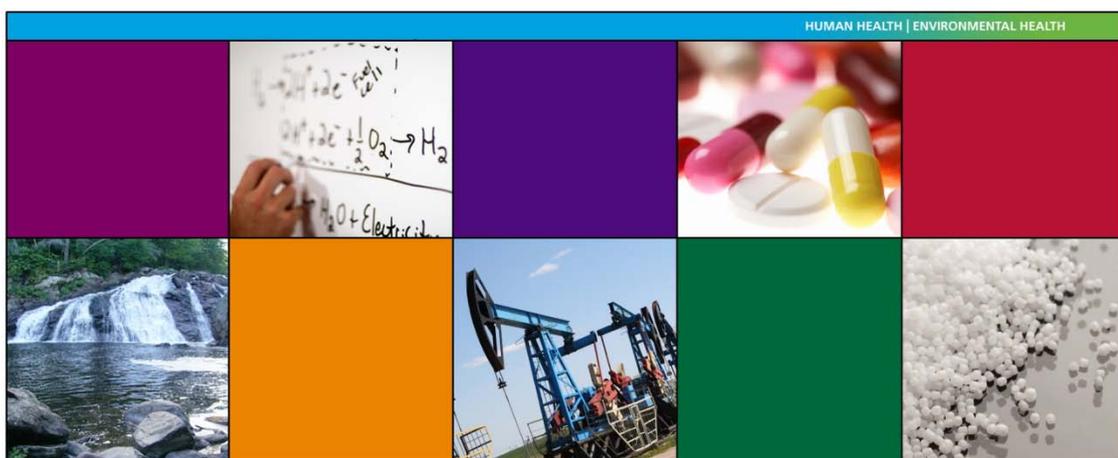


RamanMicro 200 Series



Getting Started Guide

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Introduction

About This Manual

This Getting Started Guide contains all of the information that you will need to setup your RamanMicro 200 Series Raman Microscope and start collecting spectra.

The Getting Started Guide is divided into the following chapters:

Introduction

This chapter contains an overview of the manual, and the conventions and warnings used.

Safety Practices

This chapter outlines the electrical and laser safety issues. We recommend that all users read this chapter.

Raman History, Theory and Instrumentation

This chapter contains a brief overview of the history and theory of Raman spectroscopy.

Preparing your Facility

This chapter shows you how to position your RamanMicro 200 Series instrument, and what utilities are required, for example electricity and temperature control.

Installing your RamanMicro 200 Series

This chapter contains instructions about how to assemble your RamanMicro 200 system and information on installing your instrument in the software.

Using the RamanMicro 200 Series

This chapter gives an overview of the instrument and contains an introduction to the Fiber Optic Probe. It provides a number of Standard Operating Procedures (SOPs) that describe how to perform certain tasks, for example how to collect a spectrum, or how to calibrate the spectrometer.

Maintenance and Troubleshooting

This chapter contains information on how to maintain the system, and solutions to problems you may encounter.

Conventions Used in this Manual

Normal text is used to provide information and instructions.

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.

The term RamanMicro 200 Series means the RamanMicro 200 and the RamanMicro 200F Raman Microscopes.

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.



Safety Practices

Overview

This chapter contains details of the safety precautions. Everyone who is going to install and/or use the system must read and follow the precautions before unpacking, installing or using the instrument.

Before performing any of the operations outlined in this manual, read all of the safety practices and instructions, and make sure that you understand the procedures explained.

If you do not understand any of the details regarding general safety precautions, please contact PerkinElmer before proceeding.

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General Operating Conditions



WARNING

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

The RamanMicro 200 Series spectrometers have been designed and tested in accordance with PerkinElmer specifications and in accordance with the safety requirements of the International Electrotechnical Commission (IEC). The RamanMicro 200 Series conforms to IEC 61010-1 (Safety Requirements for electrical equipment for measurement, control and laboratory use) as it applies to IEC Class 1 (earthed) appliances and therefore meets the requirements of EC directive 2006/95/EC.

Only use the RamanMicro 200 Series spectrometers indoors and under the following conditions:

Temperature 15 °C to 30 °C

Relative Humidity 80% maximum (non-condensing).

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

The instrument has been designed to be safe under the following conditions:

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

Whenever it is likely that the RamanMicro 200 Series instrument is unsafe, make it inoperative. The RamanMicro 200 Series may be unsafe if it:

- Shows visible damage
- Fails to perform the intended measurement
- Has been subjected to prolonged storage in unfavorable conditions
- Has been subjected to severe transport stresses.

Fire Safety

- Ensure that there is at least 15 cm (6") between any cooling vents and adjacent equipment or walls.
- Ensure that the power supply unit (PSU) vents or fan do not become obstructed.

Electrical Safety

The spectrometer module comes assembled within a protective housing to prevent exposure to electrical hazards. There is also a number of exterior service panels that you should remove only if you are cleaning the air filters. For more information see *Cleaning the Air Filters* on page 91.



WARNING

Removal of the protective housing or external panels, other than for maintenance procedures specifically described in this manual, is dangerous and is not recommended under any circumstances.

If the protective covers or service panels on the instrument appear to be damaged, turn the instrument off, secure the instrument against any unauthorized operation and contact PerkinElmer for advice before using it again.

Electrical Protection

Insulation: Class I rating for external circuits. Only connect equipment that meets the requirements of IEC 61010-1, IEC 60950 or equivalent standards.

Power Supplies

The RamanMicro 200 is powered by one external PSU. The PSU used with the spectrometer module can adjust automatically to a variety of AC power sources as outlined in Table 1.

Table 1 PSU input voltages and mains fluctuations information

Input Voltage	Mains Fluctuations
100–230 V AC	±10% of the nominal voltage



WARNING

Under no circumstances should you attempt to disassemble the PSU and carry out repairs. If the PSU has a fault, please contact your PerkinElmer Service Representative. The entire unit must be replaced.

Connect the PSU to a three-wire (live, neutral and ground/earth) socket. The ground must be a non-current carrying wire that is connected to the earth ground at the main distribution box. The power point (socket) for the instrument PSU should be easily accessible for when the instrument is serviced.



WARNING

Check the line voltage and frequency of the power point (socket) before the instrument is connected or the power is switched on. This should be carried out prior to the installation of the instrument and at any time the instrument is plugged into a different power point (socket).

Power Cords (Between PSU and Power Outlet Socket)

The power cord supplied to connect the PSU to the power point (socket) should be appropriate for the country listed as the shipping destination.



WARNING

If the power cord is not appropriate for your country it must be removed from use immediately and replaced.

If the power cord has any physical damage it must be removed from use immediately and replaced.

To avoid electrical hazards, the ground pin on the power cord should not be interfered with in any way.

Fuses

The only fuses that you may have to replace are those in the mains power cords (UK-type plug only) and the stage controller box fuse.

EMC Compliance

EC directive

The RamanMicro 200 and RamanMicro 200F have been designed and tested to meet the requirements of the EC directive 2004/108/EC. The RamanMicro 200 Series 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).

Laser Safety

Details of the RamanMicro 200 Series laser source are summarized in Table 2.

Table 2 Laser Specifications

Class	3B
Wavelength	785 nm
Power output	250 mW (max)

The RamanMicro 200 Series complies with the requirements of IEC 60825-1:2007 and 21CFR 1040.10 FDA performance standards for laser products, except for deviations pursuant to Laser Notice No. 50, dated 26 July 2001.



WARNING

Use of controls or adjustments or the performance of any procedures other than those specified in this document may result in exposure to hazardous laser radiation.



WARNING

Removal of the protective housing or external panels is dangerous.

If the protective covers or service panels on the instrument appear to be damaged, turn the instrument off, secure it against any unauthorized operation and contact PerkinElmer for advice before using the instrument again.

The RamanMicro 200 emits a focused beam of laser radiation at 785 nm (250 mW max.), and is classified as a Class 3B laser system.



WARNING

Avoid direct exposure to the laser beam. Do not look at the laser light or scattered laser light.



WARNING

During normal operation laser radiation is accessible from the microscope objective lens. The output energy should be enclosed whenever possible (using an enclosure or a beam attenuator) to avoid unnecessary exposure to laser radiation.

If you do not have an enclosure, you should use protective laser safety goggles, with an optical density (OD) of at least 3 at 785 nm, as a precaution against accidental exposure to the direct or reflected laser light. Suitable goggles are available from PerkinElmer (part number L1323518).

Class 3B Safety Precautions

You should follow Class 3B laser safety precautions such as the use of door interlocks and the wearing of laser safety goggles. For information on these precautions refer to an appropriately trained laser safety officer within your own facility. Laser safety is very important. Inappropriate use of laser emitting devices may result in permanent injury. In the United States the precautions are described in the ANSI Z136.1-2000 Standard. For information on the ANSI Z136 standard please consult your laser safety officer and/or consult <http://www.z136.org>. Outside the United States the guidelines are described in PD IEC TR 60825-14 (Safety of laser products – Part 14: A user's guide). You should also be familiar with the guidelines published by the national authorities in your country.

NOTE: In certain circumstances when the probe is permanently fixed in a closed environment, for example inside a sealed reaction vessel, the device can be defined as Class 1. **Please refer to an appropriately trained laser safety officer within your own facility.**

NOTE: The RamanMicro 200 Raman Microscope can be fitted with an optional light-proof enclosure, which is fitted with interlock sensors to ensure that the microscope cannot be used with the enclosure doors open. The Raman system is NOT reclassified to a Class 1 instrument.

The following information is provided as a general overview only. This information is rudimentary and is supplied to assist in the interpretation of this manual. Under no circumstances use this information as the basis of a laser safety code of behavior:

1. Class 3B lasers may emit visible and/or invisible radiation up to a maximum of 0.5 W continuous wave (CW is longer than 0.25 s). They present a hazard to the eye if the direct beam or spectral reflections are viewed without appropriate protection. You must only operate Class 3B lasers in a designated area.
2. You must fit each Class 3B device with a captive key control switch, such that the key cannot be removed from the lock except in the OFF position. The key should be removed from the laser when it is not in use and kept in a safe place in the custody of a nominated person. Keys must never be issued to unauthorized persons.
3. Each Class 3B device should be assessed by a suitably trained laser safety officer to determine the accessible radiation emitted by the device. The laser safety officer should consider laser power, wavelength and beam divergence when determining if the Class 3B device's remote interlock connector should be connected to an interlock switch at the entrance. For certain laser systems the laser safety officer may require the shutter to be interlocked with the laboratory entrance in order to prevent inadvertent exposure of persons entering to very hazardous laser radiation.
4. Rooms in which Class 3B devices are used should have notices affixed to the doors so that they conform to the requirements of appropriate Laser Safety Regulations.

General Laser Safety Issues

Laser Keyswitch

There is a captive laser key control switch on the front panel of the spectrometer. This key is required to operate the laser. A laser safety officer or laboratory manager should control the use of this key.

Laser Warning Labels

Laser warning labels are attached to the instrument for your safety. Please observe the necessary precautions. For more details see *Warning Labels* on page 25.

Microscope Safety

The laser should also be turned off when the system is not in use. **AVOID DIRECT EXPOSURE TO THE BEAM.**

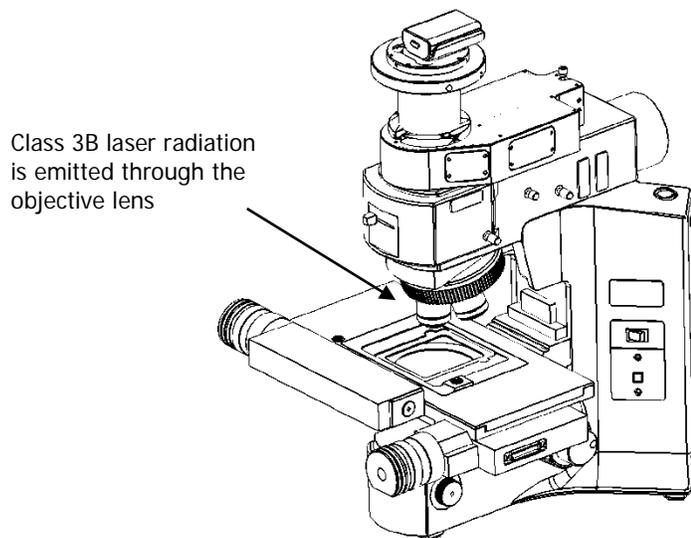


Figure 1 Schematic of the microscope

When using the microscope, the following precautions must always be observed:

- Never look directly into the laser beam or at the laser light scattered from a reflective surface or sample.
- Avoid direct exposure to the laser light.
- Place warning signs near the laser operating areas.
- Control laser areas and limit access to individuals trained in the safe operation of lasers.
- Use protective laser safety goggles as a precaution to accidental exposure to the direct, or reflected, laser light.
- Use an interlock switch on the door to the laboratory, if required.

**WARNING**

Do not make any adjustments to the microscope or any part of the system when experiments are in progress.

In particular:

- *Do not remove the objective lens from the microscope*
- *Do not insert or remove samples*
- *Do not disconnect the Raman module*
- *Do not fit binocular eyepieces to the microscope module.*

These adjustments may deflect laser light from the safe light path through the system.

**WARNING**

Do not leave blank positions in the microscope turret when performing your experiments. Ensure that all blank positions have protective covers in position at all times.

If your system includes any Fiber Optic Probes, ensure that protective covers are in place when FOPs are not in use.

Fiber Optic Probe Safety

NOTE: This section is only applicable to RamanMicro 200F systems equipped with a fiber optic probe.

The fiber optic probe body incorporates a number of important safety features, including:

- Beam attenuator/cover
- Emission indicator/LED.

When attached to the spectrometer, the fiber optic probe provides a focused beam of Class 3B laser radiation. During normal operation, laser radiation is accessible from the end of the fiber optic probe. The LED emission indicator (Figure 2) at the probe head is configured so that the LED lights up when the laser is turned on, indicating that laser radiation is emitting from the probe aperture. The LED emission indicator is visible through laser safety goggles. The output energy should be enclosed whenever possible (using a beam attenuator or cover) to avoid unnecessary exposure. The laser should be turned off and the fiber optic probe attenuator cap replaced when the system is not in use. **AVOID DIRECT EXPOSURE TO THE BEAM.**

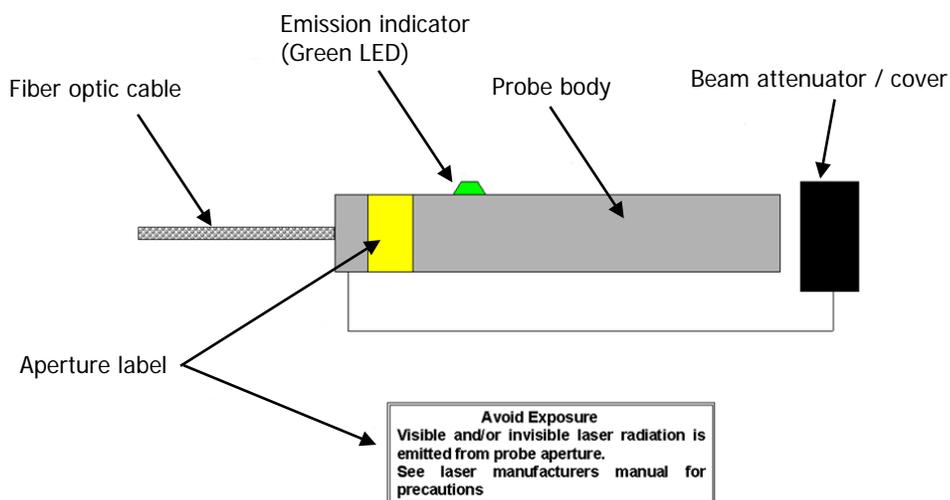


Figure 2 Schematic of the fiber optic probe (not to scale)

When using the fiber optic probe, the following precautions should be observed:

- Never look directly into the laser beam or at the laser light scattered from a reflective surface.
- Avoid direct exposure to the laser light.
- Use an interlock switch on the door to the laboratory and/or the reaction vessel.
- Place warning signs near the laser operating areas.
- Controlled access areas, limited to individuals trained in the safe operation of lasers, are suggested for laser operations.
- Use protective laser safety goggles, with an OD (optical density) of at least 3 at 785 nm, as a precaution against accidental exposure to the direct or reflected laser light. Suitable goggles are available from PerkinElmer (part number L1323518).
- Ensure that the attenuator cap is attached.

Triggered Fiber Optic Probe Safety

When attached to the RamanMicro 200, a triggered fiber optic probe (TFOP) provides a focused beam of Class 3B laser radiation. During normal operation, laser radiation is accessible from the end of the probe.

The following precautions must be observed at all times:

- Keep the protective cover in place on the probe tip when the probe is not in use. If you are using the TFOP with a plastic spacer, always rotate the spacer so that the screw is beneath the probe barrel when not in use; when in this position the spacer acts as a lens cap.
- Never look directly into the laser beam or at the laser light scattered from a reflective surface or sample. Permanent eye-injury may result. Do NOT attempt to examine the probe tip when the Path Active light on the probe is lit.
- Post warning signs near the laser operating areas.

- Controlled access areas, limited to individuals trained in the safe operation of lasers, are suggested for laser operations.
- When the probe is in use, always ensure that no stray laser radiation is directed at people in the vicinity.
- Use protective laser safety goggles, with an optical density (OD) of at least 3 at 785 nm, as a precaution against accidental exposure to the direct or reflected laser light. Suitable goggles are available from PerkinElmer (part number L1323518).
- Do not use the TFOP if it shows any sign of damage.
- If repair or servicing is needed, call your PerkinElmer Service Representative for advice.

