place the thin, flat, sample onto a window (or across an aperture) and use the joystick to bring the sample into visible focus.

   If necessary, use the **Auto-Focus** option to focus the image.

2. Set the apertures to the appropriate size for the sample area, and mark this position using the **Add Marker** option from the **Microscope** menu.

3. Use the joystick to move the sample away from the center of the viewing screen, and focus on the top surface of the window.

   Use the **Monitor** function (from the Measurement toolbar) to monitor the energy throughput. Either manually adjust the lower cassegrain for maximum energy using the up ^ or down v buttons of the **Correction** function, or use the **Maximize Energy** facility.

4. Once the maximum energy through the window (or aperture) has been achieved, click **Halt** on the Measurement toolbar.

5. Run a background spectrum through this point on the window (or aperture) using the correct aperture size as selected previously.

6. From the **Stage Move** menu in the **Microscope** menu, select **To Selected Marker** and record the sample spectrum.

This procedure for correction of window materials should be done for single point, mapping and imaging experiments.

If the same window is used repeatedly for analysis, this height correction of the lower cassegrain need only be done once.

Preparing Samples

Preparing Samples

The Spotlight 200 enables you to examine the sample in the Camera View window to choose 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; otherwise, 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 200. 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.

Tools for Sample Preparation

This section lists the tools you need for preparing samples:

- Tools provided with the Spotlight 200;
- Tools in the microsampling toolkit;
- Materials to have available;
- Specialized accessories you may want to purchase.

Tools Provided with the Spotlight 200

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 assembly	Reflection measurements
KBr windows (2)	Supporting samples

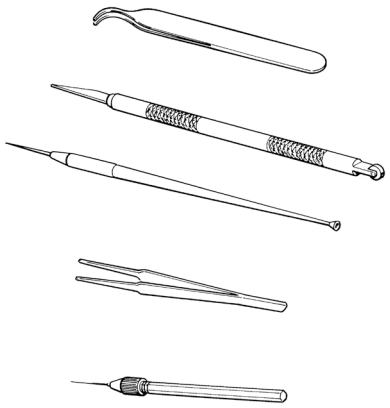


Figure 16 Some of the Tools in the Microsampling Toolkit

The following tools are provided in the Microsampling toolkit (see Figure 16).

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

The following 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 58):

- 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 200, 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

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.

- BaF_2: 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.

Techniques for Preparing Samples for Transmittance Measurements

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. As 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 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 *Diamond anvil cell* on page 60 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 BaF_2 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.

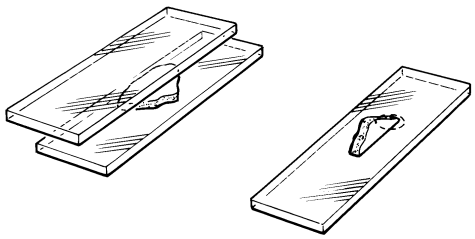


Figure 17 Cutting a Wedge-Shaped Sample

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 microscope so that the infrared beam goes through the thin end of the wedge (circled in Figure 17).

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

CAUTION *One diamond can damage another.*

5. Press down on the cell with your thumb to thin the sample.
6. 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.

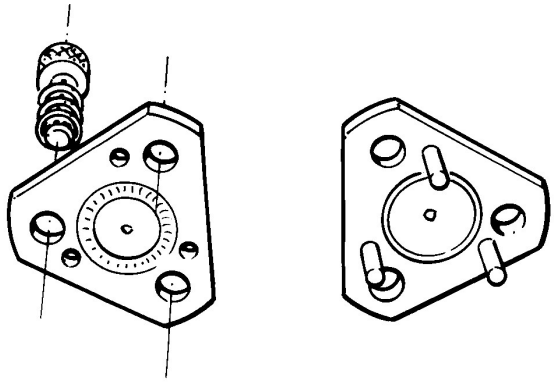


Figure 18 The Miniature Diamond Anvil Cell

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:

1. Press on the windows with a probe, flattening the sample.
2. 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 66) 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 Camera View 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 (shown by the red dashed lines) 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 microscope.

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 58), or they can be pressed in a diamond anvil cell (see *Polymers* on page 60).

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 substrate is opaque, scrape off a sample of the coating; use the roller knife to scrape a small piece on to a KBr or BaF_2 disk.
- Coatings on reflective or opaque substrates can be measured using the micro-ATR objective. See *Collecting Spectra With the ATR Objective* on page 78 for details.

Liquids

Solutions of samples

Although liquids are seldom analyzed with the Spotlight 200, 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 Spectra

Techniques for Collecting Spectra

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

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 microscope. Windows with thicknesses equal to 1 mm and 2 mm, and outer diameter equal to 13 mm can be used with the cell. Two KBr windows (2 mm thickness) are included with the system. The cell can apply pressure without rotating the windows, and therefore avoids scratching them.

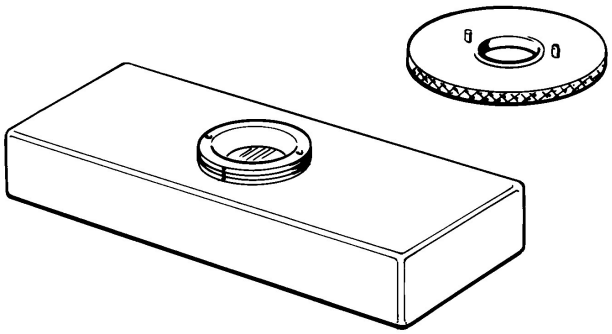


Figure 19 The Compression Cell

Although samples can be thinned using 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 a Hot/Cold Stage

An optional hot stage enables you to study temperature-dependent phenomena in microsamples. A 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.

A hot stage can heat samples in 1 degree increments. The maximum temperature that can be achieved depends upon the type of hot stage being used; see *Accessories* on page 119 for details. A target temperature can be selected and maintained.

A hot stage is held in the slide clip on the sample stage of the microscope. The microscope requires no modifications to accept the hot stage.

A 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.

NOTE: Some types of hot stage can be used with a cooler. The cooler uses liquid nitrogen to reduce the temperature of the stage down to −196 °C. See *Accessories* on page 119 for details

Collecting the Spectrum 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 method can be used to view the sample and collect spectra.

To collect the spectrum of a thick sample

1. Click **Zero** on the Setup Microscope Advanced tab.

   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. Click **Park** on the Setup Microscope Advanced tab.

   The lower cassegrain bracket moves down to a lower position.

4. Release the locking lever located on the right side on the back of the lower cassegrain assembly (Figure 20).

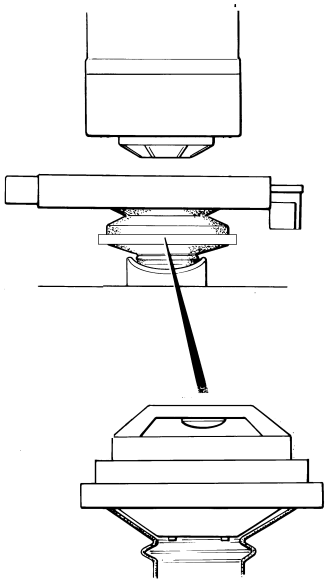


Figure 20 The Lower Cassegrain

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

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

6. Click **Lower Park**.
   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 21).

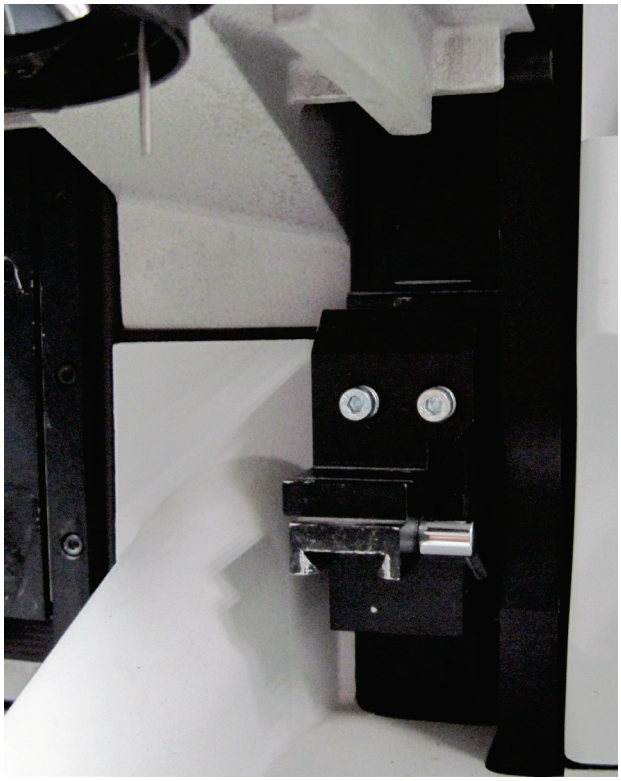


Figure 21 Location of the dovetail connector behind the stage

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 Setup Microscope Advanced tab.
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 a Spectrum 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 an inert atmosphere.

WARNING

Do not use flammable gases for purging.

Parts of the purging system

Some parts of the purging system are shown in Figure 22.

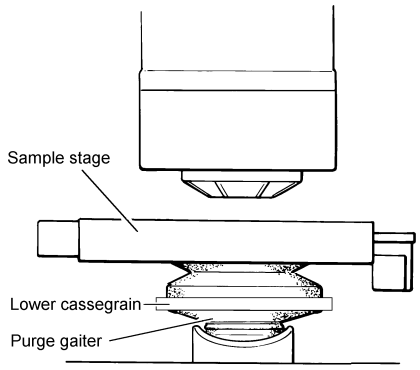


Figure 22 Parts of the Purge System

The purge system consists of:

- The gas inlet connector on the metal plate at the rear of the microscope and the connector at the rear of the spectrometer;
- The purge coupling molding connecting the spectrometer to the microscope;
- 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 microscope.
   See *Getting Ready to Use the Spotlight 200* starting on page 41.
3. Place the sample in position.
4. Purge the microscope and spectrometer for 15 to 20 minutes at a rate of 10 l min^{-1}.
5. Collect the background spectrum and the spectrum of the sample.
   See the on-screen Help for further information about collecting spectra.

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 23.

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 23.