For further safety details, see the *Raman Triggered Fiber Optic Probe* leaflet (L1321940)

Calculation of Nominal Hazard Zones for use of Fiber Optic Probe

The following calculations show how to calculate a nominal hazard zone. These calculations are taken from ANSI Z136.1-1933. A laser safety officer uses these calculations to help define how to use your Raman probe safely in your environment.

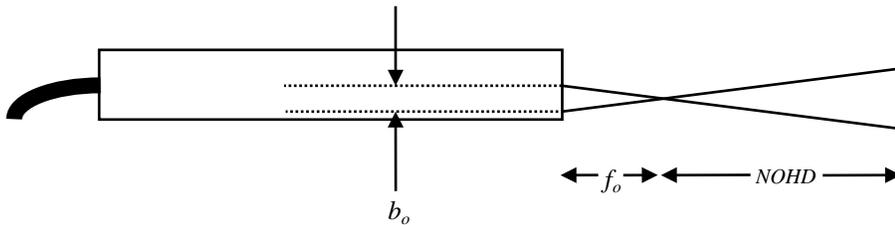


Figure 3 Probe parameters used to calculate the nominal ocular hazard distance

The equation for a focused beam as given here (Figure B5 in ANSI Z136.1-1933) is used to calculate the nominal ocular hazard distance (NOHD) for the fiber optic probe. The equation is:

$$NOHD = \left(\frac{f_o}{b_o} \right) \left(\frac{4\Phi}{\pi MPE} \right)^{1/2}$$

Where:

- f_o = Probe focal length (cm)
- b_o = Diameter of laser beam incident on probe focusing lens (mm)
- Φ = Total radiant output power of probe (watts)
- MPE = Maximum permissible exposure ($J\ cm^{-2}$)

For the FOP b_o is 0.254 cm, therefore the above equation can be simplified to give:

$$NOHD = 4.442.f_o \left(\frac{\Phi}{MPE} \right)^{1/2}$$

For the wavelength range 700–1050 nm the MPE for a 10 second exposure can be calculated from:

$$MPE = \left[(10.1)(10^{2(\lambda-0.7)}) \right] 10^{-4} J\ cm^{-2}$$

Where λ is the laser wavelength in micrometers (μm). For example, a laser wavelength of 785 nm (0.785 μm) will have an *MPE* value of $1.49 \times 10^{-2} \text{ J cm}^{-2}$ or $1.49 \times 10^{-3} \text{ W cm}^{-2}$ for a 10 second exposure or longer. Table 3 shows the calculated nominal ocular hazard distance for 785 nm Raman systems.

Table 3 Nominal Ocular Hazard Distance (NOHD) for 785 nm

Probe focal length / mm	NOHD for 100 mW exiting fiber optic probe / cm
7.5 ($\pm 10\%$)	27.3

NOTE: Other focal lengths are available; contact PerkinElmer for details. In particular, the use of an ultra-long working distance lens adaptor (part number L1320071) provides a working distance of 25 mm. Where different focal lengths are used, the NOHD should be calculated accordingly.

Internal Interlock System

There is no internal interlock system fitted to the RamanMicro 200. If instrument panels are removed collimated laser light may be visible. Under no circumstances remove any instrument panels.

External Interlock System

Class 3B laser systems have the potential to cause harm or injury to those using them or others in the vicinity of the instrument. For more information refer to *Attaching the External Interlock* on page 40.

Warning Labels



When this label is attached to an instrument it means refer to the manual to find out the nature of the potential hazard and any actions which have to be taken.

Labels on the Exterior of the Spectrometer

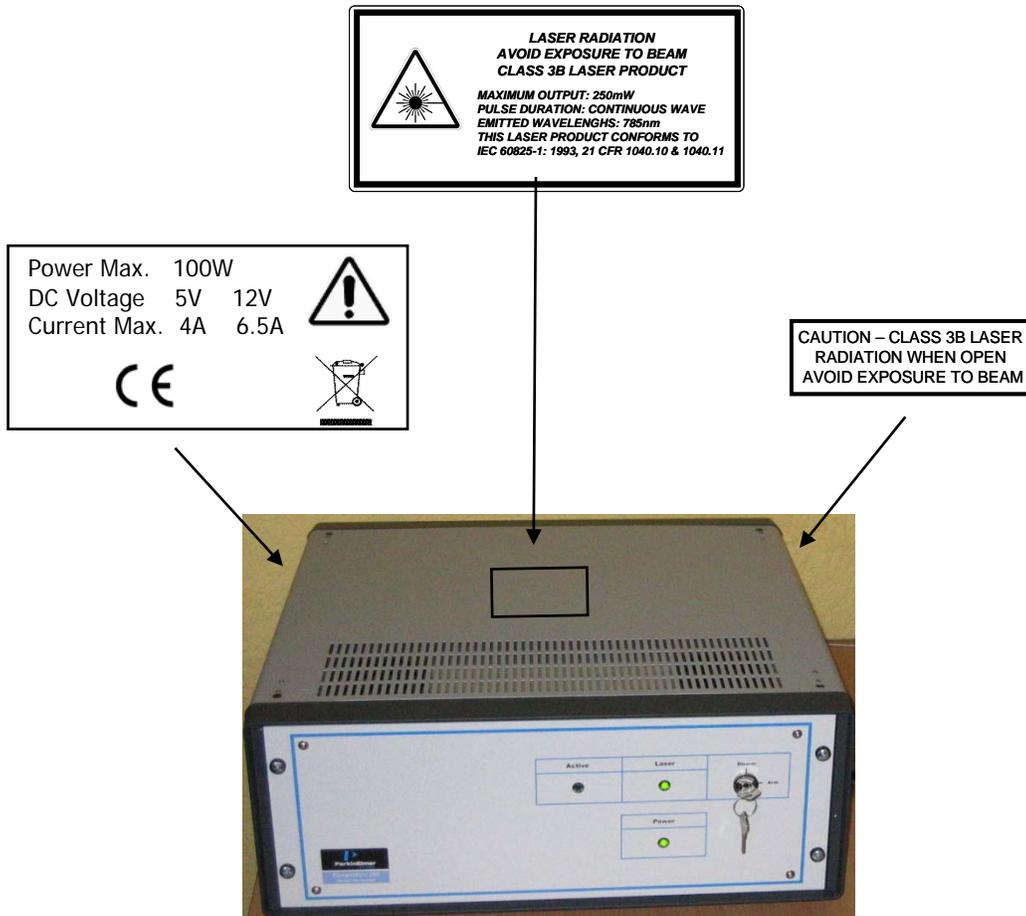


Figure 4 Labels on the spectrometer module

Labels on the Exterior of the Microscope

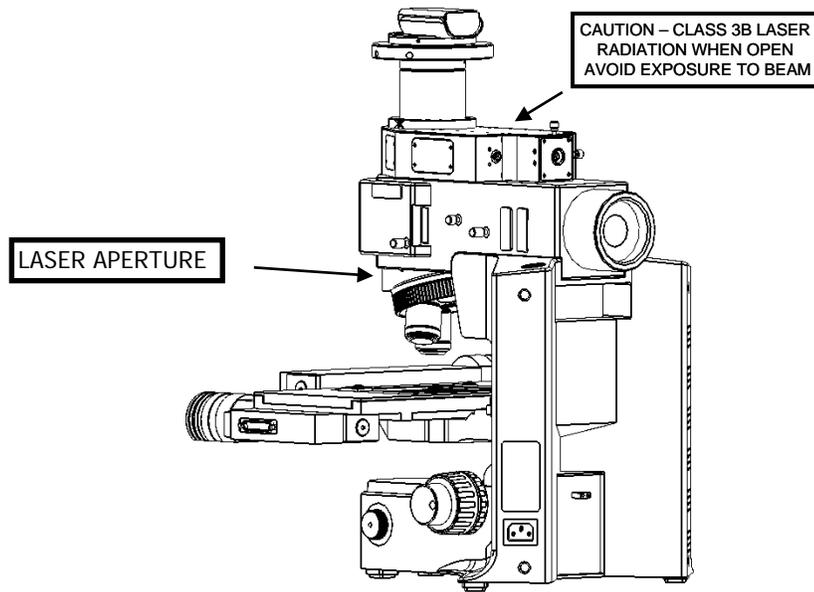


Figure 5 Labels on the microscope

Raman History, Theory
and Instrumentation

The History of Raman Spectroscopy

Inelastic light scattering was first predicted in 1923 by A. G. Smekal in Germany. Research carried out by A. H. Compton discovered inelastic scattering of X-rays, which led the Indian scientist C. V. Raman to investigate this phenomenon. Raman and his assistant Krishnan observed what came to be known as the Raman effect (Figure 6) for the first time in 1928. They separated the blue light from sunlight and observed the scattering from water and alcohol visually. In addition to the blue incident light, a faint green light also scattered, later named Stokes scattering. By the 1930s much of the theory underlying Raman scattering was understood, but due to the complexity of the instrumentation the initial interest in Raman spectroscopy was not maintained. Improvements in charge-coupled device (CCD) detectors, filters and lasers over the past 25 years have radically simplified Raman instrumentation, and interest in Raman spectroscopy has been renewed. Lasers are ideal excitation sources for Raman because they give highly monochromatic radiation and provide high irradiances at the sample.

Raman Theory

When photons from a monochromatic light source impinge on a sample, a small fraction are scattered in all directions. Some of the scattered photons are of the same energy as those of the incident beam, and hence the same frequency, giving rise to Rayleigh scattering (Figure 6).

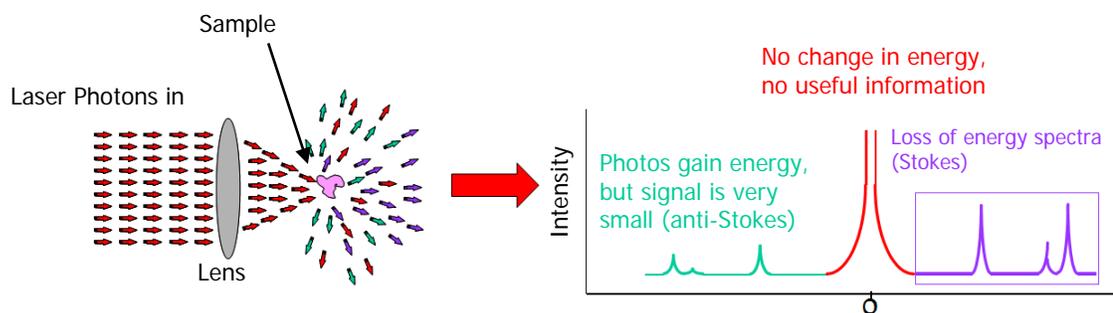


Figure 6 The Raman effect: irradiation of sample and subsequent scatter

Some photons will be inelastically scattered and will be detected at either longer wavelengths (lower frequencies, ν' , Stokes scattering) or shorter wavelengths (higher frequencies, ν'' , Anti-Stokes scattering). With Stokes scattering, some of the incident photons give up a portion of energy to the molecules and are detected at a lower frequency (longer wavelength) than the Rayleigh radiation (Figure 7).

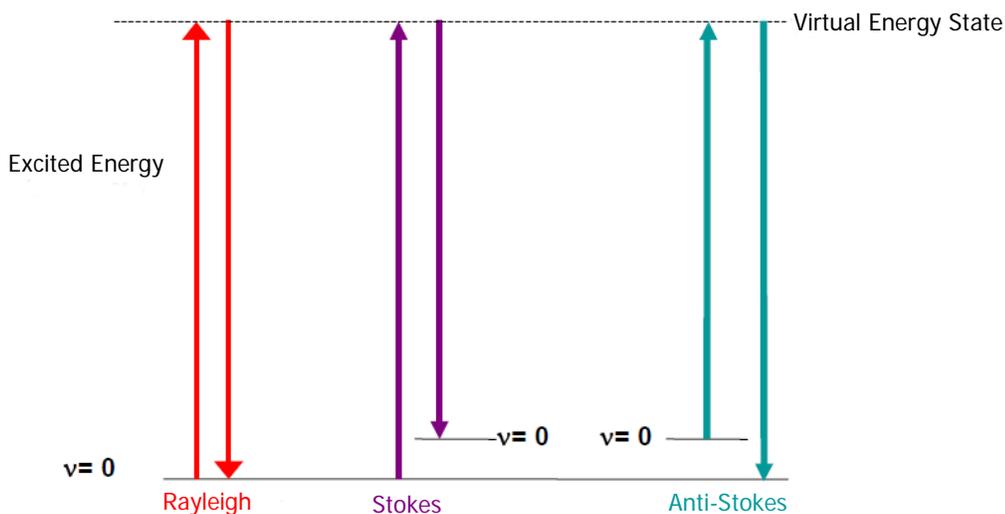


Figure 7 Rayleigh, Stokes and Anti-Stokes scattering

The change between the incident radiation and the Stokes/Anti-Stokes radiation represents a change in the vibrational and/or rotational energy of the molecule. The frequency shifts from the frequency of the incident beam are displayed on the x-axis of a Raman spectrum, generally in relative wavenumbers (otherwise known as Raman Shift) and the intensity of the bands are displayed in arbitrary units on the y-axis.

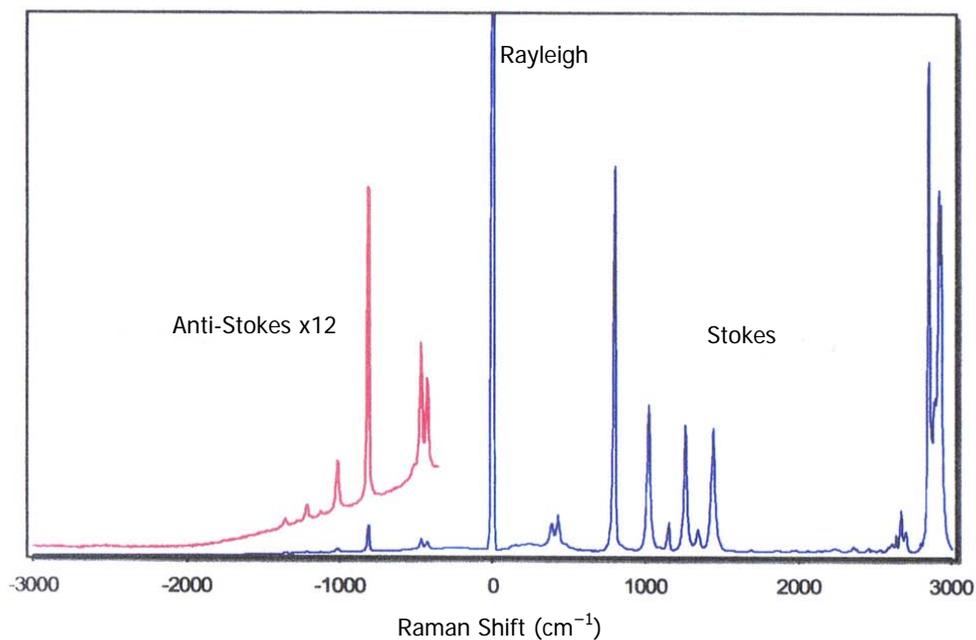


Figure 8 Raman spectrum of cyclohexane, showing Stokes, anti-Stokes and Rayleigh scattering

The RamanMicro 200

The RamanMicro 200 is a Raman microscope system with laser excitation wavelength at 785 nm.

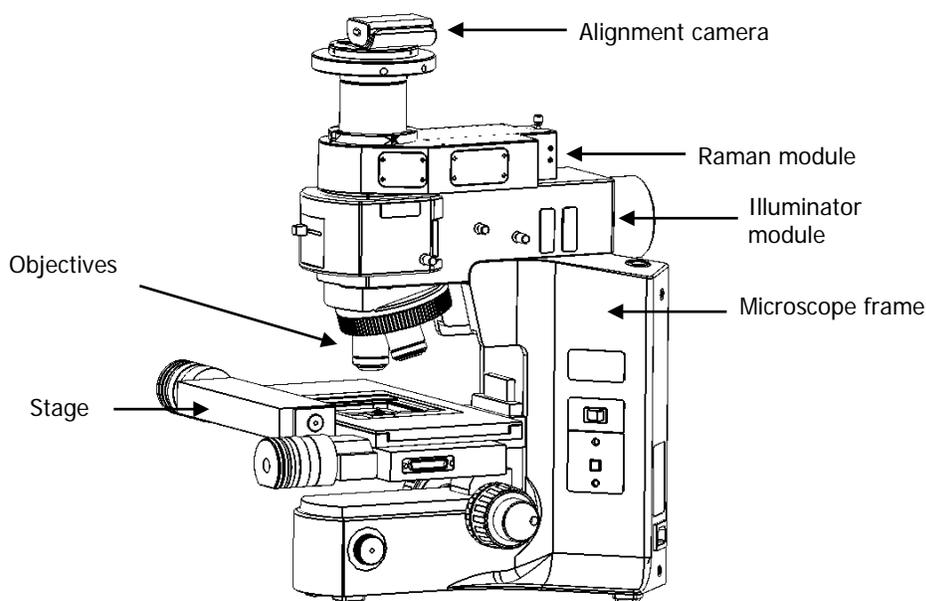


Figure 9 The RamanMicro 200 microscope

The microscope (Figure 9) is composed of a frame, illumination module, Raman module and camera module. This is connected to the spectrometer module by two fiber optic cables. The spectrometer module contains the spectrograph and CCD detector. The laser supplies light through an objective lens onto the sample. The scattered light from the sample is collected with the same objective lens and transmitted back to the spectrometer. A schematic of the system configuration is provided in Figure 10.