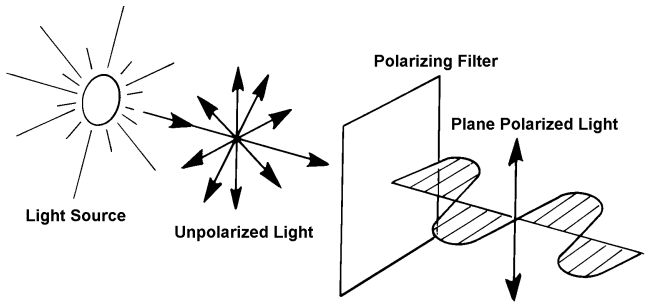


Figure 23 Representation of Unpolarized Light (left) and Polarized Light (right)

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 24), the polarized light can pass straight through.
- However, if the second polarizer 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 24).

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

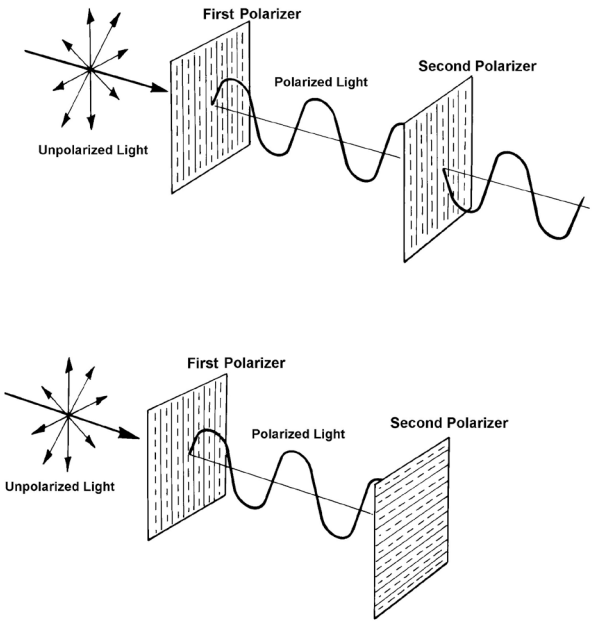


Figure 24 Polarizers Parallel (top) and Polarizers Crossed (bottom)

Some materials are *anisotropic* (or *birefringent*), that is, 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 a separate infrared spectrum from each layer. This is useful for identifying the materials used to create the structure.

Inclusions in polymer films

Although they may appear identical when using non-polarized light, the film and inclusion present in a polymer film may exhibit different degrees of birefringence when 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 of the different regions of interest.

Equipment

The equipment for visible polarization studies consists of two parts: the polarizer and the analyzer (Figure 25).

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 Spotlight 200, and are automatically switched into the beam when the analyzer is inserted.

Figure 25 The Analyzer

Operating the Analyzer

1. Insert the analyzer into the slot in the right side of the front cover (Figure 26). 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.

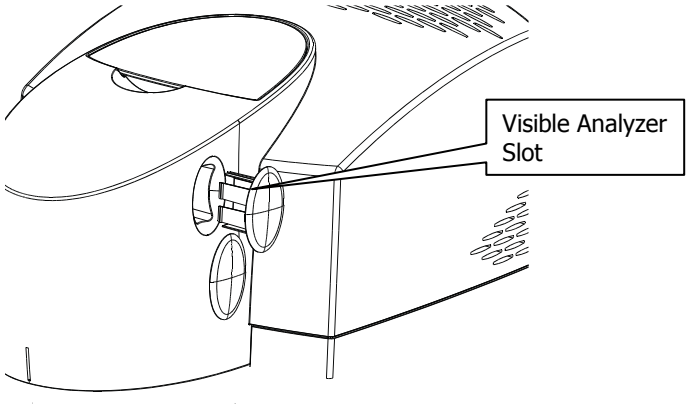


Figure 26 Analyzer Position

2. When the polarizing element is in the beam, rotate the wheel while viewing the Camera View window.

Collecting IR Spectra 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 200 enables you to collect polarization spectra of very small samples. These include:

- Single filaments (typically 14 x 70 µm);
- Films;
- Single crystals;
- Liquid crystals.

Equipment

The optional polarizer has a rotatable silver bromide element in an aluminum mount (Figure 27).

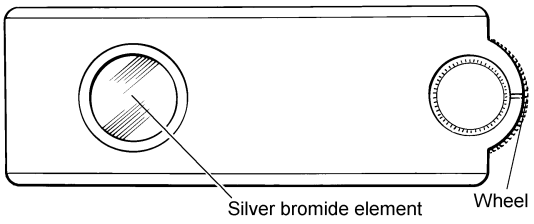


Figure 27 The Infrared Polarizer

Using the Polarizer

1. Remove the snap-in cover that masks the aperture for the infrared polarizer (Figure 28).

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 microscope, and the wheel facing outwards.

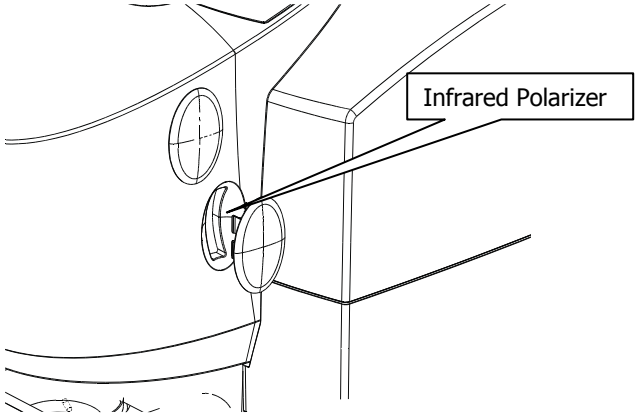


Figure 28 Infrared Polarizer Position

3. Push the analyzer into the slot until it stops.

4. Turn the wheel to orient the polarizing element.

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.*

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

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.

Spectrum software enables you to automatically map a sample using the 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.

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 (for germanium 4.0)

λ is the wavelength of the radiation

θ is the angle of 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.

Two versions of the ATR objective are available. The **manual ATR objective** requires you to raise and lower the crystal using a toggle bar on the ATR assembly. The **automated ATR objective** not only controls the crystal automatically, but also includes a weighbridge to measure the force applied to the sample by the crystal, which helps you obtain more repeatable spectra.

NOTE: The weighbridge communicates with the microscope using a small infrared transmitter located behind a window at the rear of the platform. Take care not to cover this window with any part of your sample as this will prevent the weighbridge from transmitting the value of the force applied by the crystal.

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 microscope.

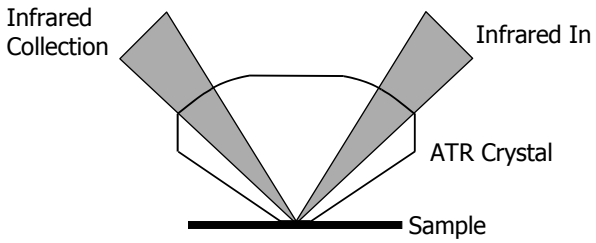


Figure 29 Infrared Radiation Optical Path

ATR Objective Specification

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

ATR crystal materials

Germanium (Ge), Silicon (Si), and Diamond Coated Germanium

Range of measurement

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

Ge/Di: 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 Microscope* on page 87), cleaned (see *Cleaning the ATR Objective* Crystal on page 90), and replaced and aligned by the user (see *Fitting the ATR Crystal Holder to the Microscope* on page 81).

Fitting the ATR Crystal Holder to the Microscope

1. Switch off the microscope at the electricity supply.
2. Stop any laser radiation from entering the microscope 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 30).
   The assembly is aligned if the adjusting levers have not been moved.
5. If fitting the automated ATR objective, insert the jack plug into the socket at the rear of the cassegrain (Figure 31).
   Lower the stage if necessary to improve the access to the socket.
6. Direct the infrared beam to your microscope.
7. Switch on the microscope.

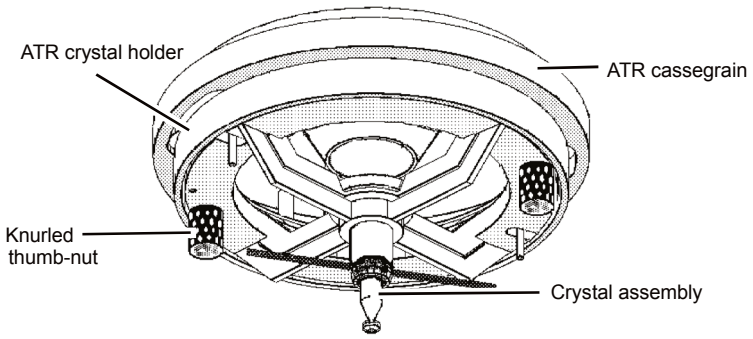


Figure 30 The Manual ATR Crystal Holder and Cassegrain

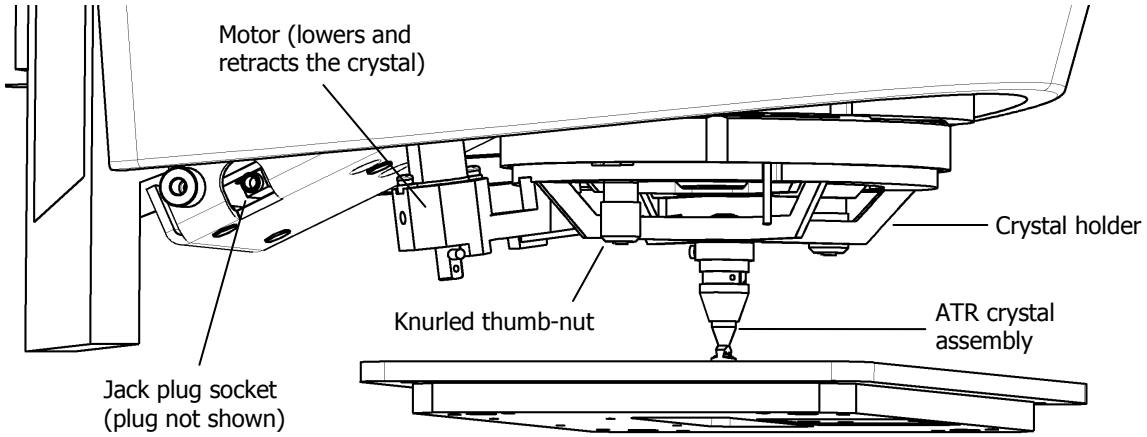


Figure 31 The Automated ATR Crystal Holder and Cassegrain

Measuring the Maximum Energy in Reflectance Mode

The following procedure allows you to determine the maximum energy reaching the detector when the ATR crystal is retracted. The value obtained here can be used to check that the crystal is aligned correctly; see *Checking the Infrared Alignment* on page 86 for details.

1. Make sure that your spectrometer is switched on, and the source has warmed up.
2. Make sure that the microscope, PC, and stage controller are switched on and that Spectrum software is running on your PC.
3. Ensure that the infrared beam is directed through the microscope and not the spectrometer by using the **Setup Instrument BeamPath** tab.
4. Place the slide holding the reference mirror (supplied with the microscope) on the sample stage.
5. Select the **Reflectance** sampling mode on the **Setup Microscope Basic** tab.
6. Look at the image in the Camera View pane and focus the beam on the surface of the mirror using the Z-control on the joystick, or the **Auto-Focus** option.

   There are usually dust particles or scratches on the surface that you can use to focus on.
7. Enter **100** in the Aperture **Width** and **Height** text boxes on the **Setup Microscope Advanced** tab.
8. Monitor the energy reaching the detector by selecting **Monitor** from the Measurement toolbar and choosing the **Energy** option.
9. Record the energy level measured.
10. Click **Halt**.

Adjusting the Height of the Crystal

NOTE: The crystal is mounted on a bayonet assembly similar to that on some light bulbs.

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 manual ATR crystal onto the reference mirror by turning the bar clockwise and slowly lowering the toggle bar.

   You must always support both ends of the toggle bar when lowering or raising the crystal.

   OR

   Click the  button to lower the automated ATR crystal onto the reference mirror.

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 and Figure 33).

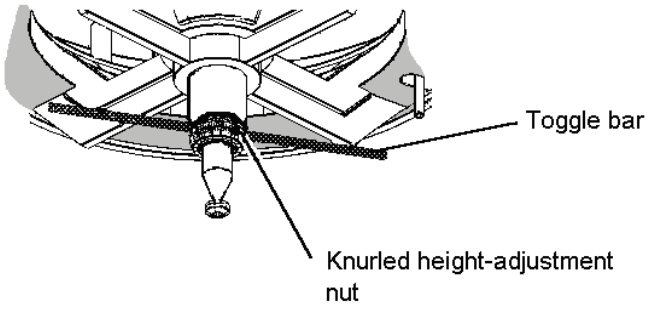


Figure 32 Adjusting the Crystal Height (Manual ATR Objective)

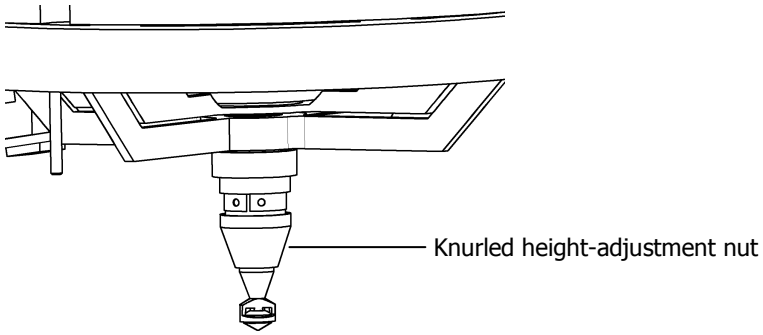


Figure 33 Adjusting the Crystal Height (Automated ATR Objective)

Centering the Crystal

1. Retract the manual ATR crystal using the toggle bar, by lifting the bar then twisting counterclockwise.

   OR

   Click the  button to retract the automated ATR crystal.

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.

   If the crystal height is adjusted correctly, it will just touch the tape and make a shallow impression in it.

5. Retract the crystal.

   If there is no impression on the tape, the crystal height should be lowered slightly by turning the knurled height-adjustment nut left to right a quarter of a turn.

   If there is a deep impression on the tape, which does not disappear within a few seconds, the crystal height should be raised slightly by turning the knurled height-adjustment nut right to left a quarter of a turn.

6. If appropriate, repeat steps 4 and 5 until the crystal just touches the tape.

7. Once the crystal height has been corrected, 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 34).

NOTE: The centering adjustments are the same for both the manual and automated ATR objectives.

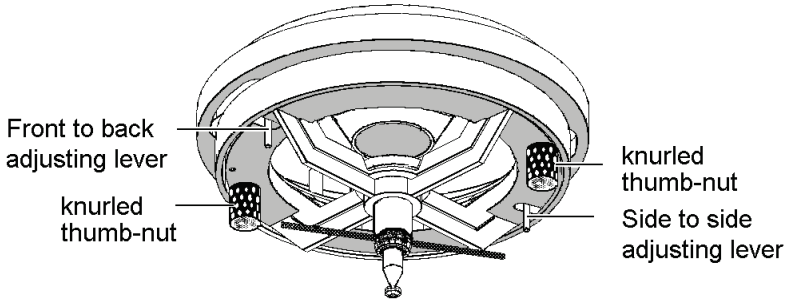


Figure 34 Centering the ATR Crystal

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.
7. Look through the microscope at the impression of the crystal on the tape. 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

The following procedure allows you to determine the energy reaching the detector when the ATR crystal is in place. If the crystal is aligned correctly, the energy level measured should be at least 20% of the maximum energy measured in reflectance mode; see *Measuring the Maximum Energy in Reflectance Mode* on page 83 for details.

1. Place the slide holding the reference mirror (supplied with the microscope) on the sample stage.
2. Look at the image in the Camera View pane and focus the microscope on the surface of the mirror with the Z-control on the joystick, or the **Auto-Focus** option.
   There are usually dust particles or scratches on the surface that you can use to focus on.
3. Lower the crystal on to the reference mirror.
   You should lower the crystal and then raise the stage slowly with the manual ATR objective. For the automated ATR objective, click the **Contact Sample** button on the Setup Microscope Basic tab once the crystal is lowered.
4. Enter **100** in the Aperture **Width** and **Height** text boxes on the Setup Microscope Advanced tab.
5. To monitor the energy reaching the detector, select **Monitor** from the Measurement toolbar and choose the **Energy** option.
6. Record the level of energy measured and compare it with the value measured when the crystal is retracted.
   If the energy is less than 20% of the maximum energy measured, repeat the alignment procedure on page 85.
7. Click **Halt**.

Alternatively, you can measure the energy in reflectance mode before and after lowering the ATR crystal on to the mirror. The software displays the maximum energy with the crystal retracted, and then shows the current energy when ithe crystal is lowered.

Removing the ATR Crystal Holder from the Microscope

To use the microscope for conventional microspectroscopy, 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 microscope at the electricity supply.
2. Stop any laser radiation from entering the microscope 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. For the automated ATR objective, remove the jack plug from its socket.
6. While holding the crystal assembly, unscrew the two adjustment thumb-nuts 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.
7. Store the assembly carefully for future use.

   When refitted, the assembly will still be aligned, provided that the adjusting levers have not been moved.
8. Direct the infrared beam back to the microscope.

Fitting a Crystal Assembly to the ATR Objective

Manual ATR Objective

1. Remove the ATR crystal holder, as described on page 87.
2. Place the crystal holder on a bench, with the crystal upwards.
3. While holding the crystal assembly in place, unscrew the knurled height-adjustment 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-adjustment nut.
   Take care not to damage the crystal.
6. Refit the ATR crystal holder to the microscope, as described on page 81.
7. Align and adjust the ATR crystal holder, as described on page 84.

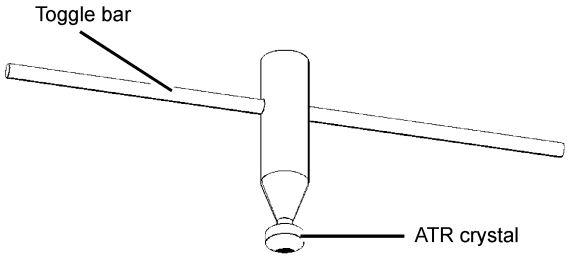


Figure 35 The Manual ATR Crystal Assembly

Automated ATR Objective

1. Remove the ATR crystal holder, as described on page 87.
2. Place the crystal holder on a bench, with the crystal pointing upwards and the motor pointing away from you.
3. Unscrew the knurled height-adjustment nut.
4. Pull the crystal assembly out of the holder.
5. Hold the new crystal assembly with the thin alignment groove facing you (Figure 36).

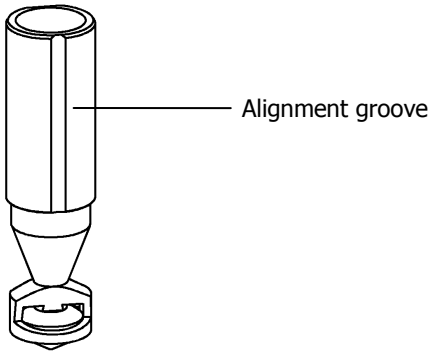


Figure 36 The Automated ATR Crystal Assembly

6. Slide the crystal assembly into the holder.
   You will feel it click into place as it comes into contact with a magnet inside the holder.

7. Rotate the crystal assembly until the alignment groove is in line with the sprung-loaded ball bearing in the wall of the holder (Figure 37).
   You will feel the ball bearing slide into the alignment groove and lock the crystal assembly in position.

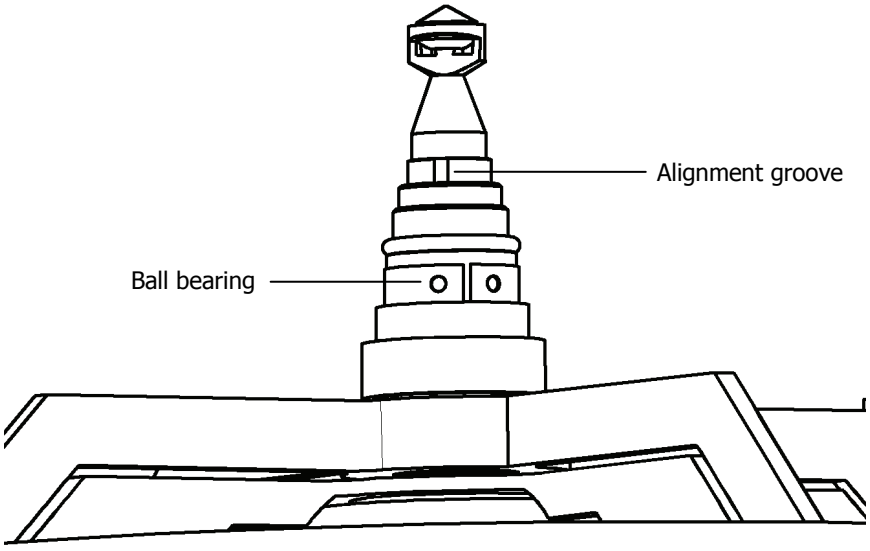


Figure 37 Crystal Assembly aligned in holder

8. Refit the height-adjustment nut.
   Take care not to damage the crystal.

9. Refit the ATR crystal holder to the microscope, as described on page 81.

10. Align and adjust the ATR crystal holder, as described on page 84.

Cleaning the ATR Objective Crystal

Because the ATR objective crystal is in contact with the sample under test, 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 of 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. Lower the crystal.
4. 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.