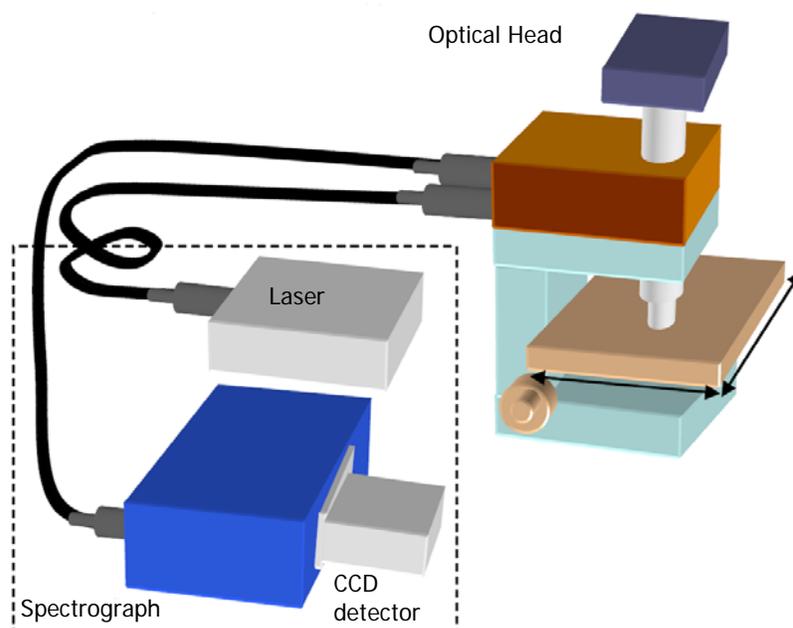


Figure 10 Schematic of the RamanMicro 200 system

The Czerny–Turner Spectrograph

The RamanMicro 200 is equipped with a Czerny–Turner spectrograph. The Czerny–Turner spectrograph is the most commonly used spectrograph design in Raman spectroscopy. Here, light is focused into the spectrograph, collimated and shone on a grating which disperses that light in one dimension. The light is then focused onto a photo-sensitive CCD detector (typically 1024 pixels wide), which detects the Raman spectrum.

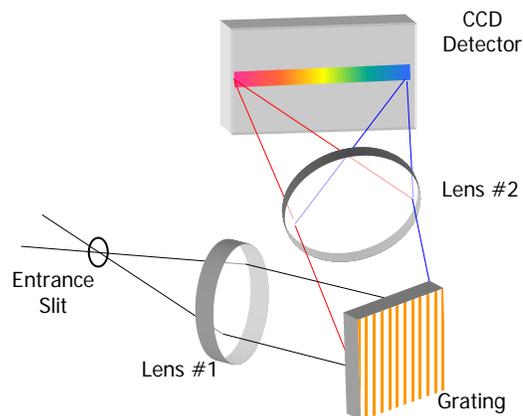


Figure 11 Schematic of the Czerny–Turner spectrograph

The Czerny–Turner spectrograph is equipped with a fixed grating, and the range of light falling on the CCD can be factory configured to be approximately $95\text{--}3200\text{ cm}^{-1}$ at a resolution of about 8 cm^{-1} .

Preparing Your Facility

Specifications

	Spectrometer	Microscope	Stage Controller (for motorized XYZ stage only)
Weight	20 kg	20 kg	2.8 kg
Height	161 mm	520 mm	90 mm
Width	530 mm	370 mm	220 mm
Depth	600 mm	430 mm	184 mm
Power Supply	100–230 V \pm 10%, 50–60 Hz	100–120 V/220–240 V 50–60 Hz	100–240 V, 50–60 Hz
Primary Fuse	–	See Olympus manual	1.6 A T (time-lag)
Laser Class	Class 3B	–	
Operational Temperature	15 °C – 30 °C		
Recommended Temperature	20 °C – 25 °C		

If the electricity supply does not conform to these specifications, please consult PerkinElmer prior to installation. If the supply of power is erratic please use an uninterruptible power supply (UPS). Incorrect shutdown, power fluctuations or brown-outs may damage the spectrometer.

Finding a Suitable Location for the Instrument

The following conditions should be observed:

- The environment must be suitable for a Class 3B laser system.
- There must be a minimum of 4 power points (1 for the microscope, 1 for the spectrometer and 2 for the PC). If your system has a motorized stage you will also require 1 power point for the stage controller.
- The workbench must be flat and level.
- The workbench where the spectrometer and PC are to be located should be at a suitable height to allow the user to work comfortably.
- Use conducting floor coverings to minimize static discharge.
- Floor vibrations or noise from heavy manufacturing equipment can affect the performance of the instrument.
- Intense magnetic fields should be at least 5.5 meters (18 feet) away from the spectrometer.
- Never place the instrument near a window that gets direct sunlight.

Ensure all users understand laser safety issues before locating and using the instrument. Consult your Laser Safety Officer and perform a risk assessment before installing the equipment.

Instrument Dimensions

The RamanMicro 200 spectrometer module weighs approximately 20 kg. The microscope module weighs approximately 20 kg. Laboratory benching must be strong enough to support the combined weight of the microscope, spectrometer module and any ancillary equipment without warping or wobbling. The bench must be at least 610 mm (26") in depth.

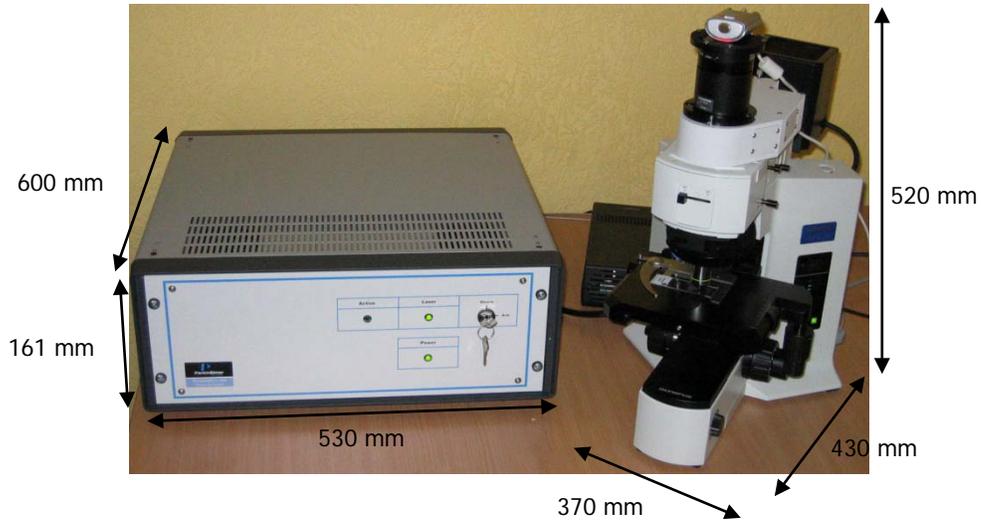


Figure 12 Dimensions of the RamanMicro 200 system

**Installing Your
RamanMicro 200 Series**

Unpacking the Instrument

When the instrument has been delivered, please move it with care and avoid shaking or dropping the spectrometer. Allow the instrument to equilibrate to room temperature for at least 12 hours before opening. The instrument may have been stored at low temperature prior to delivery and you should allow it to equilibrate to stop moisture condensing on the internal electronics.

When unpacked, check that you have the following parts and check for any physical damage. If there is any damage, do not continue with the installation. Contact your PerkinElmer Service Representative immediately.

Part Number	Description
	RamanMicro 200 Series Contains the microscope, the Raman module, the alignment camera module, and the spectrometer module.
LX108870	Spectrum Std for Raman Software Kit Contains the Spectrum Std Software CD (LX100877), the Raman Instrument Configuration Disk (LX108869), the Raman Spectral Libraries CD (L1321877), and the Spectrum Insight Software CD (LX108872)
L1323503	Laser key (x2)
L1321802	Mains PSU
L1320821	USB Cable
L1320264	External Interlock Override
L1323055 OR L1323011	Manual Stage Motorized Stage
L1865749	RS232 Cable (systems with motorized stages only)
L1860289	Stage Controller (systems with motorized stages only)
L1865723	Joystick and cable assembly (systems with motorized stages only)
L1181228	Polystyrene Sample
LX108897	SpectrumIMAGE Software Kit (systems with motorized stages only)
N1870151	Microscope Tools Kit

NOTE: Your instrument is intended for installation by a PerkinElmer Service Representative. There are no user-serviceable parts in the RamanMicro 200 Series. The following sections are provided for your information only.

Lifting the Instrument

The RamanMicro 200 spectrometer and microscope modules each weigh 20 kg. To avoid injury or damage to the instrument, please use proper lifting techniques.



WARNING

To lift and/or move the instrument safely requires two people.



WARNING

Always hold the instrument under the base when you lift the spectrometer.

Attaching the External Interlock Override

The RamanMicro 200 Series has an external interlock connection located on the rear panel of the spectrometer module. To operate the instrument, the external interlock must be satisfied.

You must connect an external door interlock to the 7-way DIN connector labeled **INTERLOCK** on the rear panel of the spectrometer, or attach the External Interlock Override (L1320264) supplied with the instrument.

If your laser safety officer determines that an interlock is required on the door to the room, you will need to purchase the Raman Laser Safety Kit (L1320262). For more information contact your PerkinElmer representative.

Assembly of the Microscope

The Olympus BX51 microscope is designed to enable you to analyze microscopic samples. You can purchase a manual stage or a motorized stage.



WARNING

To prevent potential injury to yourself and damage to the instrument, switch OFF all components in the system and disconnect them from the line power supply before you perform any of the procedures described in this section.

Fitting the Stage on the Microscope

To fit the stage:

1. Unpack the stage from the shipping material.
2. Remove the objective nosepiece from the microscope by unfastening the grub screw on the right-hand side of the nosepiece.
3. Carefully locate the stage above the stage support ring and then lower the stage into position.
4. Ensure that the stage is level, and then fasten the stage in position by tightening the screw at the front of the stage (Figure 13).

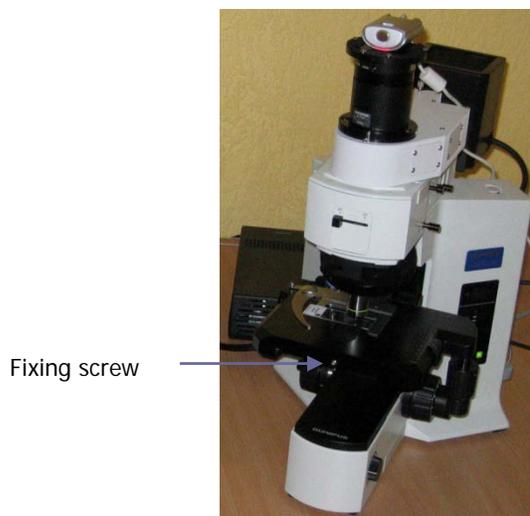


Figure 13 Fitting the stage (manual stage shown)

5. Replace the objectives and tighten the securing grub screw.

If you have a manual stage, you can upgrade to a motorized stage at any time. For more information contact your PerkinElmer representative.

Connecting the Motorized Stage

The motorized stage and joystick (L1865723) are powered by the stage controller box (L1860289).



Figure 14 Stage controller and joystick

To connect the motorized stage:

1. Connect the cable from the joystick to the socket labeled **JOYSTICK** on the rear panel of the stage controller box (Figure 15).



Figure 15 Rear panel of the Stage Controller

2. Connect the cable from the socket on the right side of the stage to the **MOTOR X** and **MOTOR Y** sockets on the rear panel of the stage controller box.
3. Connect the RS232 cable (L1865749) from the PC to the socket labeled **RS232** on the rear panel of the stage controller.
4. Connect the power cable from the stage controller box to the mains supply.

Attaching the Z-axis Focus Motor and Sleeve

NOTE: If you have purchased the optional microscope light-proof enclosure, attach the z-axis focus motor and sleeve before fitting the enclosure. See the *Raman Microscope Light-Proof Enclosure* leaflet (L1321873) for more information.

1. Slide the focus motor sleeve over the z-axis drive control knob on the left side of the microscope, and then tighten the connections using a hexagonal key.
2. Push the focus motor into the sleeve until it touches the drive control, and then tighten the screw to finger tight.
3. Connect the serial connector from the focus motor into the connection at the rear of the stage control box labeled **MOTOR Z** (Figure 15).

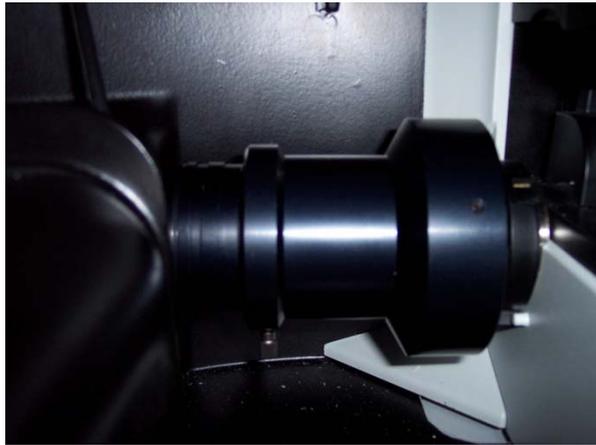


Figure 16 Z-axis focus motor and sleeve attached to the microscope inside the optional light-proof enclosure

NOTE: Ensure that the pre-focusing lever on the coarse adjustment control is on the off position.

Attaching the Illumination Source

To attach the illumination source to the illuminator module:

1. Hold the illumination source in position against the rear of the illuminator module.
2. Using the allen key supplied (Figure 17), fasten the two screws so that the source is supported.
3. Connect the black cable from the illumination source to the microscope frame.

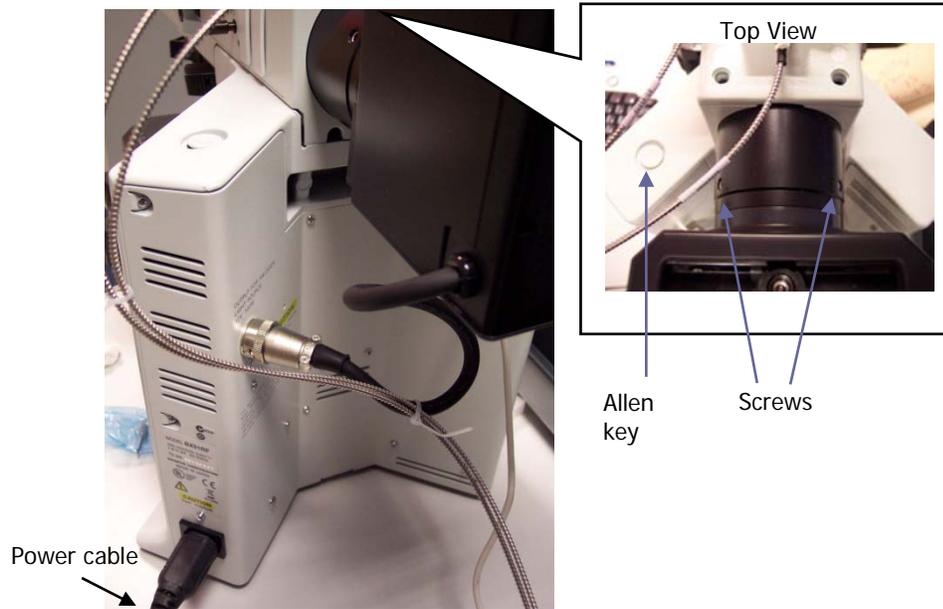


Figure 17 Attaching the illumination source to the microscope

Connecting the Raman Module to the Microscope

The Raman module is positioned on top of the illuminator module so that the connector bracket is aligned above the mounting hole. Ensure that the side of the Raman module is aligned with the illuminator module and then tighten the grub screw.

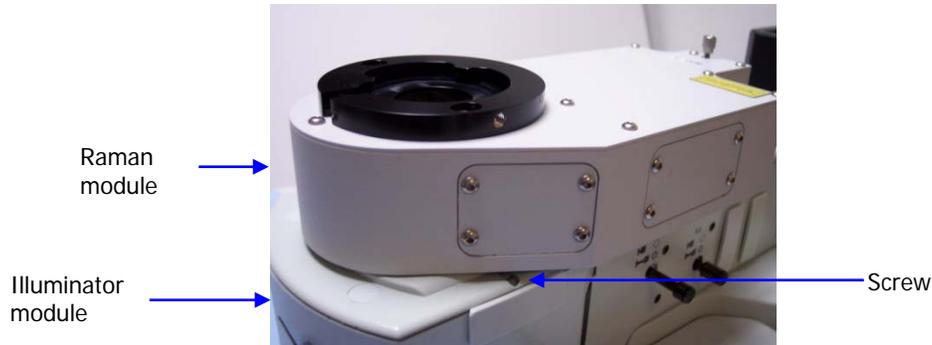


Figure 18 Connecting the Raman module

Connecting the Alignment Camera to the Raman Module

The alignment camera module is then connected into the mounting bracket on top of the Raman module, and the screws tightened.

NOTE: Do not attach more than two alignment cameras in the Raman system.