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

Auto ATR Cleaning Procedure

If you should see intermittent failures to raise or lower the crystal while running, it is recommended you clean your Auto ATR Accessory to keep it in good working order. Regardless of performance we recommend you clean the accessory at least once every 10,000 cycles or per the schedule below:

- Heavy User (25,000 cycles per year) – Every 4-5 months
- Medium User (15,000 cycles per year) – Every 8 months
- Light User (5,000 cycles per year) – Every 12 Months

Materials Needed

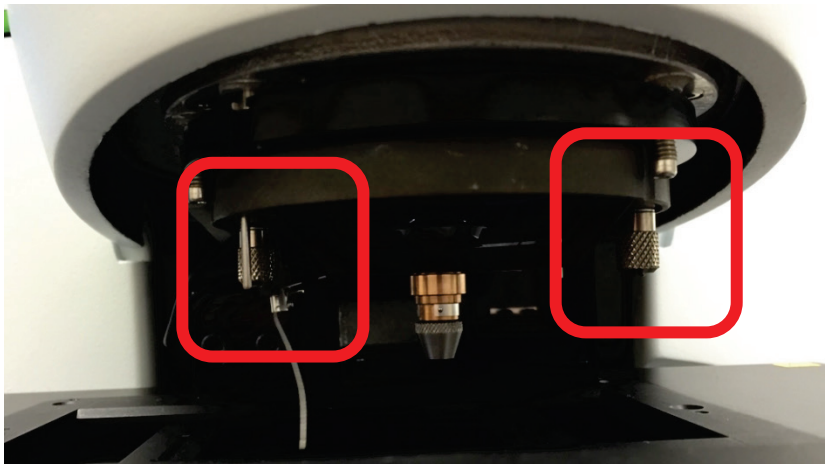
Methanol in a squirt bottle

Small Foam Head Swab
(Recommend VWR P/N 89022-992)

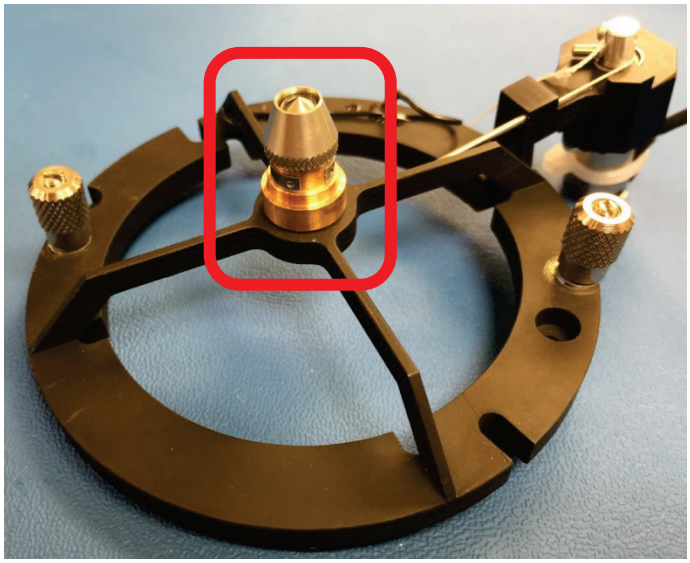
- Kimwipes or similar low lint cleaning wipes

Cleaning Procedure

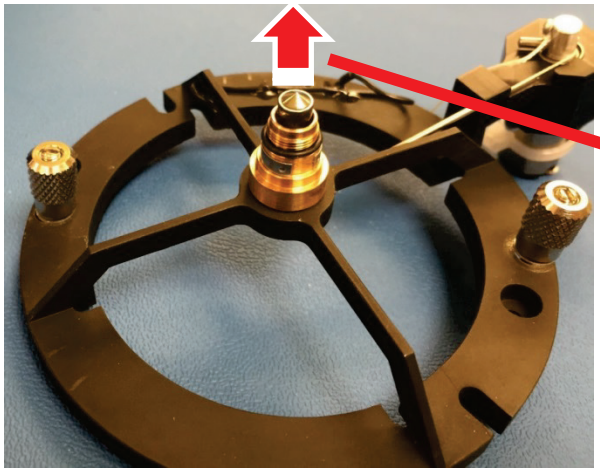
1. If not already done, use Spectrum 10 to raise the crystal into the assembly.
2. Disconnect the motor wire from the left side of the transceiver board.
3. Lower the sample stage, loosen the thumb screws, and carefully pull Auto ATR accessory from Spotlight microscope to remove it from the sample area.



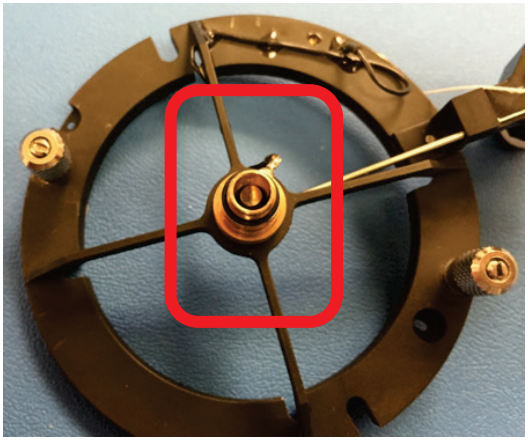
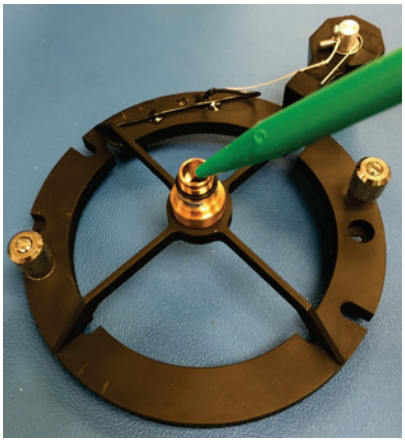
4. Lay the assembly, crystal up, on a clean work surface. Remove the coned knurled collar to expose the crystal holder.



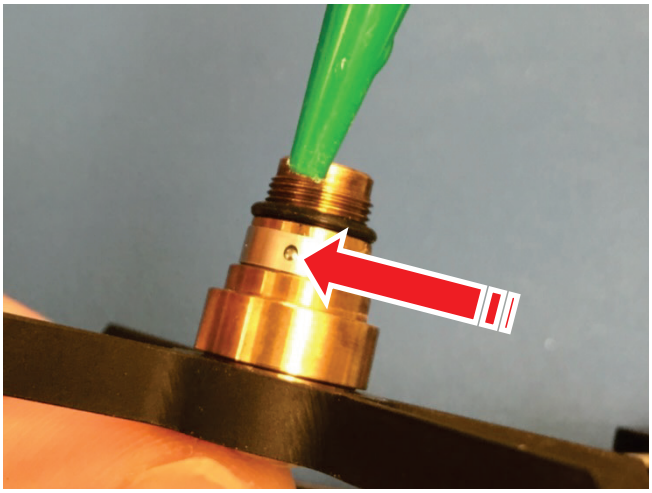
5. Carefully grab the crystal holder and remove it from the ATR assembly. Set the crystal holder aside and be careful not to damage the crystal.



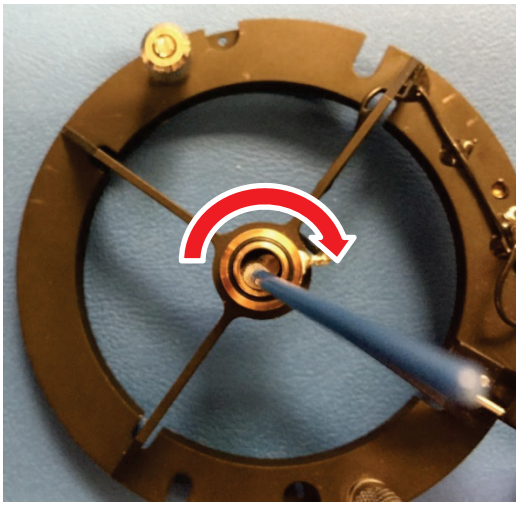
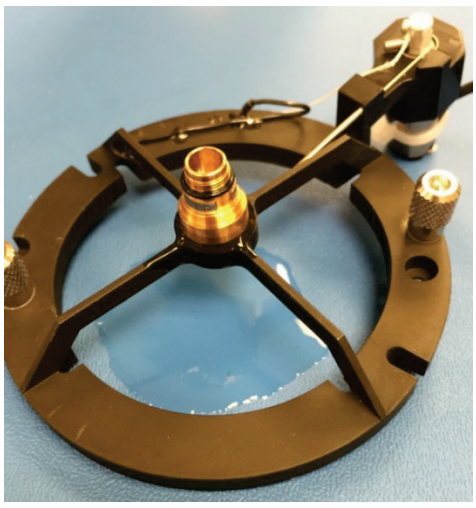
6. Look down into the center of the crystal bearing, notice the compression spring and the plunger retracted and off to one side. Spray methanol into the bore and allow it to drain from the assembly.

7. Spray methanol in hole above alignment bearing to flush out any free particles.



8. Insert a methanol moistened swab into the larger gap between the bearing and the retracted plunger and work it all the way around the bore of the bearing.