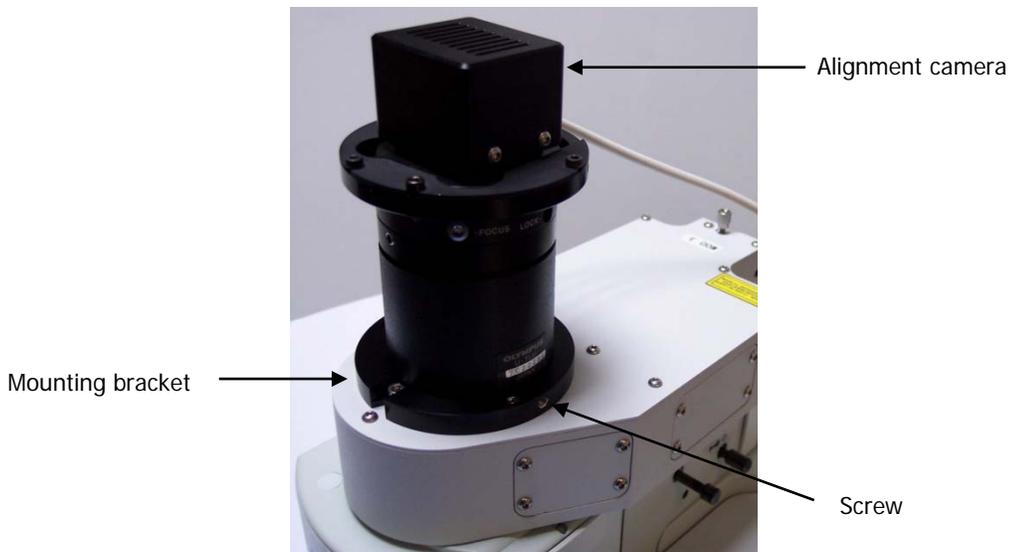


Figure 19 Connecting the alignment camera

NOTE: Do not connect the USB from the camera to the PC until after the software is installed. See *Installing the Instrument in Spectrum Software* on page 49.

Connecting the Microscope to the Spectrometer Module

The microscope is connected to the spectrometer module by two fiber optic cables. The excitation fiber transmits the laser energy from the spectrometer to the microscope, and the collection cable transmits the collected Raman data back to the detector.

1. Connect the excitation cable (L1323331) from the Raman module to the **EXC** coupler under **LED FIBER 1** on the rear panel of the spectrometer.
Ensure that the end of the fiber with the white cable marker is attached to the Raman module.

CAUTION

Try to bend the fiber as little as possible. If you bend the fiber through a tight angle it can be damaged.

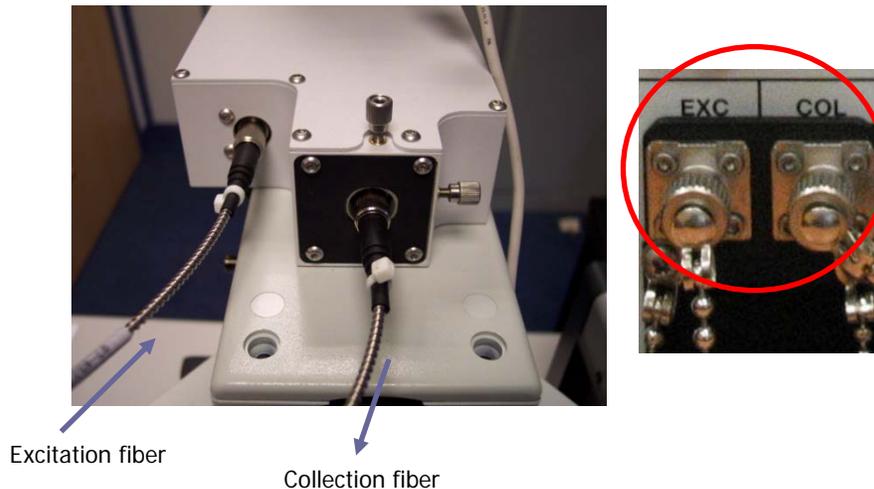


Figure 20 Fiber optic cables connecting the Raman module to the spectrometer

Make sure that the polarizing ridge on the fiber optic cable is aligned with the slot in the coupler casing before carefully pushing the cable into the fiber bracket.

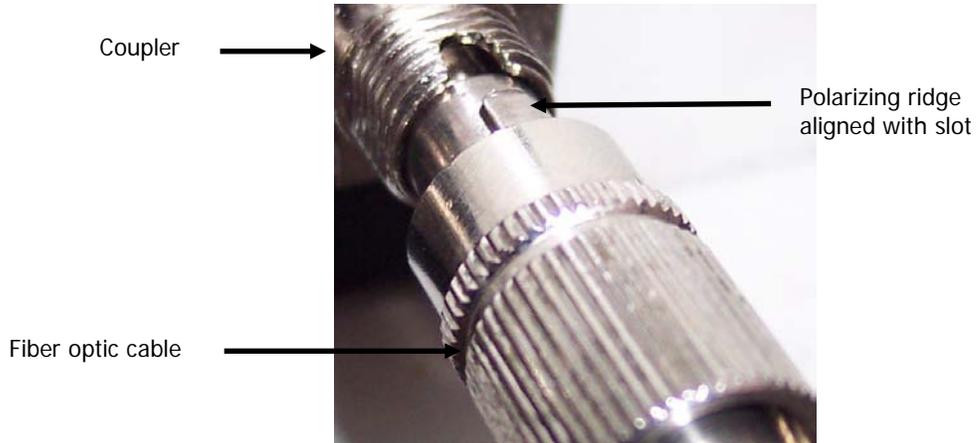


Figure 21 Connecting the fiber optic cable

2. Connect the collection cable (L1323332) from the Raman module to the **COL** coupler under **LED FIBER 1** on the rear panel of the spectrometer.
Ensure that the end of the fiber with the white cable marker is attached to the Raman module.
Make sure that the polarizing ridge on the fiber optic cable is aligned with the slot in the coupler casing before carefully pushing the cable into the coupler (Figure 21).

CAUTION

Try to bend the fiber as little as possible. If you bend the fiber through a tight angle it can be damaged.

3. When you have connected both cables, ensure that they do not touch the illumination source.
You could use a cable tie to direct the cables round the source.

Switching on the Instrument

NOTE: Do not connect the USB from the alignment camera to the PC until after the software is installed. See *Installing the Instrument in Spectrum Software* on page 49.

1. Insert the key into the laser power keyswitch on the front panel of the spectrometer module and ensure that it is turned to the ARM position.
The RamanMicro 200 is supplied with two keys. One should be placed in the instrument and the other should be stored in a safe place. Replacement keys (L1323503) can be obtained from PerkinElmer. A laser safety officer or lab manager can control use of the instrument with these keys.
2. Connect the instrument power supply unit (L1321802) to the RamanMicro 200 spectrometer **Main Power** socket and then switch on.
Do not connect to the **Aux Power** connector.
3. Connect the power cable from the microscope to the mains supply (Figure 17), and then switch on the microscope.
4. If you have a motorized stage, switch on the stage controller box (Figure 15).

Installing the Instrument in Spectrum Software

You must not connect the instrument USB cable or the microscope alignment camera USB cable to the PC before the Spectrum software has been installed.

NOTE: A PerkinElmer Service Representative will install the Spectrum software on the PC and add set up your instrument in the software. However, details of the software installation can be found in the *Spectrum Administrator's Guide* (part number L1050095).

After the software is installed, you should switch on your Raman instrument. Wait a few moments for your instrument to initialize and then connect the microscope alignment camera USB cable to the PC.

The instrument then needs to be added to the software. See "Installing a Raman Instrument" in the *Spectrum Administrator's Guide* (part number L1050095). You can add multiple FT-IR spectrometers to Spectrum on the same PC as a Raman instrument. However, you can only add one Raman instrument to Spectrum.

You should now continue to connect the fiber optic cables for any fiber optic probe or triggered fiber optic probe to the RamanMicro 200 Series instrument, as described in *Attaching and Removing the Fiber Optic Probe* on page 59.

NOTE: For details of how to use a triggered fiber optic probe, see *Raman Triggered Fiber Optic Probe User's Guide* (part number L1321940).

Alignment of the Microscope



WARNING

Do not disconnect the microscope from the spectrometer. Only trained PerkinElmer Service Representatives should attach and remove the fiber optic cables that connect the two modules.

The RamanMicro 200 system consists of four primary components:

- The spectrometer module (containing the laser and the detector)
- The microscope
- The Raman module
- The alignment camera module.

Please read and understand the general safety precautions before proceeding. If you do not understand any of the details regarding general safety precautions, please contact PerkinElmer before performing any of the following procedures.

Aligning the Microscope Objectives

NOTE: Do not touch the glass of the objective lenses when you select the appropriate magnification. Do not push on an objective to rotate the nosepiece, but use the revolving plastic ring.

The microscope objective nosepiece can fit up to 4 objectives.

1. Select the highest magnification objective lens on the microscope (either the 100x or 50x) by rotating the plastic ring on the objective nosepiece.
2. Select the Bright Field (BF) switch position on the microscope.
3. Place a slide with an easily recognizable feature on it (for example, a graticule slide).
4. In the Spectrum software, select **Raman Instrument** from the Setup menu. The Setup Instrument tabs are displayed.
5. On the Setup Instrument Basic tab, ensure that **Microscope** or **Microscope XYZ Stage**, as applicable, is selected as the Accessory.
6. Select the Setup Microscope or Setup Microscope XYZ Stage tab, as applicable, and then select **Live Video**.
7. Move the stage until the feature is in focus and in the center of the crosshairs in the Live Video. Ensure that the image remains sharp and focussed.
8. Select the next highest magnification objective.

9. Insert the adjuster keys supplied with the microscope into the nosepiece and move the adjusters until the crosshairs are aligned with the same feature as for the previous objective lens.



Figure 22 Microscope objective adjusters

10. Repeat Steps 8–9 until all the objectives are aligned.

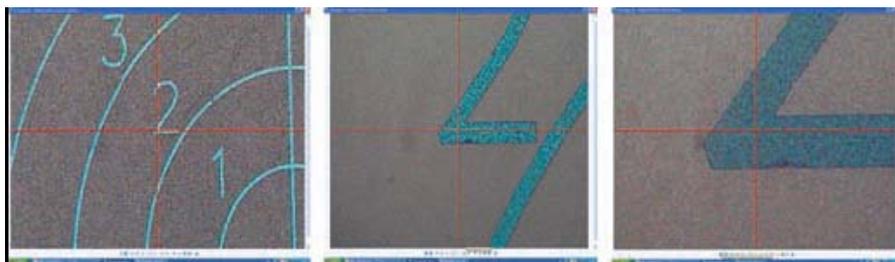


Figure 23 Feature in center of cross hairs at three objective magnifications

Aligning the Raman Module

There are three levels of optical alignment in the Raman module:

- (i) Alignment of the laser path
- (ii) Alignment of the video camera
- (iii) Alignment of the Raman coupler

Level (ii) and level (iii) may be carried out without removing any instrument paneling. Level (i) requires removal of instrument paneling and must only be carried out by a PerkinElmer Service Representative, and in a Class 3B environment.

Aligning the Laser Path

The laser path only needs realignment if the beam is observed to move grossly in the *xy*-plane when moving into and out of focus. If the laser path is aligned correctly, then the laser spot should get larger in off-focus positions without appreciable lateral movement.

NOTE: The requirement for laser alignment is NOT indicated by a situation where the laser spot and the center of the crosshairs are non-coincident (this scenario requires realignment of the video camera with the laser spot).

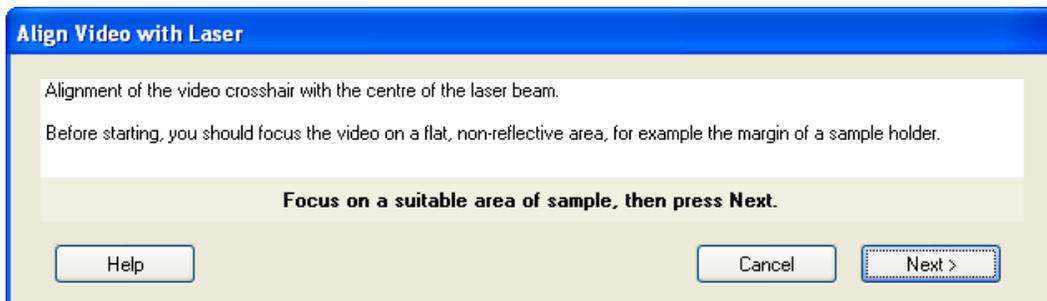
If the system is misaligned, we strongly recommended that you contact a PerkinElmer Service Representative. Changes to the laser path alignment will have a dramatic impact on the performance of your instrument.

NOTE: Movement of the laser spot in the xy -plane may be caused if the surface of the sample is not flat or not mounted correctly. Ensure that the stage is level and that the sample is flat. PerkinElmer recommends the use of a flat silicon surface.

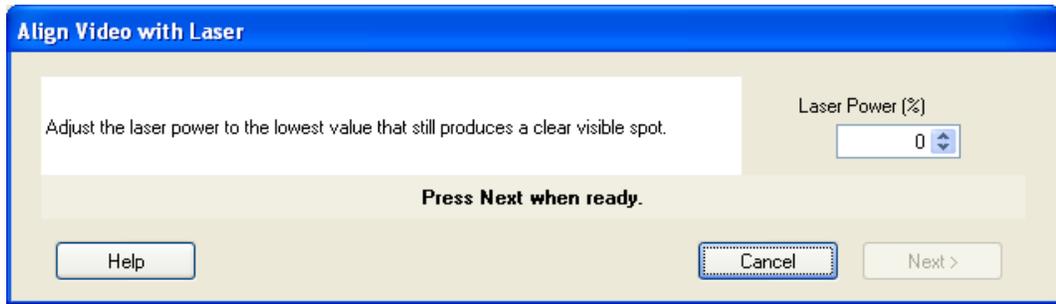
Aligning the Video Camera with the Laser Spot

The crosshairs displayed on the Setup Microscope or Setup Microscope XYZ tab in the Spectrum software represent the position of the laser. If the microscope is knocked, then this crosshair may not correspond to the true position of the laser. Minor corrections of the crosshair position can be made in the software using **Align Video with Laser**. If the laser spot is very far from the crosshair, then a complete realignment of the video camera is required:

1. Select the 20x objective lens on the microscope.
2. Place a flat sample on a microscope slide.
We recommend that you purchase the sCAL Raman Calibration Standard (L1321843) for this alignment procedure.
We recommend that you do not use a colored polymer sample.
3. In the Spectrum software, select **Raman Instrument** from the Setup menu.
The Setup Instrument tabs are displayed.
4. On the Setup Instrument Basic tab, ensure that **Microscope** or **Microscope XYZ Stage**, as applicable, is selected as the Accessory.
5. Ensure that the correct objective magnification is selected in the **Microscope Objective** drop-down list.
6. Select the appropriate **Sample Holder** in the drop-down list and load the sample holder onto the sample stage, following the instructions on-screen if you have a motorized stage.
7. Select the Setup Microscope or Setup Microscope XYZ Stage tab, as applicable, and then select **Live Video**.
8. Focus on a flat, non-reflective area of sample, and then select the Dark Field (DF) switch position on the microscope and switch off the illumination.
9. Select  **Toolbox** and then select **Align Video with Laser** from the sub-menu.
The Align Video with Laser wizard starts.



10. Ensure that you have followed the instructions on the screen, and then click **Next**. The following dialog is displayed.



11. Adjust the **Laser Power** until you can see a clear visible spot.
12. If the laser spot is visible and close to the cross hairs, you do not need to physically adjust the camera module. Continue at step 16 to perform a fine adjustment to align the cross hairs with the laser spot.

OR

If no spot is visible, a physical adjustment of the camera module is required. Continue at step 13.

13. Loosen the 4 screws on the top plate of the Raman module until the camera is just free to move (Figure 24).

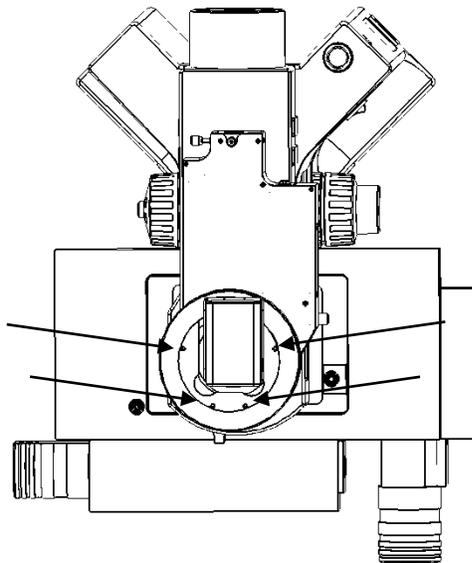
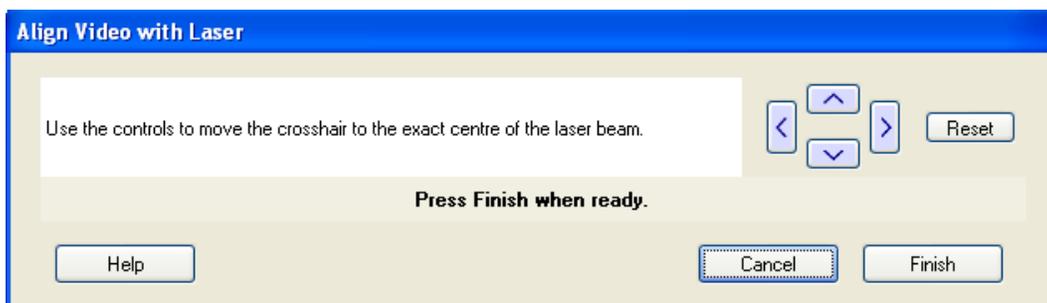


Figure 24 Top view of the microscope showing the screws used to adjust the position of the camera module

14. Move the camera module until the crosshairs line up as closely as possible with the center of the laser spot.
15. Re-tighten the 4 screws.

16. Click **Next**.

The following dialog is displayed.



17. Use the arrows in the Align Video with Laser dialog to fine-tune the position of the crosshairs to be in the center of the laser spot.

You will need to repeat the fine adjustment of the cross hairs using the **Align Video with Laser** function for each microscope objective, but do not adjust the camera module.

Aligning the Raman Coupler

CAUTION

Adjustments to the Raman coupler can have a dramatic impact on the performance of your Raman instrument. We recommend that you contact your PerkinElmer Service Representative.

You should only adjust the Raman coupler if the signal throughput has decreased. This may occur if you have moved the unit. The only adjustment to the Raman is the *xy*-adjuster at the rear of the Raman module (see Figure 25). The procedure for alignment is as follows:

1. Place a flat sample of silicon on the sample stage.
We recommend that you purchase the sCAL Raman Calibration Standard (L1321843) for this alignment procedure.
2. Loosen the 4 retaining screws at the coupler so that about 0.5 mm of thread is showing.
3. Ensure that the Bright Field (BF) switch position is selected on the microscope, and that the illumination is turned on.
4. In the Spectrum software, select **Raman Instrument** from the Setup menu.
The Setup Instrument tabs are displayed.
5. On the Setup Instrument Basic tab, ensure that **Microscope** or **Microscope XYZ Stage**, as applicable, is selected as the Accessory.
6. Select the appropriate **Sample Holder** in the drop-down list.
7. Select the lowest magnification objective on the microscope.
8. Ensure that the correct objective it is selected in the Microscope Objective drop-down list on the Setup Instrument Basic tab.
9. Select the Setup Microscope or Setup Microscope XYZ Stage tab, as applicable, and then select **Live Video**.

10. Ensure that the sample is in focus.
11. Repeat steps 7–10 for each objective until the highest magnification is selected.
12. Switch to the Dark Field (DF) switch position on the microscope, and turn off the illumination.
13. Cover the microscope with a dark cloth or, if fitted, close the light-proof enclosure doors and ensure that caps are placed on any external fiber optic probes or accessories, to minimize stray light from interfering with the calibration.

14. Click .

The RamanMicro 200 will start collecting data in the Live display.

15. Observe the data displayed in the Live display.
16. Very slowly move one of the coupler knobs clockwise and counter-clockwise until the Raman signal is maximized.

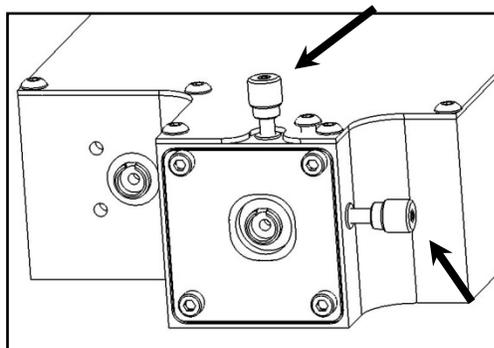


Figure 25 Adjustment of the XY Raman coupler

17. Move the other knob in a similar manner until the Raman signal has maximized.
18. Return to the first knob and move again to maximize the Raman signal (the movement should be much less than used the first time).
19. Return to second knob and do likewise.
20. Tighten each retaining screw a little and move the *xy*-coupler knobs to compensate each time.
21. Tighten screws until the plate at the rear coupler is secure – do not over tighten.

Moving the Instrument



WARNING

The RamanMicro 200 spectrometer weighs about 20 kg. The RamanMicro 200 microscope module weighs about 20 kg. Two people are needed to lift the instrument.

Please contact PerkinElmer for advice about removing the fiber optic cables that connect the spectrometer and microscope modules. If you need to ship your instrument you must use the original supplied packing materials to prevent damage to the system.

When the instrument has reached its destination and has been reassembled, you should perform the appropriate calibration/verification procedures as described in the *Calibration and Performance Verification SOPs* on page 78.

Using the
RamanMicro 200 Series

Spectrometer Front Panel

The front panel of the spectrometer module contains 3 LED indicators and 1 key switch (Figure 26).



Figure 26 Spectrometer module front panel

LED	Description
Power	If the Power LED is illuminated, this indicates that the instrument is receiving power.
Laser	This LED indicates the power status of the laser. If the LED is illuminated then the laser is receiving power. This does not mean that laser radiation is present throughout the optical path of the instrument, only that the laser power is on.
Active	This LED indicates the status of the laser shutters. If the LED is illuminated then the shutters may be activated (open) and laser radiation present throughout the optical path of the instrument.

NOTE: If you open the microscope light-proof enclosure (if fitted) when the Active LED is illuminated, the safety interlocks will close the shutters.

NOTE: The shutter on the RamanMicro 200 instrument will be open continuously during acquisition. Care should be taken when scanning and monitoring not to damage your sample.

Fiber Optic Probe

NOTE: This section is only applicable if you have a RamanMicro 200F that is fitted with a fiber optic probe.

NOTE: For details of how to use a triggered fiber optic probe, see the *Raman Triggered Fiber Optic Probe* leaflet (L1321940).

General Overview of the Fiber Optic Probe

The fiber optic probe (FOP) is designed to enable the remote measurement of samples. It is available in a range of different configurations, depending on the end-user's application. However, it should be noted that compatibility of the working environment with the probe should be verified with PerkinElmer prior to use.

The probe body contains the filtering optics and is interfaced with two fiber optic cables (an excitation fiber and collection fiber). Care should be taken when handling the probe body so that the optical window is not soiled or damaged.

NOTE: If you use the probe body in any chemical or aggressive environment you must use an immersion sleeve to protect the probe body. **DO NOT ATTEMPT TO USE THE PROBE BODY IN SUCH AN ENVIRONMENT WITHOUT THE USE OF AN IMMERSION SLEEVE** (L1320003). If required, contact PerkinElmer for further details.

Attaching and Removing the Fiber Optic Probe

The standard fiber optic probe is constructed such that a single armored cable bifurcates into an **excitation** cable and a **collection** cable. The excitation fiber transmits the laser energy, whereas the collection cable is a conduit for the Raman energy. Both fibers terminate in FC style connectors. If you have a standard probe, there is also a DIN connector attached to the end of two wires which connects the fiber optic LED to the instrument. If you have a triggered probe, there is an Ethernet cable that connects to a USB extender which connects the probe to the PC.

Attaching the FOP

1. Ensure the attenuator cap is attached to the FOP.
2. Turn off the instrument and disconnect the main power cable and, if appropriate, the stage controller power cable.
3. Carefully move the spectrometer module to permit easier access to the rear panel.
4. Remove the two retained screw-in caps from the two coupler ports marked **EXC** and **COL** below the **FIBER 2** connector on the rear panel (Figure 27).



Figure 27 Attaching the FOP

5. Connect the excitation cable of the fiber optic probe to the coupler marked **EXC**.
Make sure that the polarizing ridge on the fiber optic probe cable is aligned with the slot in the coupler casing before carefully pushing the cable into the coupler (Figure 28).

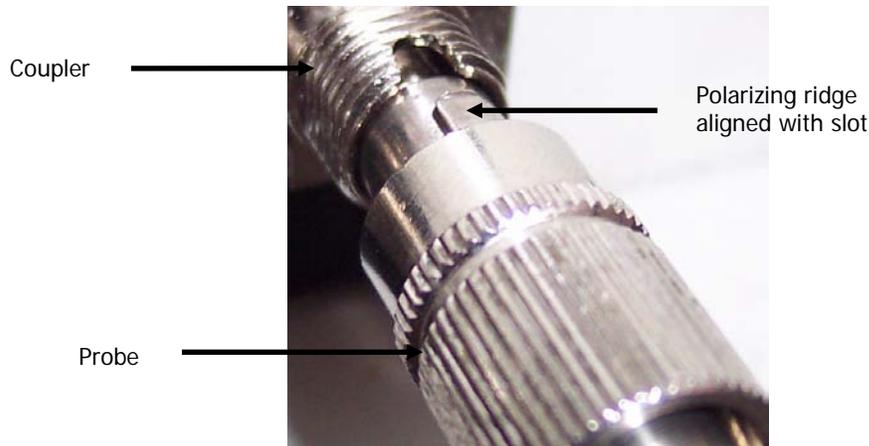


Figure 28 Connecting the fiber optic probe

6. Connect the collection cable of the fiber optic probe to the coupler marked **COL**.
Make sure that the polarizing ridge on the fiber optic probe cable is aligned with the slot in the coupler casing before carefully pushing the cable into the coupler.
7. Screw the screw caps from the fiber optic cables onto the coupler casings.
8. If you have a standard probe, connect the DIN connector to the nearby socket marked **LED FIBER 2**.

OR

If you have a triggered probe, connect the USB extender cable to a USB port on your PC.

9. Reconnect the power cables.

Removing the FOP

If it is necessary to remove the FOP, for example, for replacement or transportation, please follow this procedure carefully:

1. Ensure the attenuator cap is attached to the FOP.
2. Switch off the instrument and remove the main power cable.
3. Carefully move the spectrometer module to permit easier access to the rear panel.

4. If you have a standard probe, remove the DIN connector in the socket marked **LED FIBER 2**.

OR

If you have a triggered probe, disconnect the USB extender cable from the USB port on your PC.

5. Carefully remove the retained ends of the FOP by unscrewing and pulling the cables away from the couplers perpendicularly.
Be extremely careful when removing the FOP as you can easily damage the instrument and the fiber optic cables.
6. Replace the protective plastic caps on the fiber ends of the FOP that have been removed from the rear panel.
7. Fasten the retained screw-in caps onto the couplers on the rear panel.
8. Reconnect the power cables.

Acquiring Spectra with the Fiber Optic Probe

The Raman fiber optic probe has a tight focus spot that is approximately 7.5 mm away from the lens. The focused spot enables you to pinpoint the measurement area on the sample.

Sampling Solids

Solids can be analyzed by mounting the probe on a stand and adjusting the focal spot on the surface of the sample. Whenever possible, powders should be compacted down and thin films of materials should be folded so that the sample covers the entire depth of field. Solids can be analyzed in glass and plastic containers, similar to liquid samples.

<p>NOTE: Raman spectra are measured in the visible and near-infrared region. Room lights and sunlight will contaminate your spectra. Take care not to misinterpret room lights or sunlight features as Raman features.</p>

Sampling Liquids

Liquid samples can be analyzed by immersing the FOP in the liquid of interest or by analyzing through the container.

Immersion

The FOP can only be immersed in liquid if you have purchased an immersible fiber probe sleeve. Do not totally submerge the probe. It is only acceptable to immerse the probe up to 1 inch from the top of the sleeve. It may be useful to focus the sample by sliding the FOP inside the immersion sleeve to obtain the best spectrum. Note that immersion sleeves contain either quartz or sapphire windows. These windows can yield a detailed Raman spectrum – do not confuse this with the spectrum of the sample.

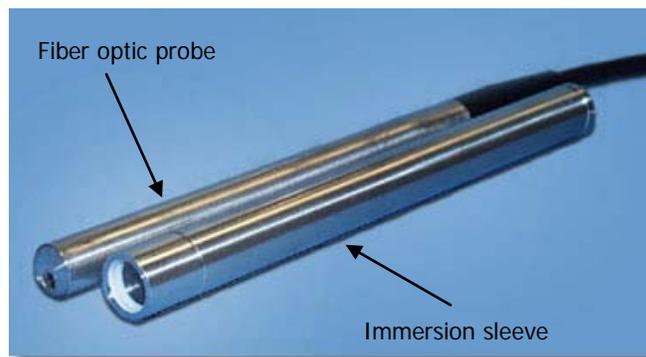


Figure 29 Fiber optic probe with the immersion sleeve detached

Non Immersion

When using 785 nm lasers, many types of glass exhibit fluorescent bands between 1400 – 1600 cm^{-1} . Glass spectra of the container should be obtained before analyzing the sample to ensure that glass-derived fluorescence bands do not pose a problem. With 785 nm excitation, fused silica (quartz) should be used instead of glass where possible as this does not yield fluorescent peaks. Pyrex is also excellent for Raman analysis. When working through containers it is best to try different probe positions. If the liquid of interest is not absorbing it may be best to focus through the container by placing the FOP in contact with the container.

If this glass fluorescence is a problem and it is not possible to use quartz containers, then it is possible to acquire a spectrum of the container, and spectrally subtract this from any spectra containing this fluorescent signature.

Sampling Slurries/Highly absorbing species

Please refer to *Sampling Liquids*. For sample immersion care taken in focusing the probe within the sleeve can result in a dramatic improvement in spectral quality.

Sampling Gases

Although Raman scattering from gas samples is very weak, the FOP can be used to measure gas phase under long acquisition times.



WARNING

The standard FOP is not designed for immersion use. The FOP is sealed against minor splashes. However, immersion in solvents will cause serious damage. Ensure an immersion sleeve is in place before immersing your FOP; see Figure 29. Please contact PerkinElmer if you have any questions in relation to sampling using the FOP.

General SOPs

The RamanMicro 200 combined with the Spectrum software is a very flexible instrument. This chapter demonstrates how to perform a number of tasks. The procedures provided may not be the most straightforward or simple for the task you wish to perform, so treat these Standard Operating Procedures (SOPs) as a starting point and with time you may wish to develop your own SOPs which better suit your specific mode of use. These procedures also act as a “learn by doing” section allowing you to explore the functionality of the software and spectrometer.

The General SOPs demonstrate how to collect data when your samples are on the microscope stage. For SOPs that demonstrate how to collect data using a fiber optic probe, please refer to *Fiber Optic Probe SOPs* on page 76. For step-by-step instructions for calibrating the spectrometer, see *Calibration and Performance Verification SOPs* on page 78.

NOTE: The RamanMicro 200 system can be purchased with either a manual stage or with a motorized stage.

SOP-1: Turning the Instrument On

1. Switch on the PC and monitor.
2. Login to Windows.
3. Ensure the laser power key on the front panel of the spectrometer is in the ARM position.
4. Ensure the power supply is connected to a mains outlet and that the outlet is switched on.
5. To turn on the spectrometer, press the PSU power switch ON (Figure 30):

| - ON

○ - OFF



Figure 30 The PSU power switch

6. Check that the Power LED on the front panel is On.
The Laser LED and the Active LED should be off. If the Power LED is not on, please turn the instrument off at the PSU and check all of the connections, and any power socket switches and then switch the PSU on again.
7. Switch on the microscope and the microscope illumination.
8. If you have a motorized microscope stage, switch on the stage controller box.
9. From the Start menu select **All Programs**; the **PerkinElmer Applications** group; the **Spectrum** sub-group and then the **Spectrum** application.
The Spectrum start-up screen is displayed; followed by a dialog that may require your login details:



10. If required, enter your **User name** and **Password**, and then click **OK**.
An instrument selection dialog is displayed.
11. Select the **Instrument** you want to use.

OR

If you want to work with data that has been collected previously, without connecting to an instrument, select **Work Offline**.
12. Click **OK**.
Spectrum starts.
If you selected to connect to the instrument, it will prepare for use by turning on the laser and cooling the detector to its operating temperature (usually $-50\text{ }^{\circ}\text{C}$). Messages will be displayed in the Status bar at the bottom of the workspace, and in the Scan icons on the Measurement bar.

NOTE: The CCD detector will become more stable the longer it is cooled.

If you have a motorized XYZ stage and have just switched on the stage controller box you will be required to reset the stage before you can use the stage.

To adjust the height of the stage and set the limits of the stage travel, refer to SOP-3.

SOP-2: Turning the Instrument Off

1. Shut down Spectrum software by selecting **Exit** from the File menu to return to the Windows operating environment.
2. Switch off the PC and monitor.
3. Switch off the spectrometer by pressing the power switches on the PSUs to OFF:

| - ON O - OFF

4. Switch off the microscope illumination, and then the microscope.
5. If appropriate, switch off the stage controller box.
However, we recommend that you leave the stage controller box switched on, even if you switch off the instrument and microscope. If the stage control box is switched off, you will need to reset the stage upon restarting the software.
6. If appropriate, ensure that all sample holders have been removed from the stage in preparation for the next time the instrument is used.

After Spectrum closes, if you do not switch off the instrument it will remain active for 5 minutes. This is so that you do not have to wait for the CCD detector to cool and the laser to stabilize should you need to exit Spectrum and then restart the software immediately, or when changing methods in the AssureID software application.

NOTE: For information on how to use AssureID with a triggered fiber optic probe, refer to the AssureID on-screen help.

The instrument will shut down automatically after the appropriate time period. You can shut down the instrument by right clicking on the Raman instrument icon in the Notification area of the Windows Taskbar and accessing the shortcut menu.



You can shut down your PC when the Raman instrument icon is displayed. The system will shut down.

NOTE: If Spectrum software stops responding, refer to *Troubleshooting* starting on page 94 for details of how to shut down the instrument and restart the software.

SOP-3: Setting the Limits of the Motorized Stage

NOTE: If you move the stage, you may also have to check the alignment of the microscope objectives. Refer to *Aligning the Microscope Objectives* on page 50.

You may need to adjust the height of the stage, for example, when you want to measure thicker samples.

CAUTION

After moving the stage, you will need to reset the limits in the software.

1. Ensure the instrument is switched on by following SOP-1.
If the stage controller box was switched off, you will be required to reset the stage before using the motorized stage.
2. Select **Raman Instrument** from the Setup menu.
The Setup Instrument tabs are displayed.
3. Select the Setup Instrument Basic tab.
4. Ensure that **Microscope XYZ Stage** is selected in the Accessory drop-down list.
The options available will depend upon your instrument configuration.
5. Ensure that the correct **Microscope Objective** is selected in the software.
6. Ensure that the pre-focusing lever on the coarse adjustment control is on the off position.
The pre-focusing lever restricts the travel of the coarse adjustment control, and is located on the left-hand side of the microscope. The lever is in the off position when it is rotated counter-clockwise away from you when facing the microscope. Refer to the Olympus manual provided with your microscope for further information.
7. Loosen the fixing screw on the side of the microscope (Figure 31).