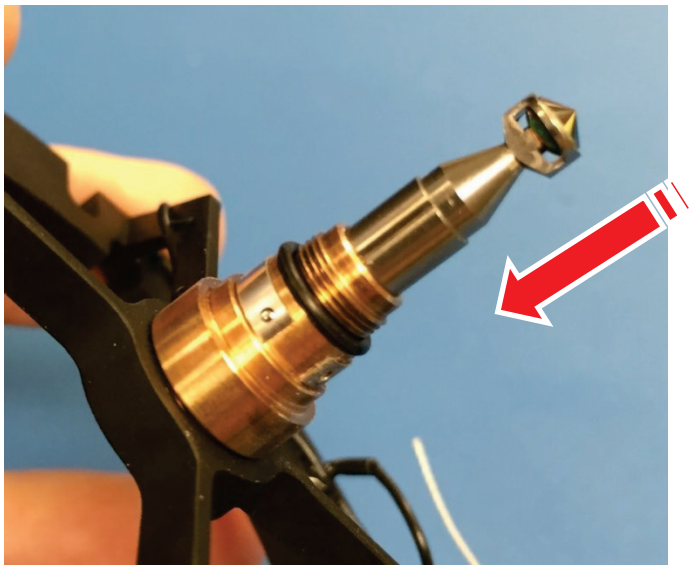



9. Use a methanol moistened Kimwipe to clean the outside bearing surface, alignment groove and contact flat opposite the groove on the crystal holder.

It may be necessary to use a non-marring implement to clean any debris on the sliding surfaces (I use a finger nail). Take care not to damage the crystal. This is a good time to clean the crystal itself, refer to the User Guide for suggestions.

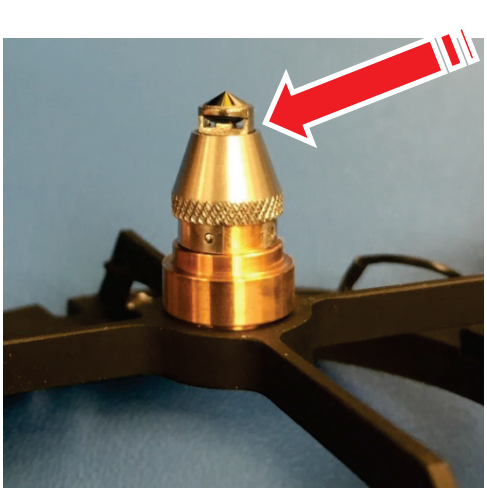
10. Once all of the methanol has evaporated, it's time to reassemble the system. Insert the crystal holder into the bore of the bearing being careful to line up the groove with the ball in the crystal holder.

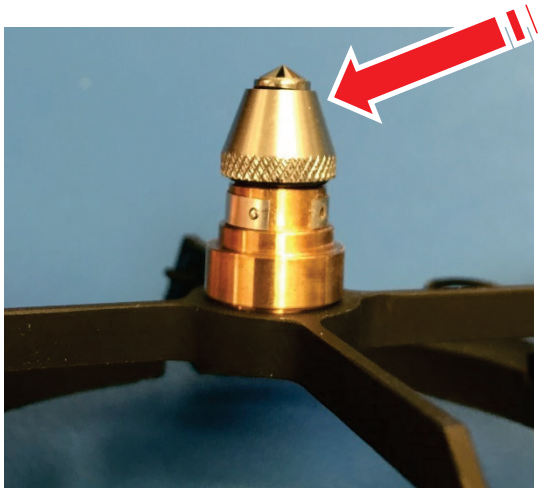
11. Gently press down on the crystal holder until it stops. You may need to twist the holder a small amount from side to side to seat it on the retracted plunger. **Do not force it**, you may damage the stop contact.



12. Using another swab, clean any debris from the knurled stop collar. Notice the black spots in the image below.

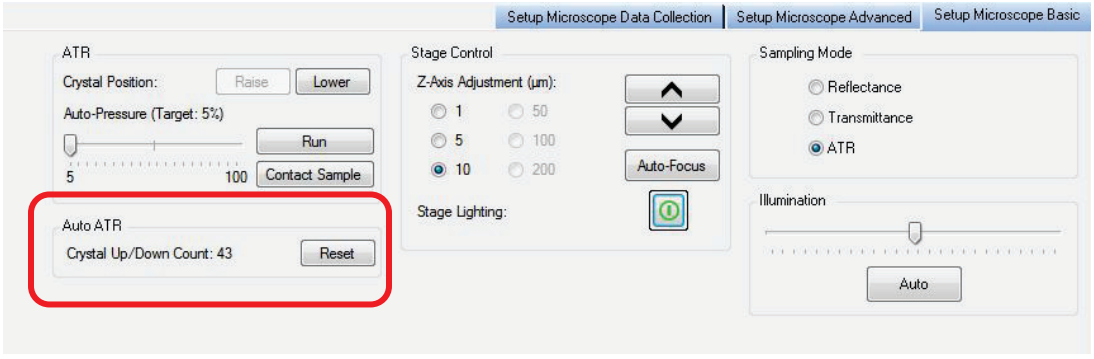
13. Screw the knurled collar on all the way then back off one and a half (1.5) full revolutions. This will set the lower limit of travel for the crystal.





14. Reinstall the Auto ATR accessory in your Spotlight system and perform the crystal alignment and focusing procedure to maximize IR energy.

15. Reset the counter in the Spectrum software on the **Setup Microscope Basic** tab, click the **Reset** button to reset the **Auto ATR Crystal Up/Down** counter.



Changing the Weighbridge Battery

Figure 38 The Weighbridge for the Automated ATR Objective

The weighbridge supplied with the automated ATR objective allows you to apply a known and repeatable force to the sample when you are collecting spectra. This relies on the weighbridge communicating with the microscope using a wireless infrared transmitter powered by a battery. When the battery power starts to decrease, Spectrum displays a symbol in the Status bar at the bottom of the screen. You should change the battery at this point. If the symbol changes to , the weighbridge will not function until the battery is replaced.

To change the weighbridge battery:

1. Remove the four screws from the corners of the weighbridge and lift it off the microscope stage.

2. Turn the weighbridge over and place it on a clean, flat surface.

3. Loosen the three captive screws in the cover on the underside (Figure 39).

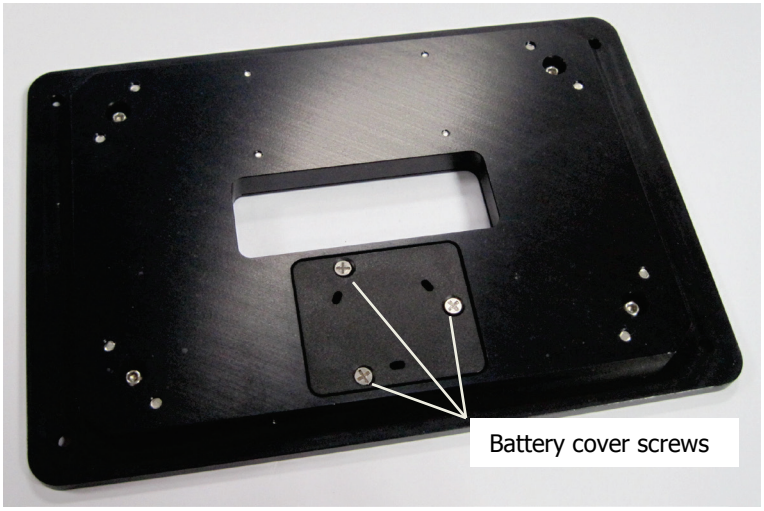


Figure 39 Weighbridge battery cover

4. Remove the battery from inside the cover lid and replace it with a new battery (Figure 40).
   A 3V lithium coin cell (size CR2450) is required (L9004212).

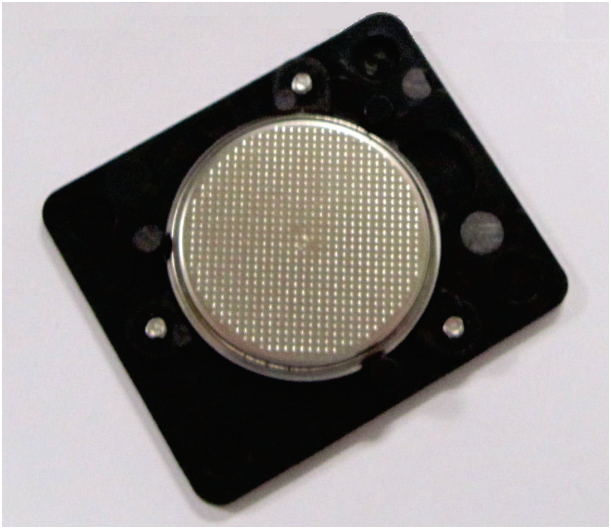


Figure 40 Weighbridge battery

5. Replace the battery cover and tighten the screws firmly.

6. Place the weighbridge on the stage and fix it in place with the four corner screws.
   The PerkinElmer logo should be at the front of the weighbridge.

NOTE: The battery status indicator in Spectrum will update the next time you perform an operation with the weighbridge. If the battery is correctly fitted, a symbol will be displayed.

Collecting Spectra

Manual ATR Objective

Collecting a Background Spectrum

1. Select the **ATR** sampling mode on the Setup Microscope Basic tab.
2. Set the infrared aperture to a suitable size and rotation.
   For information on how to do this see 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 x 100 µm.
3. Choose the **Scan Settings** you want on the Setup Instrument Basic tab.
4. Click  .
   The stage will be lowered so that the background spectrum can be collected in air.
5. When prompted, lower the ATR crystal to the working position, and click **Retry**.
   The spectrometer starts to scan and the spectrum is displayed as it is collected.
6. When the scan is completed, retract the ATR crystal.

Collecting a Sample Spectrum

Once you have collected a background spectrum, follow the procedure below to collect a spectrum of your sample.

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. Make sure that the ATR crystal is retracted.
2. Place the sample on the stage, either on the slide holding the reference mirror or on its own slide.
3. Move the sample to the center of the field of view.
   Even though the microscope 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.
4. Look at the image in the Camera View and focus on the sample using the Z-control on the joystick or the **Auto-Focus** option, and then move the area of interest into the center of the field of view.

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.

5. Lower the ATR crystal to the working position, and slowly raise the stage until the sample touches the crystal.

CAUTION	*To prevent accidental damage to the ATR crystal, always bring the sample slowly up to the crystal, rather than lowering the crystal directly on to the sample.*

6. Click  on the Measurement toolbar.
   The Live tab is displayed.

7. Select **Sample**.

8. Make sure that the spectrum is satisfactory.
   If your sample is soft or there is poor contact, you may need to increase the contact of the crystal with the sample. To increase the contact, raise the stage slightly using the **Adjust Up**  button on the accessory toolbar. If the stage position is too high, lower it using the **Adjust Down**  button.

9. When the spectrum is satisfactory, click  .
   The spectrometer starts to scan and the spectrum is displayed as it is collected.

CAUTION	*Always retract the ATR crystal immediately after data collection has been completed, in order to avoid accidental damage.*

Scanning a Line or Map

Spectrum software enables you to collect spectra at marked points using the manual ATR objective. These points can be individual markers, or groups of markers arranged in lines or maps across the surface of the sample.