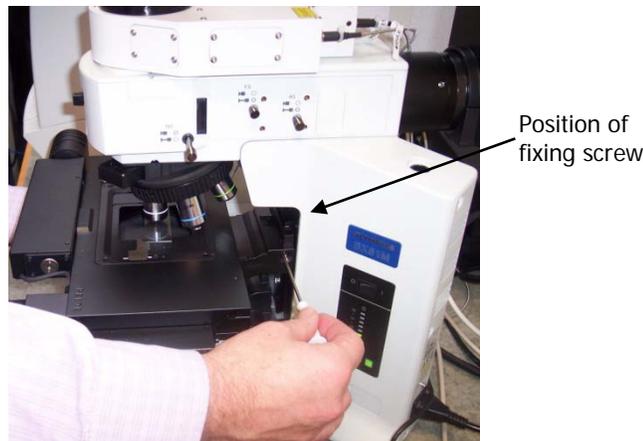


Figure 31 Adjusting the height of the motorized stage

8. Manually move the stage down until the stage is at its lowest position.

9. Re-secure the fixing screw to fix the height of the stage.
10. Select the Setup Microscope XYZ tab.

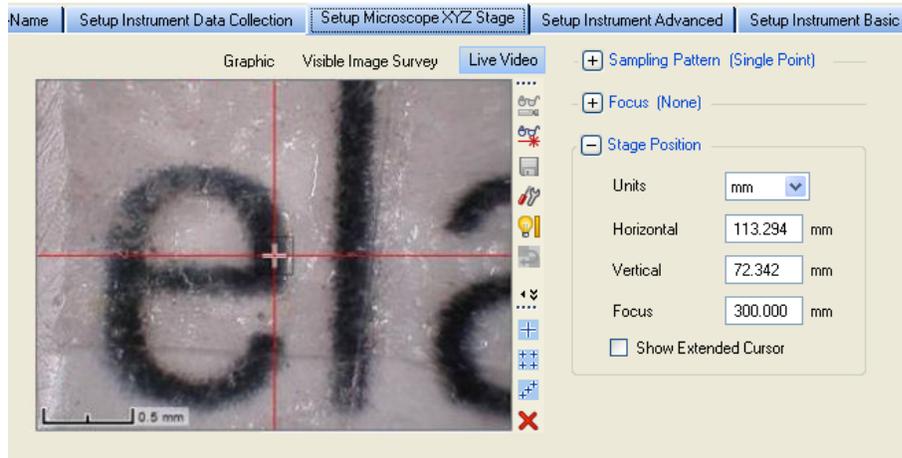


Figure 32 Setup Microscope XYZ Stage tab showing the Live Video view

11. Select  **Toolbox** and then select **Reset Stage** from the sub-menu.
12. Follow the prompts on-screen.
During the Reset Stage procedure, you will be prompted to use the joystick to move the stage to the point of lowest travel. If you place your hand carefully on the coarse adjustment control, you will feel it move (as it starts to counter-rotate with the fine focus control) when you have reached the lower limit. Stop using the joystick at this point.
13. When the stage has been reset, use the joystick or the stage position controls in the software to move the microscope stage up to 0 mm (approximately 25 mm travel).
The stage will stop at some point between 0 mm and 1 mm, at the maximum length of travel for the z-axis motor.
14. Ensure that you are now at the maximum point of travel for the stage by turning the coarse adjustment control.
It may move slightly, but should be nearly at the maximum.
15. Place your sample on the stage.
Using a hexagonal key, loosen the fixing screw on the side of the microscope (Figure 31).
16. Carefully, move the stage up manually until it is at the highest position possible without the sample touching the objective.

CAUTION

Be very careful not to touch the objective.

The performance of the Raman microscope will be affected by dirt on the surface of, or damage to, the objectives.

NOTE: When using long working-distance objectives (for example, Long Working Distance Objective Lens Kit, L1323347), the working distance depends on the objective (usually, 2–10 mm).

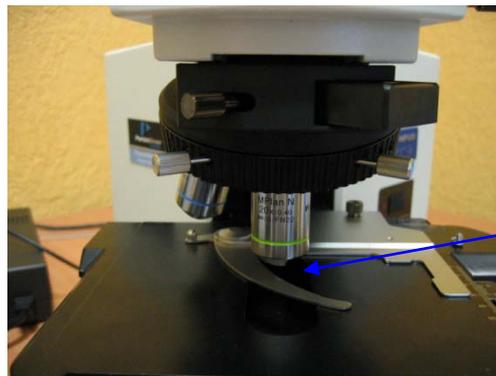
17. Re-secure the hexagonal fixing screw to fix the height of the stage.
18. Select  **Toolbox** and then select **Reset Stage** from the sub-menu.
19. Follow the prompts on-screen, as before.

You will need to repeat this process every time you adjust or install the stage.

CAUTION	<p><i>Once you have reset the stage you should use the joystick ONLY to raise or lower the stage. DO NOT try to adjust the stage manually, or you will have to reset the stage.</i></p> <p><i>When using the joystick, take care not to continue using the motors when the stage has reached the lower or upper limits of travel. To see if you have moved outside the limits, check the z-axis focus dials on the right-hand side of the microscope. If the two wheels counter-rotate, you will need to reset the stage. If you continue to move the stage, the cable can become trapped and the motor could be damaged.</i></p>
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SOP-4: Sample Positioning using a Manual Stage

1. Lower the microscope sample stage.
2. Place the sample on a microscope slide and place it on the stage.



Place your
sample here

Figure 33 Positioning the sample under the objective lens

3. Select the 5x objective lens on the microscope.

<p>NOTE: Do not touch the glass of the objective lenses when you select the appropriate magnification. Do not push on an objective to rotate the nosepiece, but use the revolving plastic ring.</p>
--

4. Move the stage so that the sample is approximately lined up below the objective lens.
5. Select the Bright Field (BF) switch position on the microscope.
6. Switch on the illumination source.
The switch is located on the right side of the microscope frame.
7. Set the required light intensity using the adjustment knob on the right side of the microscope frame base.

8. Select **Raman Instrument** from the Setup menu.
The Setup Instrument tabs are displayed.
9. Select the Setup Instrument Basic tab.

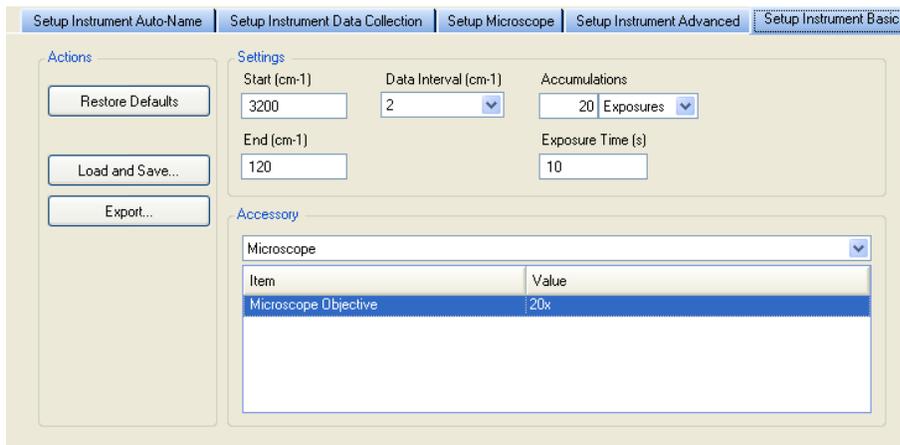


Figure 34 Setup Instrument Basic tab with the Microscope accessory selected

10. Ensure that **Microscope** is selected in the Accessory drop-down list.
The options available will depend upon your instrument configuration.
11. Select **5x** from the **Microscope Objective** drop-down list.
The microscope is supplied with 5x, 20x and 50x objective lenses.
12. Select the appropriate **Sample Holder** from the drop-down list.
13. Select the Setup Microscope tab.

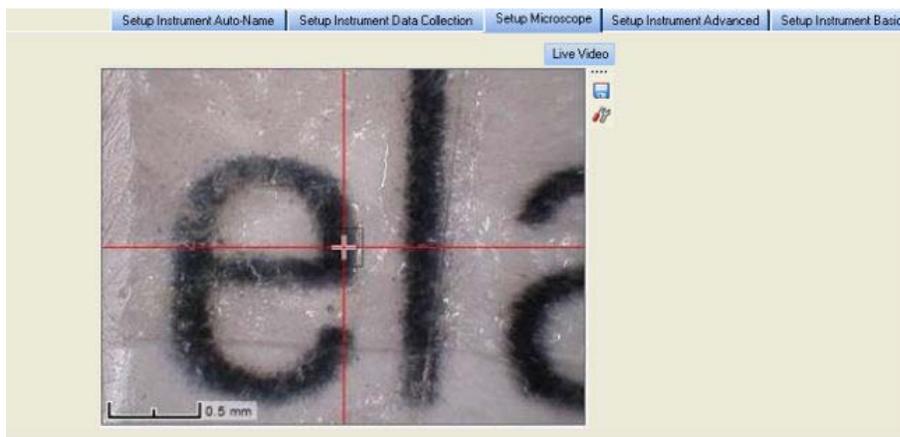


Figure 35 Setup Microscope tab

14. Focus on the sample using the microscope z-axis control knobs.
Take care not to crush the sample or dirty the objectives. The working distances of the objective lenses supplied as standard are 20 mm (5x), 1.3 mm (20x) and 0.38 mm (50x).
15. If higher magnification is required, select the next most powerful objective lens on the microscope.
16. Select the appropriate objective lens from the **Microscope Objective** drop-down list.

17. Focus on the sample and again move the sample in the x and y directions, if necessary. The crosshairs indicate the spot where analysis takes place.

NOTE: If there is an offset between the laser position and the cross hairs, use the **Align Video with Laser** tool to adjust this. See the Spectrum on-screen help and *Aligning the Video Camera with the Laser Spot* on page 52 for more information.

18. When the sample is correctly positioned, switch off the illumination source.
19. Select the Dark Field (DF) switch position on the microscope.
20. If the microscope light-proof enclosure is fitted, close the doors.

21. Click .

The RamanMicro 200 will start collecting data in the Live display.

You can use Monitor to help you optimize the Exposure time and Laser power for your sample, and adjust the focus of your sample to maximize the Raman signal. The data displayed on-screen when monitoring cannot be saved. To save data you must collect spectra.

Click  to exit the Live display.

You are now ready to acquire data. For more information see SOP-6.

The most appropriate microscope objective for your analyses will depend upon your samples. High-magnification objective lenses give the most efficient Raman collection from thin samples, such as films or wafers, but low-magnification objectives are better for macro samples such as tablets, bulk plastics and tissue samples.

NOTE: Raman spectra are measured in the visible and near-infrared spectrum. Room lighting and sunlight will contaminate your spectra. Take care not to misinterpret room lighting or sunlight features as sample features.

SOP-5: Sample Positioning using a Motorized Sample Stage



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.

1. Select the 5x objective lens on the microscope.
2. Select the Bright Field (BF) switch position on the microscope.
3. Switch on the illumination source.
4. Select **Raman Instrument** from the Setup menu.
The Setup Instrument tabs are displayed.

5. Select the Setup Instrument Basic tab.

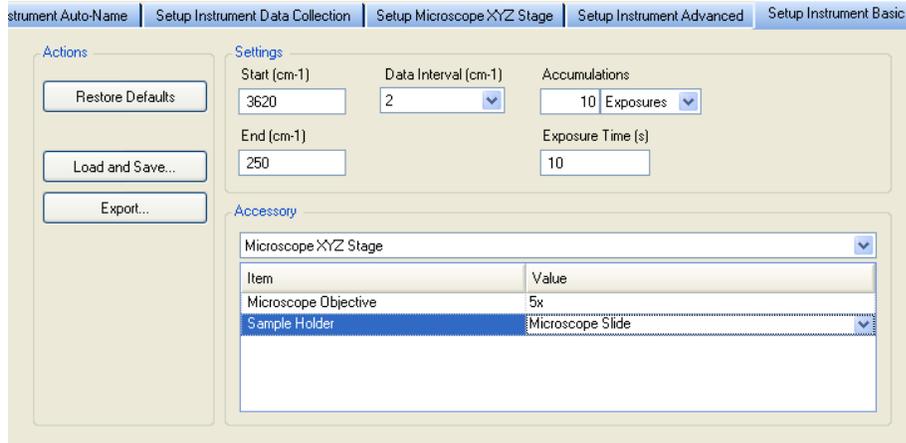


Figure 36 Setup Instrument Basic tab with the Microscope XYZ Stage accessory selected

6. Ensure that **Microscope XYZ Stage** is selected in the Accessory drop-down list. The options available will depend upon your instrument configuration.
7. Select **5x** from the **Microscope Objective** drop-down list. The microscope is supplied with 5x, 20x and 50x objective lenses.
8. Load your sample into a suitable sample holder.
9. Select the appropriate Sample Holder in the drop-down list, and follow the instructions on-screen to load the sample holder onto the sample stage.

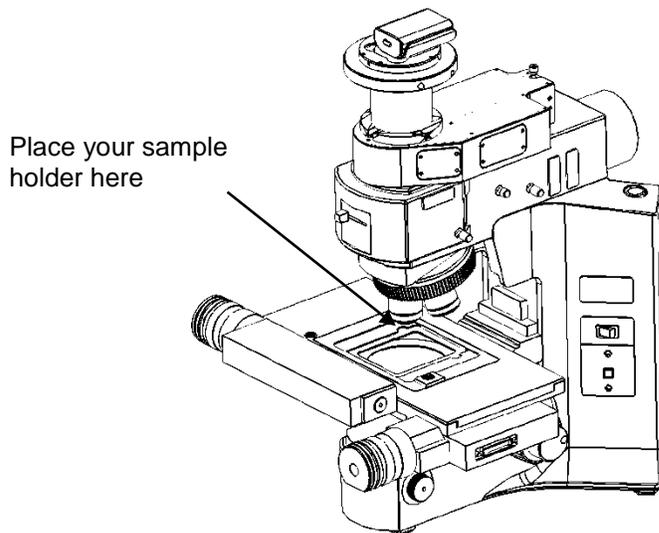


Figure 37 Sample position on motorized stage

10. Select the Setup Microscope XYZ tab, and then select the Live Video view.

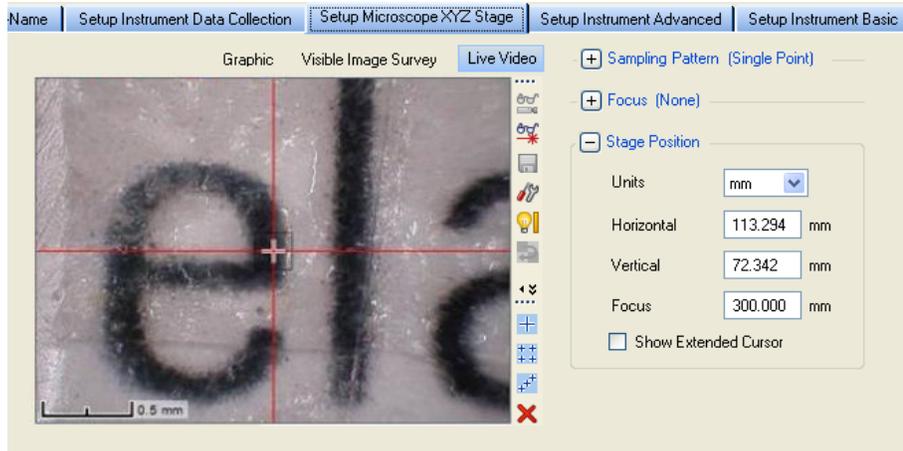


Figure 38 Setup Microscope XYZ Stage tab showing the Live Video view

11. Use the joystick, or click **Stage Position** and then use the controls, to move the stage so that the sample is approximately lined up below the objective lens and then focus on the sample.

The crosshairs indicate the spot where analysis takes place.

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 (if purchased):
Left twist (counter clockwise) gives upward stage movement;
Right twist (clockwise) gives downward stage movement.

NOTE: You can also click  **Video Autofocus** to perform an automatic video focus when you are close to the sample surface (when the microscope illumination is on and the switch position set to Bright Field). Alternatively, select the Dark Field switch position on the microscope, and click  **Raman AutoFocus** to focus using the strongest Raman signal.

12. If higher magnification is required, select the next most powerful objective lens on the microscope.

The microscope is supplied with 5x, 20x and 50x objective lenses.

NOTE: Do not touch the glass of the objective lenses when you select the appropriate magnification. Do not push on an objective to rotate the nosepiece, but use the revolving plastic ring.

13. Select the appropriate objective lens from the **Microscope Objective** drop-down list.
14. Focus on the sample and, if necessary, move the stage in *x* and *y* directions to accurately position the sample.
15. When the sample is correctly positioned, switch off the illumination source.
16. Select the Dark Field (DF) switch position on the microscope.

17. If the microscope light-proof enclosure is fitted, close the doors.

18. Click  .

The RamanMicro 200 will start collecting data in the Live display.

You can use Monitor to help you optimize the Exposure time and Laser power for your sample. The data displayed on-screen when monitoring cannot be saved. To save data you must collect spectra.

19. Adjust the **Laser power (%)** in the Live display to an appropriate value for your sample.

20. Enter an **Exposure time (s)**.

Changing the exposure time will automatically acquire another background.

21. Click  to exit the Live display.

You are ready to acquire data. For more information see SOP-6.

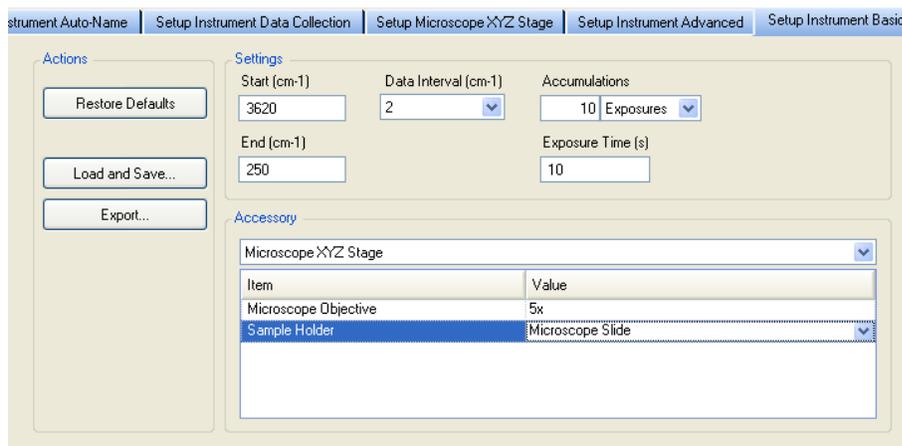
The most appropriate objective for your analyses will depend upon your sample. High-magnification objective lenses give the most efficient Raman collection from thin samples, such as films or wafers, but low-magnification objectives are better for macro samples such as tablets, bulk plastics and tissue samples.

NOTE: Raman spectra are measured in the visible and near-infrared spectrum. Room lighting and sunlight may contaminate your spectra. Take care not to misinterpret room lighting or sunlight features as sample features.

SOP-6: Setting-Up and Saving Experimental Parameters

This SOP is designed to act as a quick guide to help you start collecting data. The exact setup for optimized spectral collection is sample dependent, and we recommend that you modify these settings to suit your needs.

1. Start the Spectrum software and select **Raman Instrument** from the Setup menu.
The Setup Instrument tabs are displayed in the Dialog pane. They enable you to choose the accessory, and setup and save the experimental parameters.
2. Select the Setup Instrument Basic tab.



Instrument Auto-Name | Setup Instrument Data Collection | Setup Microscope XYZ Stage | Setup Instrument Advanced | Setup Instrument Basic

Actions

Restore Defaults

Load and Save...

Export...

Settings

Start (cm-1) 3620

End (cm-1) 250

Data Interval (cm-1) 2

Accumulations 10 Exposures

Exposure Time (s) 10

Accessory

Item	Value
Microscope XYZ Stage	
Microscope Objective	5x
Sample Holder	Microscope Slide

Figure 39 Setup Instrument Basic tab

3. Select **Microscope** or **Microscope XYZ Stage** as appropriate from the **Accessory** drop-down list.
Depending on the Accessory you have selected, further options may become available.
4. Ensure that the appropriate **Microscope Objective** and **Sample Holder** are selected.
5. Using the Instrument Settings toolbar, check and set the instrument parameters, such as the **Start** and **End** points of the scan range (in Raman Shift, for example, 3200 cm^{-1} to 100 cm^{-1}), and enter a unique **Sample ID** and **Description**.
6. If required, adjust the **Data Interval** in Raman Shift (cm^{-1}).
The default data interval is 2 cm^{-1} . The Data Interval can be set on the Setup Instrument Basic tab.
7. Set the **Exposure Time** and number of **Accumulations**.
You can define the Accumulations as a number of Exposures, or as a number of minutes or seconds.
Typical values for Exposure Time and Accumulations are 5 seconds and 5 exposures, respectively. Spectra may be optimized by increasing or decreasing the Exposure Time and number of exposures. To obtain a good signal-to-noise ratio, you should co-add a number of spectra. This can be done by choosing a suitable number of exposures. A minimum of two exposures is recommended for cosmic ray removal.
8. If you wish to edit the data collection parameters, such as how background scans are collected, select the Setup Instrument Data Collection tab.
To display the Setup Instrument tabs, select **Raman Instrument** from the Setup menu. The tabs are displayed in the dialog pane.
9. In the Background Exposures section, we recommend that you select **As samples**.
This sets the number of background scans to the same value as the number of sample scans. You may also choose one of the Background Collection options to define when a background will be taken.
10. If required, select any Auto Processing options.
These options, if selected, will be applied to all spectra collected, and cannot be undone.
11. If you select **Save after each measurement**, your spectra will automatically be saved using the Sample ID.
12. If you have a motorized stage, select the Setup Microscope XYZ Stage tab, and then select the **Sampling Pattern**.
Select the sampling pattern type from **Single Point**, **Super Macro Point**, **Custom Grid** and **Custom Line**. Any spectra collected using SuperMacro, Custom Grid or Custom Line sampling patterns are coadded to produce one spectrum.
For more information, see the Spectrum on-screen Help.
13. If you have a motorized stage, select any **Focus** options.
You can select to perform a **Raman AutoFocus** or **Video AutoFocus** before each sample measurement.
14. When you have finished defining your experimental parameters, position and optimize conditions for your sample as described in SOP-4 and SOP-5.
15. Click  .

Spectra are always background corrected. If there is not a valid background in memory, a background will be acquired at this point. You will see a progress bar for the background collection on the Live tab. You can enter the number of backgrounds on the Setup Instrument Data Collection. When the collection of backgrounds is complete, the software will automatically begin collecting spectra. The upper progress bar shows the progress of the current scan (%), while the lower progress bar shows the overall progress of the measurement (for example, **1 of 2** scans) if, for example, you have set up Repeat collections.

If you exit Spectrum software the instrument settings will be remembered when you restart Spectrum, providing that they have been used to collect data. If you want to return to the default instrument settings, click **Restore Defaults** on the Setup Instrument Basic tab. If you wish these parameters to be stored as a new set of instrument settings, select **Load and Save** on the Setup Instrument Basic tab.

NOTE: If you have a motorized stage, for information on how use the Setup Microscope XYZ Stage tab to set up Markers, Cell Markers, Maps and Line Scans, and to collect a Visible Image Survey, refer to the Spectrum on-screen help.

NOTE: If you are using a multi-well plate on the motorized stage on the microscope, then you must insert the multi-well plate holder (L1321675) onto the stage before adding the plate.

Fiber Optic Probe SOPs

NOTE: The SOPs included on the following pages are only appropriate for a standard fiber optic probe. For details of how to acquire data using a triggered fiber optic probe, see the *Raman Triggered Fiber Optic Probe* leaflet (L1321940).

SOP-7: Selecting the Fiber Optic Probe

1. Ensure that the RamanMicro 200 is switched on and that the fiber optic probe is connected to the instrument.
If not follow SOP-1.
2. Select **Raman Instrument** from the Setup menu in Spectrum.
The Setup Instrument tabs are displayed in the Dialog pane.
3. Select the Setup Instrument Basic tab.

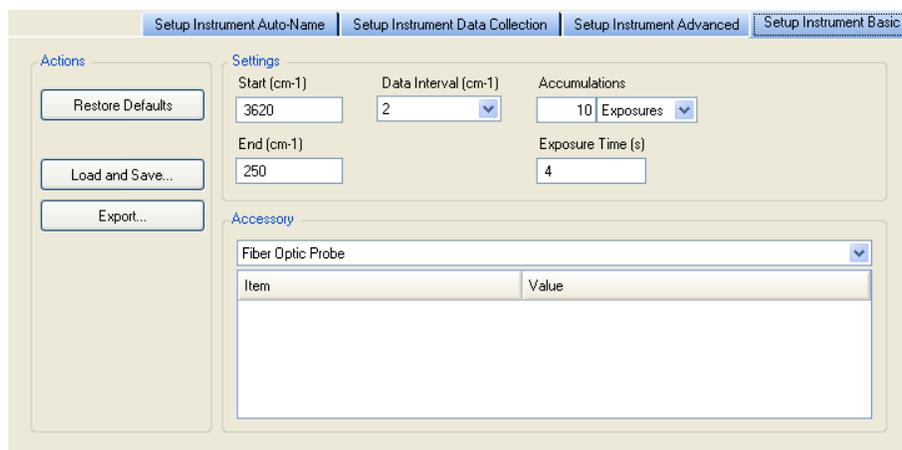
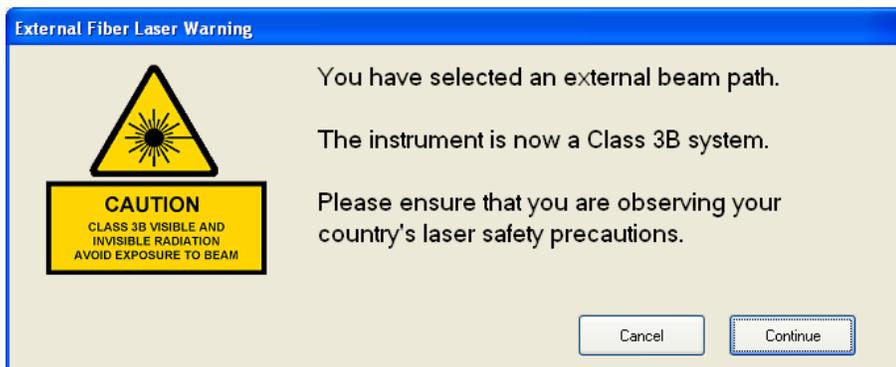


Figure 40 Setup Instrument Basic tab

4. Select the **Fiber Optic Probe** you want to use from the Accessory drop-down list.
The following warning message is displayed.



5. Ensure that laser safety precautions are being observed, and then click **Continue**.
If you have any doubts about the required safety precautions click **Cancel**.
When the laser is on and the shutter is open the Laser Status icon on the Measurement bar will be updated to Class 3B Laser Status . Laser light will be emitted from the probe during data collection and when the software is Monitoring.

The probe can now be positioned so as to achieve the best spectrum. See SOP-8.

SOP-8: Sample Positioning when using a Fiber Optic Probe

Monitor or Preview modes allow you to collect spectral data while optimizing the probe position and the laser power and exposure time, for example, to enhance the signal-to-noise ratio of spectral data. When measuring samples inside a container (for example, a bottle) it can be used to ensure that you are focused on the material of interest and not the container.

The data that is displayed on the Live tab when monitoring cannot be saved.

NOTE: To see the Live display, the **Show live display** option must be selected on the Setup Instrument Data Collection tab.

1. Click .
The Live tab will be displayed and real-time spectral acquisition will commence.
2. Adjust the **Laser power (%)** on the Live tab to an appropriate value for your sample.
3. Adjust the **Exposure time (s)**.
This will automatically acquire another background.
The sample may now be positioned and aligned correctly for optimized data collection. The spectral data can be optimized by adjusting the distance between the fiber optic probe and the sample.

NOTE: When positioning your sample, you should ensure that you are not collecting data from any substrate used to support your sample. It is advisable to have 1–2 cm of free space behind the sample. Alternatively, you can subtract the spectrum of your substrate from your sample spectrum.

4. Click  to exit the Live display.

You can now collect and save spectral data. To set up an experiment and collect data using the FOP please refer to SOP-6.

NOTE: If you have **Show Live Display** selected on the Setup Instrument Data Collection tab and you select **Preview** on the Sample Table, then you can preview each sample in the Live display after pressing **Scan**. When you have adjusted the settings, click **Scan** again to collect data.

Calibration and Performance Verification SOPs

Several different automated calibration tests are included in the software:

- Wavelength (x-axis) calibration uses the position of precisely known lines in a neon emission spectrum to calibrate the detector.
- Intensity (y-axis) calibration is performed using coated glass with a well characterized emission spectrum. This is used to correct for non-linearity in detector response.
- Laser frequency determines the exact laser wavelength.

An additional wavelength calibration verification routine has been included in the software, which compares a series of peaks for polystyrene (ASTM E 1840) with values collected using a polystyrene sample. These calibration/verification tests allow for in-house instrument performance verification.

Intensity calibration is only performed if the instrument has been moved, or as part of an annual service, or following a service repair of the instrument or accessory, or if there have been appreciable temperature variations in the local environment.

Backgrounds are always collected during a calibration procedure. The background measures the bias and dark signal over the selected area of the CCD. This is a necessary part of the calibration routines, so it is always performed.

The spectrometer has no moving parts in its detection system; therefore frequent calibration is not necessary.

We recommend that you calibrate the instrument in accordance with the following SOPs.

SOP-9: Calibration/Performance Verification

We recommend that wavelength calibration verification is performed on a regular basis to ensure that the instrument is performing within specification. This can be once a day or once a week depending on your requirements and the laboratory environment.

When carrying out the calibration/verification procedures, the instrument laser power should be set to 100% and the Data Interval to 4 cm^{-1} or smaller.

On completion of the wavelength calibration verification, the results of the verification (or calibration) procedure is displayed. A spectrum detailing the outcome of the verification procedure is saved to the folder `...\\pel_data\\config\\Raman\\Calibration Files\\Logs` with the filename Calibration Report [Name of calibration][Date].sp

IMPORTANT: If the instrument passes the wavelength calibration verification procedure, the instrument is optimized for performance and does not require wavelength calibration.

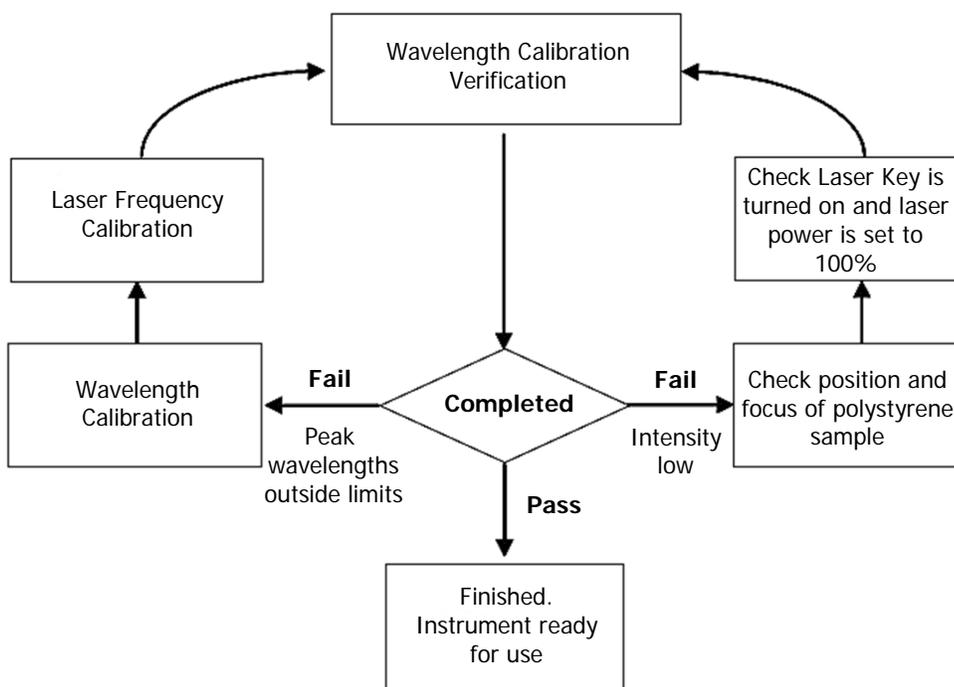


Figure 41 System verification / calibration flow diagram

If wavelength calibration verification fails and the Description of the calibration report spectrum states that peak wavelengths are outside limits, then wavelength and laser frequency calibrations should be performed. When these calibrations have been completed successfully, repeat the wavelength verification to ensure that the instrument has been brought back within specification.

If the wavelength calibration verification fails because the spectral intensity is low, then this may also be due to the calibration sample holder not being placed in the sample accessory, or that it is not correctly positioned, optimally focused or because the laser key is not switched on, or the laser power is too low. Then repeat the wavelength calibration verification to ensure that instrument has been brought back within specification. If the instrument fails the wavelength calibration verification again, please contact PerkinElmer.

NOTE: Stray light could cause the wavelength calibration verification to fail. If a test fails, particularly if you are using the microscope without an enclosure, ensure that no light is entering the Raman system.

SOP-10: Wavelength Calibration Verification

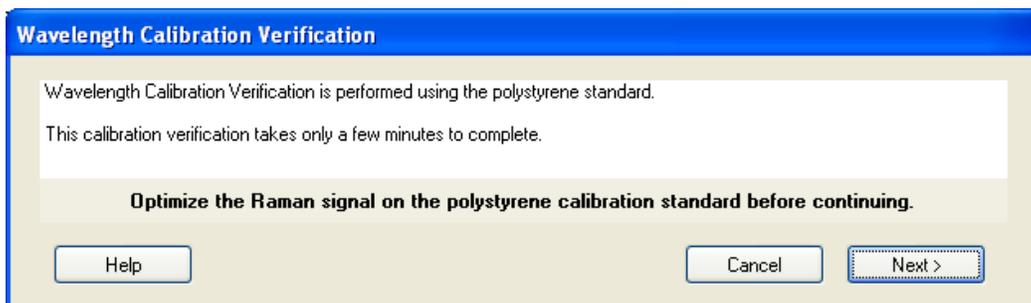
To perform the Wavelength Calibration Verification with the Microscope 50x objective selected:

1. Ensure that the RamanMicro 200 is switched on.
If not follow SOP-1.
2. Select the 50x objective lens on the microscope.
3. Place a polystyrene sample (L1181228) in a suitable sample holder.