1. Collect a background spectrum.
   Refer to *Collecting a Background Spectrum* on page 98.

2. Ensure that the sample is flat and securely fixed to the microscope sample holder or window.
   This can be done by placing adhesive tape around the edge of the sample.

3. Define a sample area that is bigger than the area you intend analyzing using the **Stage View Range** menu in the Microscope menu.

4. Look at the image in the Camera View and focus on the sample using the Z-control on the joystick or the **Auto-Focus** option, and then move the area of interest into the center of the field of view.

5. Click  on the Stage View toolbar to collect an image survey.

6. Ensure that the **ATR** sampling mode is selected in the Setup Microscope Basic tab.

7. Use **Monitor** to find the most suitable stage height to give a staisfactory spectrum of your sample.
   Refer to *Collecting a Sample Spectrum* on page 98. Click **Halt** when completed, but do not change the stage height.

8. Place markers, lines or maps on to your image survey as required.
   For information on how to do this, see the on-screen Help. The markers will all be set at the stage height (Z-axis position) that you determined previously using Monitor.

9. Click the **Scan Markers**  button on the Measurement toolbar.

10. When prompted, lower the ATR crystal to the working position and click **Retry**.
    The stage will move to the position of each marker in turn and collect a spectrum. The results will be displayed in an Image View.

CAUTION	*Always retract the ATR crystal immediately after data collection has been completed, in order to avoid accidental damage.*

Automated ATR Objective

Collecting a Sample Spectrum

1. Select the **ATR** sampling mode on the Setup Microscope Basic tab.

2. Choose the **Scan Settings** you want on the Setup Instrument Basic tab.

3. Move the sample to the center of the Camera View.
   Even though the microscope 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.

4. Look at the image in the Camera View pane and focus on the sample using the Z-control on the joystick, or the **Auto-Focus** option, and then move the area of interest into the center of the field of view.

5. Set the infrared aperture to a suitable size and rotation.
   For information on how to do this see 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 x 100 µm.

6. Click  to collect a background spectrum.
   The stage moves downwards a small distance and the ATR crystal is lowered to collect the background spectrum. Once complete, the crystal is raised and the stage returns to its original position.

NOTE: When using the automated ATR objective, the background spectrum is always collected in air at the current stage position, regardless of the **Background Location** settings in the Setup Microscope Data Collection tab.

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 at the chosen stage position.

7. Click  on the Measurement toolbar.
   The software asks if you want the ATR crystal to make contact with the sample automatically.

8. Click **Yes**.
   The stage moves upwards until the sample just touches the ATR crystal.
   OR
   Click **No**.
   The stage remains lowered to allow you to adjust the height manually.

9. On the Monitor dialog, choose **Sample**.

10. Use the **Adjust Up**  button to move the stage upwards until a spectrum is seen in the Live tab.
    The Sample Force being applied by the crystal is shown under the Stage View pane both in units and as a percentage of the maximum possible value.

11. Adjust the stage position until a satisfactory spectrum is seen in the Live tab.

NOTE: You can change the size of each stage Z-axis adjustment step using the options in the Setup Microscope Basic tab.

12. When the spectrum is satisfactory, click  .
    The sample spectrum is collected.

Scanning Markers, Lines and Maps

If your sample contains many features that you want to scan, it is more efficient to add markers, lines or maps in the Stage View that the system can then analyze automatically. You can either add markers manually or use the **Analyze Image** options which detects regions of interest and then places a marker in each region with optimum aperture sizes. Lines and maps, which are collections of markers, can also be added, but the apertures for these markers need to be set manually. Refer to the on-screen Help for more information on setting up markers, lines and maps, and using the Analyze Image processes.

With an automated ATR objective, you can set the stage position for scanning using either stage height or applied pressure. If your sample has an uneven surface, then it is safer to use the pressure option to set the height of the sample stage and make sure that there is firm contact between the ATR crystal and the sample for all measurements. For a flat sample, you can choose to use the same stage height for all markers, which will reduce the time needed to collect the spectra.

To scan markers using a predefined marker height:

1. Focus the image of the sample and collect an image survey.

2. Use **Monitor** to find a suitable stage height (Z-axis position) at which the ATR crystal is in contact with your sample and a satisfactory spectrum is obtained.

   Refer to step 7 on page 101. Click **Halt** when completed, but do not change the stage height.

3. Add markers, lines or maps to your image survey as required.

   These will all be set at the Z-axis position that you previously determined using Monitor.

4. On the Setup Microscope Data Collection tab, select the **Use Marker Height** option.

NOTE: Make sure that all the Auto-Focus options are unchecked as these will change the stage height during the analysis.

5. Click  .

   Background spectra will be collected and the markers, lines and maps will be scanned using the specified stage height. The results will be displayed in an Image View.

To scan markers using Auto-Pressure:

1. Focus the image of the sample and collect an image survey.

2. Click  on the Measurement toolbar.

   The software asks if you want the ATR crystal to make contact with the sample automatically.

3. Click **Yes**.

   The stage moves upwards until the sample just touches the ATR crystal.

   OR

   Click **No**.

   The stage remains lowered to allow you to adjust the height manually.

4. On the Live tab, select **Sample**.

5. Use the **Adjust Up**  button to move the stage upwards until a spectrum is seen in the Live tab.

   The Sample Force being applied by the crystal is shown under the Stage View pane both in units and as a percentage of the maximum possible value.

6. Adjust the stage position until the spectrum is satisfactory.

   If necessary, make further adjustments to the stage position using the **Adjust Up**  and **Adjust Down**  buttons to modify the applied pressure.

7. Note the final percentage Sample Force reading in the Stage View tab, and click **Halt**.

8. Set the Auto-Pressure slider to the required pressure.

9. On the Setup Microscope Data Collection tab, select the **Determine Height Using Pressure** option.

10. Add markers, lines or maps to your image survey as required.

11. Click  .
    Background spectra will be collected and the markers, lines and maps will be scanned using the Auto-Pressure setting. The results will be displayed in an Image View.

Analyzing Samples Using the ATR Imaging Accessory

The ATR Imaging Accessory enables you to collect ATR spectra from an area of your sample that you have identified using the microscope. Two nominal diameters are available for the crystal in the accessory: 600 µm and 1200 µm. Using these large crystals, you can collect spectra over a relatively wide area without lifting the crystal from the sample, as would be necessary using the ATR Objective. This can help you obtain more reliable results, especially if your sample is sticky and quickly contaminates the ATR crystal. It also reduces inaccuracies with selecting the location to scan, due to the possibility that the sample could move each time the crystal is raised and lowered.

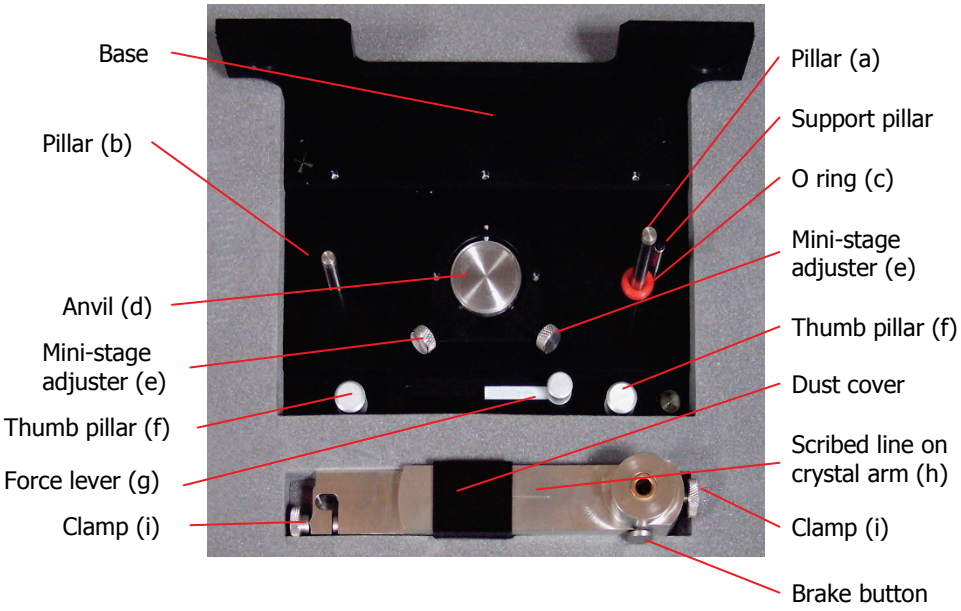


Figure 41 The ATR imaging accessory, showing the base (top) and the ATR crystal arm (bottom)

The procedure described here assumes that you are familiar with using the ATR Imaging Accessory. Further details of the installation and operation of the accessory are given in the *ATR Imaging Accessory User's Guide* (L1050048), which can be found on the *IR Manuals CD* (L1050002) supplied with the accessory.

NOTE: The collection and processing of ATR images described in the *ATR Imaging Accessory User's Guide* requires PerkinElmer's SpectrumIMAGE software. If this software application was not supplied with your Spotlight 200 system, follow the method described here which uses Spectrum software to collect the spectra.

1. Remove the lower cassegrain.
   Refer to *Collecting the Spectrum of a Thick Sample* on page 67.
2. Remove the dovetail connector underneath the stage (Figure 21).
3. Remove the ATR Objective crystal holder if it is fitted (page 87).
4. Move the stage to its lowest position.

5. Install the ATR Imaging Accessory on the sample stage, and level it.
   Refer to the *ATR Imaging Accessory User's Guide* (L1050048).

6. Clamp the ATR crystal arm to the two pillars (Figure 41) and check that there is a gap of a few millimeters between the tip of the crystal and the anvil.

7. Remove the dust cover over the crystal.

8. Select the **Reflectance** sampling mode on the **Setup Microscope Basic** tab.

9. Raise the stage, and use the joystick and the Auto-Focus option to focus the microscope on the line scribed on the crystal arm.

10. Move the stage along this line until the center of the crystal is visible in the Camera View.

11. Center, and focus on, the registration mark at the center of the crystal's surface (Figure 42).
    You may need to adjust the illumination to see the registration mark clearly.

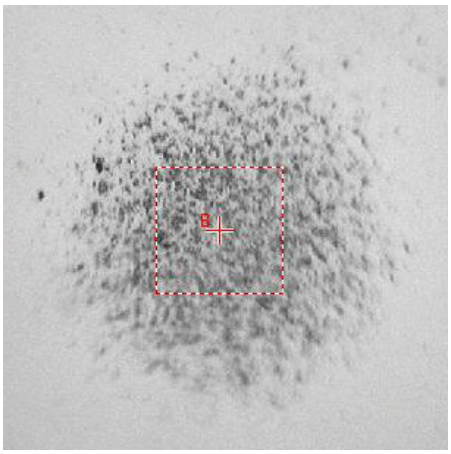


Figure 42 Image of ATR crystal registration mark

NOTE: From this point onward, do not adjust the stage X and Y positions using the joystick.

12. Select **Microscope** > **Set Stage Origin** to set the origin position of the stage.

13. Lift and swing the crystal arm away from the field of view, and place your sample on the anvil.
    We recommend that you fix your sample in position to prevent movement during the analysis, for example by using adhesive tape.

14. Without moving the X and Y position of the stage, find the area in your sample that you want to analyze.
    It is very important not to use the joystick to move the X and Y position of the sample stage. Adjust the sample position by moving the sample directly with tweezers, or use the mini-stage adjusters to center the area of interest (Figure 41).

NOTE: For best results, position the sample so that the area of interest is close to the center of the image. Remember that the area you can analyze is limited by the diameter of the ATR crystal.

15. Use the **Stage View Range** menu in the **Microscope** menu to select a suitable viewing area for your sample.

16. Click  on the Stage View toolbar to collect an image survey.

17. Note the coordinates of individual points on the image survey where you want to collect spectra.

    You will need these coordinates later to determine how far to move the stage once the ATR crystal is in position. Refer to *Correcting the Coordinates of Markers* on page 107.

    OR

    Add lines and maps to survey areas of interest on your sample.

    Refer to the on-screen Help for further information.

    You must increase the overall length or area of the lines and maps to allow for the ATR crystal's refractive index, and also reduce the space between the points to ensure that the entire surface between them is analyzed. Refer to *Setting Up Lines and Maps* on page 108.

18. Adjust the infrared apertures to account for the refractive index of the ATR crystal.

    Refer to *Adjusting the Aperture Sizes* on page 107.

19. Swing the crystal arm back over the anvil and clamp it in place on the pillars.

    Ensure that the crystal does not touch the sample.

20. Focus again on the registration mark.

21. Raise the stage position by the ATR crystal offset value.

    This value is marked in µm on the crystal arm. Raising the stage by this value focuses the infrared beam at the bottom of the crystal. You can use the joystick (taking care only to move the stage along the Z axis), or select **Microscope** > **Stage Move** > **To Coordinate** and change the Z-coordinate only.

22. Click  to collect a background spectrum.

    Ensure that the background spectrum is free from excessive noise. If necessary, increase the number of accumulations.

23. Lower the crystal arm gently on to the sample and clamp it in place.

24. Slide the force lever from right to left (Figure 41).

25. Focus again on the registration mark, and select **Microscope** > **Set Stage Origin** to set the origin position of the stage.

26. Raise the stage position by the ATR crystal offset value (step 21).

To scan a marker:

1. Select **Microscope** > **Stage Move** > **To Coordinate** and enter the coordinates for the marker you selected in step 16 multiplied by the correction factor.
   Refer to *Correcting the Coordinates of Markers* on page 107. The stage will move to the correct position.
2. Click  to scan the marker.
3. Repeat as necessary for other markers.

To scan lines or maps:

➢ Click  to collect all the required spectra automatically.

Correcting for the Effect of the ATR Crystal

It is very important to correct for the optical effects of the ATR crystal when selecting areas of the sample to analyze, and when setting the aperture dimensions. These effects arise because the refractive index of the crystal is much higher than that of air, which causes the sample image to be magnified when the crystal is in place. Therefore, the distances seen on the image survey, which was collected when passing the beam through air, will be different when the beam passes through the ATR crystal.

The ATR crystal is made of germanium, which has a refractive index of 4.0.

Adjusting the Aperture Sizes

The crystal's refractive index causes the actual area scanned on the sample to be smaller than the selected aperture area.

1. Select aperture sizes on the image survey to include the features you want to scan.
2. Increase the aperture sizes by a correction factor in both the X and Y directions to make sure that the scan covers the equivalent area that you originally selected in the image survey.
   For germanium, the correction factor is 4.

Correcting the Coordinates of Markers

The coordinates of markers on the image survey will not relate to the same position on the magnified image produced by the ATR crystal. A correction must be applied for each marker. For this reason, we recommend that you only use the following procedure to scan markers with Spectrum software:

1. Note the coordinates of the point you want to scan in your image survey.
   These coordinates must be based on the origin of the stage being set at the center of the crystal.
2. Multiply the X and Y coordinates by a correction factor.
   For germanium, the correction factor is 4.

3. When you are ready to collect a spectrum, select **Microscope** > **Stage Move** > **To Coordinate**, and enter the calculated coordinates for the marker.

4. Click  to scan the marker.

5. Repeat steps 3 and 4 for each marker.

Setting Up Lines and Maps

The ATR Imaging Accessory is particularly useful for scanning lines and maps on a sample, because it can do so without raising the crystal between each individual marker. We recommend that you use the following procedure to set up lines and maps in Spectrum software:

1. Set the stage origin at the center of the crystal before you add the lines and maps.

2. Add the required lines and maps to the image survey.

   Make sure that the areas covered by the lines and maps are sufficent to allow for movement or distortion of the sample when it comes into contact with the crystal.

3. Multiply the X and Y location coordinates of the line or map by a correction factor.

   For germanium, the correction factor is 4. This will ensure that the line or map starts at the correct position on the sample when viewed through the ATR crystal.

4. Multiply the spacing in each line or map by a correction factor.

   For germanium, the correction factor is 4. This will extend the line or map to cover the equivalent area you originally selected.

5. Add extra points or adjust the aperture sizes as required to ensure that all the area between the individual points is scanned.

You can now collect all the spectra automatically using the **Scan Markers** option.

Reflectance FT-IR Microspectroscopy

The Spotlight 200 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.

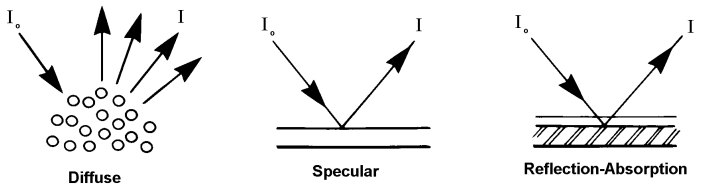


Figure 43 Three Types of Reflectance

Figure 43 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 and powders. The broad collection angle of the microscope enables it to capture a large proportion of the diffusely reflected radiation and send it to 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 44 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), micro cup (part number 01862761).

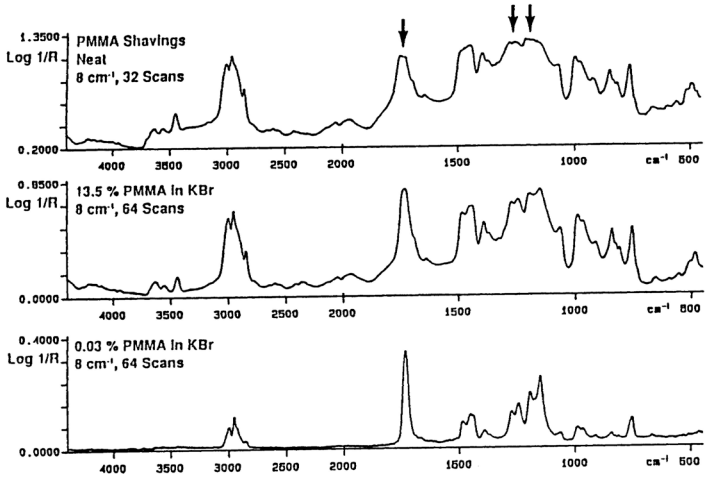


Figure 44 Diffuse Reflectance Spectra of PMMA

Specular Reflectance

Specular reflectance is reflection in one direction (Figure 43). 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.

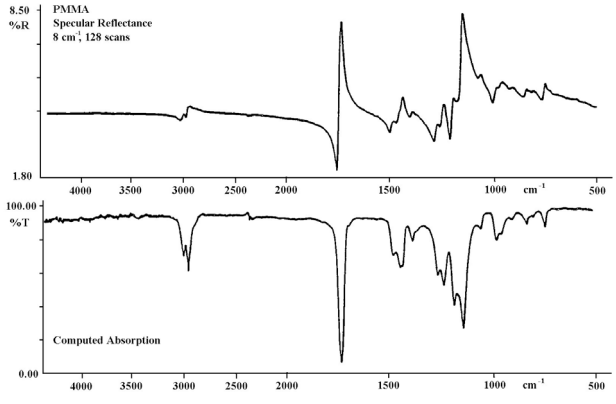


Figure 45 Specular reflectance spectrum of a PMMA plate and the absorption spectrum calculated from it with the Kramers–Kronig process in Spectrum

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

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 (see Figure 43). The absorbance spectrum of the film can be collected directly. Figure 46 shows the spectrum of residual oil on an electronic contact. The sample diameter was 100 µm.

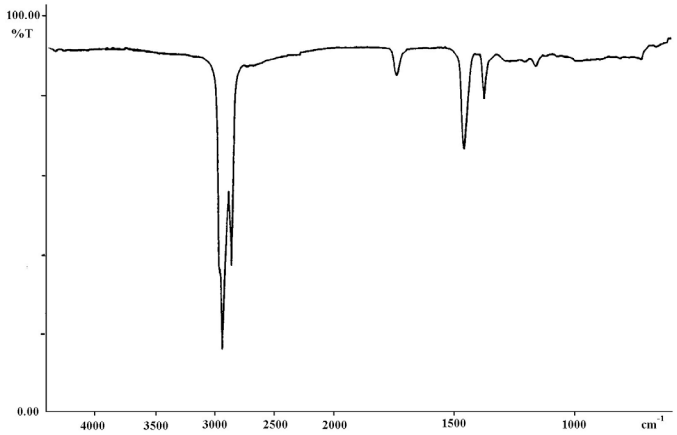


Figure 46 Reflection-Absorption Spectrum of Residual Oil on an Electronic Contact

Maintenance

Maintenance

WARNING

Switch off the mains voltage and remove the mains cord before cleaning.

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

- Inspecting the microscope;
- Protecting the microscope;
- Cleaning the optics;
- Cleaning the microscope 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 microscope.

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

CAUTION

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

Inspecting the Microscope

At least once a year, or whenever the microscope 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 *Warning Labels* on page 19.

Protecting the Microscope

The most important rule in caring for the microscope 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 microscope is not in use, cover it with a plastic cover.

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

Cleaning

The microscope 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 microscope 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 microscope 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 Microscope Fuse

The microscope is not voltage sensitive and will operate at voltages between 100–240 V and at 50–60 Hz.

1. Switch off the microscope 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-lag, 250 V 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 (counter clockwise) gives upward stage movement.
  Right twist (clockwise) gives downward stage movement.

To test the stage controller:

1. Start the Spectrum software and check that the infrared beam is directed to the microscope.
2. Click the  button on the toolbar.
3. Read the initialization message and then click **Start** when ready to proceed.
   The software initializes the sample stage.
4. 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, 250 V 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 200 is cleaned, examined, and adjusted periodically by a PerkinElmer Service Engineer.

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

Accessories

Accessories can be ordered directly from PerkinElmer at https://shop.perkinelmer.com/default.aspx

Description	Part Number
Spare fuse for Microscope (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
IR Polarizer Assembly	L1860408
Holder for 13 mm Sampling Disks	L1860409
Compression Cell	N1870185
Hot Stage Accessory (max 250 °C – 110 V)	N1870184
Hot Stage Accessory (max 250 °C – 220 V)	N1870188
Hot Stage Accessory (max 600 °C – 240 V)	L1860634
Hot Stage Accessory (max 600 °C – 110 V)	L1860635
Hot Stage Accessory (max 600 °C – 220 V, 60Hz [Korea])	L1860636
Cooler Accessory for use with L1860635 (110 V)	L1860632
Cooler Accessory for use with L1860634 and L1860636 (240 V)	L1860633
Rotatable Sample Disk Holder	N1873039
Holder for Reflection Transmission Measurements	N1873124
Manual ATR Accessory	L1860334
Auto ATR Accessory	L1862043
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 (Manual)	L1860268
Spare Silicon Crystal (Manual)	L1860269
Spare Diamond Coated Germanium Crystal (Manual)	L1862054
Spare Germanium Crystal (Auto)	L1862044
Spare Silicon Crystal (Auto)	L1862051
Spare Diamond Coated Germanium Crystal (Auto)	L1862047

Sample Preparation Tools

These are described in *Tools for Sample Preparation* on page 53.

Electrical Connections

Fitting the Plug

The power cable for the electrical supply plugs into the back of the microscope. 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
Line	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 Microscope to the Electrical Supply

The microscope 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, 50 or 60 Hz without adjustment.

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

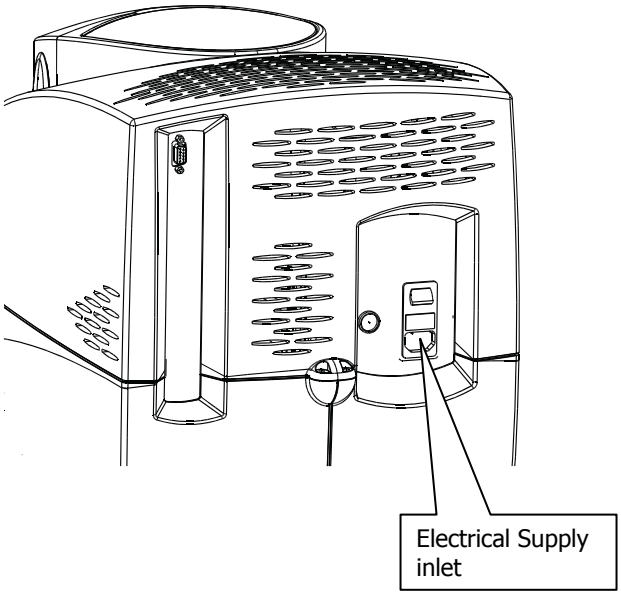


Figure 47 The Location of the Electrical Supply Inlet

Connecting the Microscope to the Spectrometer

The cable from the microscope to the spectrometer comes from the preamp inside the microscope, to a connector marked on the rear of the spectrometer.

The connections from the pre-amp in the microscope to the spectrometer are described below:

Pl 1 (PCB)	D-type Connector	Line	Power Requirement
1.	11	Output Signal	
2.	10	Output Signal (0 V)	
3.	9	0 V	
4.	12	0 V	
5.	13	+12 V	250 mA
6.	14	−12 V	200 mA

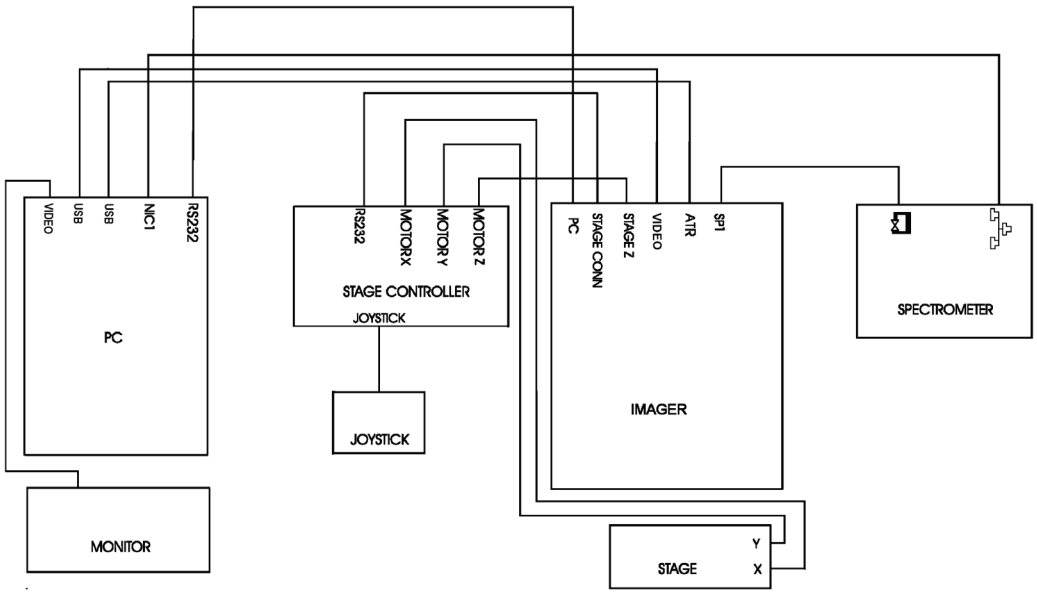


Figure 48 Electrical Connections for a Microscope Connected to a Spectrometer, PC and Stage Controller

Connections to the Stage Controller

The connection labeled X MOTOR on the stage controller plugs into the connector next to the red dot on the motorized stage.

The X, Y and Z connectors are 15-way D-type connectors. They are described below:

Pin	**Description**	**Voltage**	**Current**
1.	OUT1	24 V max	2.52 A peak
2.	OUT2	24 V max	2.52 A peak
3.	OUT3	24 V max	2.52 A peak
4.	OUT4	24 V max	2.52 A peak
5.	$X_{\_END}$; $Y_{\_END}$; $Z_{\_END}$;	+5 V max	(Input signal)
6.	$X_{\_ZERO}$; $Y_{\_ZERO}$; $Z_{\_ZERO}$	+5 V max	(Input signal)
7.	+5 V	+5 V	2.4 A maximum available
8.	OV_L	OV_L	(2.1 A short circuit)
9.	OUT1	24 V max	2.52 A peak
10.	OUT2	24 V max	2.52 A peak
11.	OUT3	24 V max	2.52 A peak
12.	OUT4	24 V max	2.52 A peak
13.	$X_{\_END}$; $Y_{\_END}$; $Z_{\_END}$;	+5 V max	(Input signal)
14.	$X_{\_ZERO}$; $Y_{\_ZERO}$; $Z_{\_ZERO}$	+5 V max	(Input signal)
15.	+24 V	+24 V	2.52 A peak

The Joystick 9-way D-type connector is described below:

Pin	**Description**	**Voltage**	**Current**
1.	$0V_L$	0 V	--
2.	$0V_L$	0 V	--
3.	X_{IN}	+5 V max	(Input signal)
4.	Y_{IN}	+5 V max	(Input signal)
5.	Z_{IN}	+5 V max	(Input signal)
6.	NC	--	--
7.	NC	--	--
8.	+5 V	+5 V	10 mA normal operation (2.4 A maximum available)
9.	+5 V	+5 V	10 mA normal operation (2.4 A maximum available)

The RS232 15-way high density D-type connector is described below:

Pin	**Description**	**Voltage**	**Current**
1.	CTSOPT	+5 V max	15 mA
2.	RXD	+5 V max	15 mA
3.	TXD	+5 V max	15 mA
4.	DTR	+5 V max	15 mA
5.	$0V_L$	0 V	--
6.	CTS	+5 V max	15 mA
7.	RTS	+5 V max	15 mA
8.	CTS	+5 V max	15 mA
9.	ATR_{stop}	+5 V max	15 mA
10.	STEPSTAGE-	+5 V max	15 mA
11.	STEPSTAGE+	+5 V max	15 mA
12.	STEPDONE-	+5 V max	15 mA
13.	STEPDONE+	+5 V max	15 mA
14.	NC	--	--
15.	NC	--	--

Appendices

Appendix 1: Decontamination and Cleaning

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.

Decontamination

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:

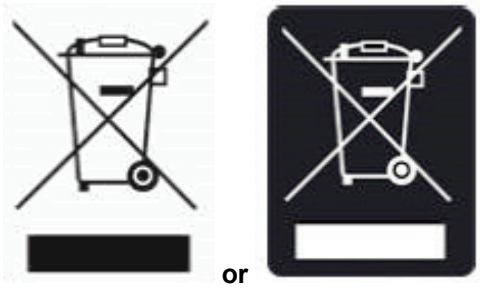
Customer Care USA:	1-800-762-4000	(inside the USA)
(8:30 a.m. – 7 p.m. EST)	(+1) 203-925-4602	(outside the USA)
Customer Care Canada:	800-561-4646	
Customer Care EU:	0800 40 858	(Brussels)
	0800 90 66 42	(Monza)

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

Cleaning the Instrument

Exterior surfaces may be cleaned with a soft cloth, dampened with a mild detergent and water solution. Do **not** use abrasive cleaners or solvents.

Appendix 2: 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.

Index

O

P

R

S

T

V

W