4. Select **Raman Instrument** from the Setup menu.
The Setup Instrument tabs are displayed.
5. On the Setup Instrument Basic tab select **Microscope** or **Microscope XYZ Stage** as appropriate from the **Accessory** drop-down list
6. Select **Microscope 50x** from the Objective drop-down list.
7. Select the appropriate **Sample Holder** in the drop-down list and load the sample holder onto the sample stage, following the instructions on-screen if you have a motorized stage.
8. Select the Setup Instrument Advanced tab and ensure that the **Laser Power** is set to 100%.
9. Select the Dark Field (DF) switch position on the microscope and switch off the illumination.
10. Cover the microscope with a dark cloth or, if fitted, close the light-proof enclosure doors and ensure that caps are placed on any external fiber optic probes or accessories, to minimize stray light from interfering with the calibration verification.
11. Focus on the polystyrene sample.
If you have a motorized stage you can use the joystick or Stage Position controls on the SetupMicroscope XYZ Stage tab. Alternatively, click  **Raman AutoFocus** to focus using the strongest Raman signal. The Focus Limits should be set to **Full Range**.
If you do not have a motorized stage, you may find it useful to Monitor your sample to view the Raman signal while manually adjusting the focus.
12. When the polystyrene sample is optimally focused for Raman signal, select **Toolbox** on the Setup Instrument Advanced tab.
The Adjustments Toolbox dialog is displayed.



13. Select .
The Wavelength Calibration Verification dialog is displayed.



14. Ensure that you have followed the instructions on the dialog, and then click **Next**.
The wavelength calibration verification routine begins. This will take a few minutes. When the verification is complete, the result is displayed.
15. Remove the polystyrene sample and store safely.

The instrument is now ready for use.

In the unlikely event that the wavelength calibration verification should fail, follow Figure 41. If the wavelength calibration verification fails for the microscope, this does not necessarily mean that the performance of any other accessories attached to your instrument is also affected.

SOP-11: Wavelength Calibration

Wavelength calibration should only be performed if the instrument has failed a wavelength calibration verification.

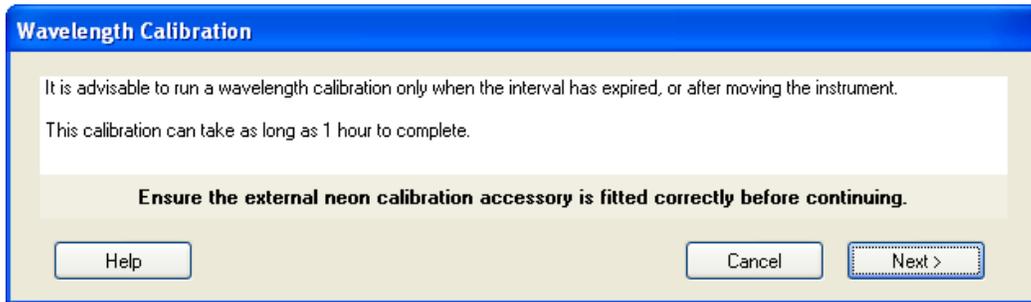
1. Ensure that the RamanMicro 200 is switched on.
If not, follow SOP-1.
2. Attach the external neon calibration accessory (L1320220) to the rear panel of the spectrometer module.
Push the large connector into the socket labeled **CALIBRATION** or **CAL ACCY** and carefully attach the small cable to the **COL** coupler below the **LED FIBER 1** connector.



Figure 42 Attaching the calibration accessory

NOTE: As wavelength calibration is not required on a regular basis, the external neon calibration accessory is not included in the basic RamanMicro 200 package. The external neon calibration accessory is included in the RamanMicro Calibration Kit (L1320266), which can be purchased separately.

3. Rotate the objective nosepiece until mid-way between two objectives.
4. Cover the microscope with a dark cloth or, if fitted, close the light-proof enclosure doors and ensure that caps are placed on any external fiber optic probes or accessories, to minimize stray light from interfering with the calibration.
5. Select the Dark Field (DF) switch position on the microscope and switch off the illumination.
6. Select **Raman Instrument** from the Setup menu.
The Setup Instrument tabs are displayed.
7. Select the Setup Instrument Advanced tab and click **Toolbox**.
The Adjustments Toolbox dialog is displayed.
8. Select .
The Wavelength Calibration dialog is displayed.



9. Ensure that you have followed the instructions on the dialog, and then click **Next**.
The instrument will now perform the wavelength calibration routine. This will take some time. When the calibration is complete, the result is displayed.
10. Disconnect the neon calibration accessory and refit the screw-in cap to the port.

The instrument is now wavelength calibrated.

SOP-12: Laser Frequency Calibration

Laser frequency calibration should only be performed if the instrument has failed a wavelength calibration verification.

To calibrate the microscope with the Microscope 50x objective selected:

1. Ensure that the RamanMicro 200 is switched on.
If not follow SOP-1.
2. Select the 50x objective lens on the microscope.
3. Place a polystyrene sample (L1181228) in a suitable sample holder.
4. Select **Raman Instrument** from the Setup menu.
The Setup Instrument tabs are displayed.
5. On the Setup Instrument Basic tab select **Microscope** or **Microscope XYZ Stage** as appropriate from the **Accessory** drop-down list
6. Select **Microscope 50x** from the Objective drop-down list.
7. Select the appropriate **Sample Holder** in the drop-down list and load the sample holder onto the sample stage, following the instructions on-screen if you have a motorized stage.
8. Select the Dark Field (DF) switch position on the microscope and switch off the illumination.
9. Cover the microscope with a dark cloth or, if fitted, close the light-proof enclosure doors and ensure that caps are placed on any external fiber optic probes or accessories, to minimize stray light from interfering with the calibration.
10. Select the Setup Instrument Advanced tab and ensure that the **Laser Power** is set to 100%.

11. Focus on the sample of polystyrene.

If you have a motorized stage you can use the joystick or Stage Position controls on the SetupMicroscope XYZ Stage tab. Alternatively, click  **Raman AutoFocus** to focus using the strongest Raman signal.

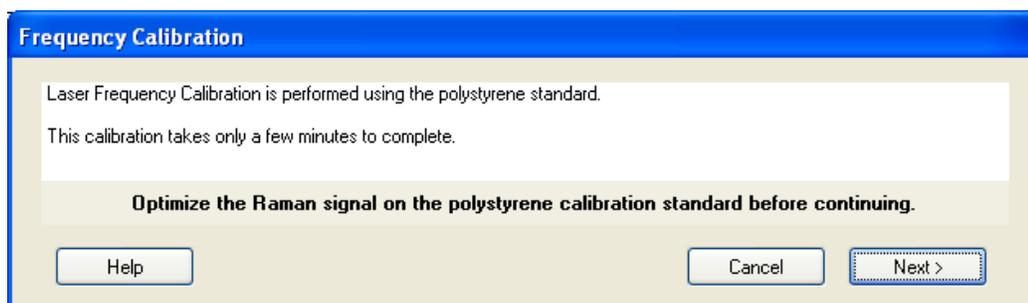
If you do not have a motorized stage, you may find it useful to Monitor your sample to view the Raman signal while manually adjusting the focus.

12. When the polystyrene sample is optimally focused for Raman signal, select the Setup Instrument Advanced tab and click **Toolbox**.

The Adjustments Toolbox dialog is displayed.

13. Select .

The Frequency Calibration dialog is displayed.



14. Ensure that you have followed the instructions on the dialog, and then click **Next**.

The instrument will now perform the laser frequency calibration routine. This will take a few minutes. When the calibration is complete, the result is displayed.

The instrument is now laser frequency calibrated.

In the unlikely event that the laser frequency calibration routine should fail, please perform a wavelength calibration and repeat the laser frequency calibration.

NOTE: If the calibration fails again, please contact your PerkinElmer Service Representative.

SOP-13: Intensity Calibration

The intensity calibration must be performed using the National Institute of Standards and Technology (NIST) Standard Reference Material. NIST SRM 2241 is the relative intensity correction standard for Raman spectroscopy when using 785 nm excitation.

NOTE: NIST calibration is temperature dependent. If you intend to perform quantitative analyses, you should ensure that your instrument is NIST calibrated at the temperature at which data is to be acquired. Otherwise, any data should be used for qualitative purposes only.

The NIST intensity correction is valid in the recommended operating temperature range 20 °C – 25 °C. If you move the instrument to an environment outside this temperature range, that is 15 °C – 20 °C or 25 °C – 30 °C, we strongly recommend that you perform a NIST intensity correction at this new temperature. Contact your PerkinElmer Raman Business Unit.

Intensity calibration should typically be performed on an annual basis – that is, during an annual instrument service – but also whenever the instrument is moved, following a service repair or when there has been significant temperature variation in the local environment.

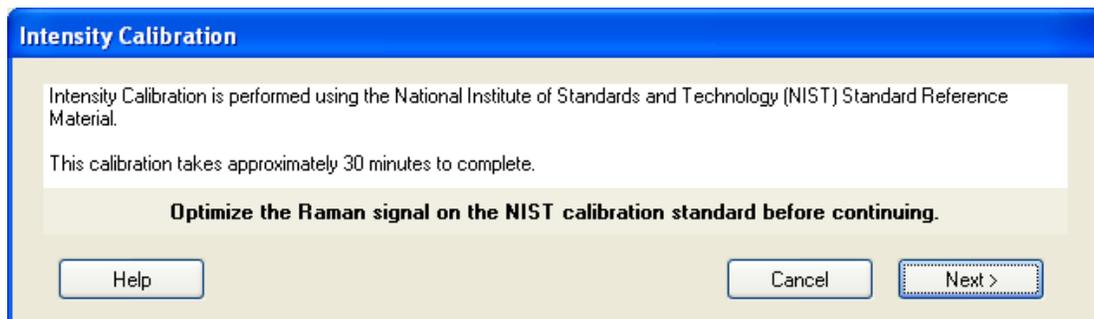
NOTE: As intensity calibration is not required on a regular basis, NIST Standards are not included in the basic RamanMicro 200 package. Standard Reference Materials can be purchased from PerkinElmer (L1321831). Your instrument will be intensity calibrated prior to shipping and during installation.

NOTE: The intensity calibration should be performed for each sampling accessory. In the case of the RamanMicro 300 Accessory, an intensity calibration is required for each objective lens.

1. Ensure that the RamanMicro 200 is switched on.
If not follow SOP-1.
2. Select the 50x objective lens on the microscope.
3. Place the NIST standard in a suitable sample holder.
4. Select **Raman Instrument** from the Setup menu.
The Setup Instrument tabs are displayed.
5. Select **Raman Instrument** from the Setup menu.
The Setup Instrument tabs are displayed.
6. On the Setup Instrument Basic tab select **Microscope** or **Microscope XYZ Stage** as appropriate from the **Accessory** drop-down list
7. Select **Microscope 50x** from the Objective drop-down list.
8. Select the appropriate **Sample Holder** in the drop-down list and load the sample holder onto the sample stage, following the instructions on-screen if you have a motorized stage.
9. Select the Dark Field (DF) switch position on the microscope and switch off the illumination.
10. Cover the microscope with a dark cloth or, if fitted, close the light-proof enclosure doors and ensure that caps are placed on any external fiber optic probes or accessories, to minimize stray light from interfering with the calibration.
11. Select the Setup Instrument Advanced tab, and ensure that the **Laser Power** is set to 100%.
12. Focus on the appropriate NIST standard with the dull surface facing upwards.
If you have a motorized stage you can use the joystick or Stage Position controls on the SetupMicroscope XYZ Stage tab. Alternatively, click  **Raman AutoFocus** to focus using the strongest Raman signal. The Focus Limits should be set to **Full Range**.
If you do not have a motorized stage, you may find it useful to Monitor your sample to view the Raman signal while manually adjusting the focus.
13. When the polystyrene sample is optimally focused for Raman signal, select **Toolbox** on the Setup Instrument Advanced tab.
The Adjustments Toolbox dialog is displayed.

14. Select .

The Intensity Calibration dialog is displayed.



15. Ensure that you have followed the instructions on the dialog, and then click **Next**.
The instrument will now perform the intensity calibration routine. This will take some time. When the calibration is complete, the result is displayed.
Any data acquired will now be intensity corrected.
In the unlikely event that the intensity calibration routine should fail, please ensure that the NIST sample is correctly positioned, and that the laser key switch is set to on (ARM).
16. Repeat steps 2–15 for each objective lens.
17. Remove the intensity correction standard and store safely.

Summary

- The only calibration procedure that needs to be performed on a regular basis is the wavelength calibration verification procedure.
- The wavelength and laser frequency calibrations should only be performed if the instrument fails the wavelength calibration verification procedure.
- We recommend that the intensity calibration is performed on a yearly basis, unless the environment (temperature and/or humidity) changes significantly, or the instrument is moved or repaired.

Additional Parts and Upgrades

Microscope Objective Lenses

Part Number	Description
L1323336	100x objective lens
L1323347	Long working distance objective lenses upgrade kit Contains 5x and 20x long working distance objective lenses
L1323343	5x long working distance objective lens
L1323344	20x long working distance objective lens
L1323345	50x long working distance objective lens
L1323346	100x long working distance objective lens

Sampling Accessories

Part Number	Description
L1323514	Quartz microscope slide, 25 × 75 × 1 mm
L1860335	Tablet holder kit for motorized stage
L1860336	Powder holder kit for motorized stage
L1321675	Multi-well plate holder for motorized stage
L1321676	Multi microscope slide holder Holds 4 standard (25 × 75 × 1 mm) microscope slides
L1321677	Universal sample holder Holds standard (25 × 75 × 1 mm) microscope slides, 50 × 75 mm glass slides, and Petri dishes up to 60 mm
L1860635/ L1860634	Linkam temperature controlled stage (110–130 V AC/220–240 V AC)
N1870184/ N1870188	Hot stage accessory (110 V AC/230 V AC) For use with long working distance objectives

SERS Consumables

Part Number	Description
L1329300	SERS gold colloid – 50 nm particles (20 ml)
L1329307	SERS gold colloid – 100 nm particles (20 ml)
L1329308	SERS gold colloid – 200 nm particles (20 ml)
L1329301	Aggregating agent – MgSO ₄ 0.16 M in milli-Q water (20 ml)
L1329303	Standard crystal violet pigment (0.25 ml)
L1329304	Disposable micropipettes – 200 µl (100 pack)
L1329305	Disposable micropipettes – 50 µl (100 pack)
L1329306	Disposable micropipettes – 2 µl (100 pack)

Safety and Calibration

Part Number	Description
L1320262	Raman laser safety kit (785 nm) Contains external interlock cable, laser safety goggles, laser detection card, and door warning label
L1320266	RamanMicro calibration kit
L1321843	sCAL Raman calibration standard
L1323503	Replacement laser keys
L1323518	Laser Glasses

Fiber Optic Probes

Part Number	Description
L1320002	Raman fiber optic probe, 7.5 mm working distance, maximum working temperature 80 °C, 5 M cabling
L1320003	Liquid immersion sleeve for Raman fiber optic probe
L1320011	Raman probe – long, 350 mm long, suitable for non-contact and liquid immersion. Maximum working temperature 200 °C, maximum working pressure 1500 psi, 5 M cabling
L1320012	Raman probe – max, suitable for non-contact and liquid immersion. Maximum working temperature 500 °C, maximum working pressure 3000 psi, 5 M cabling
L1320013	Purge Jacket for Raman probe – max, required to enable max probe operation at temperatures between 200 °C and 500 °C
L1320030	Triggered Fiber Optic Probe, 5 m cable
L1320031	Triggered Fiber Optic Probe, 20 m cable
L1320324	Raman probe fixed spacer kit (7.5 mm)
L1320325	Raman probe adjustable spacer kit
L1320070	Short immersion sleeve – for use with L1320030 and L1320031
L1320071	Ultra-long working distance lens adaptor
L1320095	Fiber probe sample holder (for measurement of samples in small vials and cuvettes)

For information about customized working distance or cable length fiber optic probes, contact your PerkinElmer Sales Representative.

Maintenance and **Troubleshooting**

Maintenance

The system does not require regular maintenance other than occasional checking and cleaning.

Cleaning the Spectrometer and Power Supply Units



WARNING

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

You can clean the outside of the spectrometer using a soft, lint-free cloth, moistened if required with a little water. Mild detergent may be used, if necessary. Do not use abrasive or solvent-based cleaning materials. Always perform a patch test on an inconspicuous area before you clean the entire instrument.

Avoid spilling liquid into the instrument or accessories. Clean all external spills immediately. If anything that is spilled enters the main body of the spectrometer, make the instrument inoperative and then contact a PerkinElmer Service Representative.



WARNING

Do not directly clean electrical contacts.



WARNING

To avoid any electrical shock hazards, do not allow liquids to run down into the external PSU. Also, do not allow liquids to run into the spectrometer or the stage controller box. Only use a soft, dry cloth to clean the spectrometer, stage controller or the PSU.

Cleaning the Air Filters

The air filters are located inside the top panel of the spectrometer module. You should clean the filters regularly.

To clean the filters:

1. Switch off the instrument power supply unit.
2. Disconnect the line power cord.
3. Remove the 4 screws holding the top cover in position and then lift off the cover.



Figure 43 Removing the top cover of the spectrometer module

4. Unclip the filter covers, remove the filters and wash in a mild detergent.
5. Dry the filters thoroughly.
6. Fit the air filters back into the spectrometer module.
7. Place the top cover in position and replace the 4 screws.

Servicing the Spectrometer

We recommend that you do not remove the protective housing or external panels of the spectrometer module except to clean the air filters (See *Cleaning the Air Filters* on page 91). There are no other user-serviceable parts in the instrument. If you require an instrument service, please contact PerkinElmer. Only PerkinElmer Service Representatives should attempt to service the spectrometer.



WARNING

Removal of the protective housing or exterior panels on any instrument will allow potentially hazardous Class 3B laser radiation to be emitted and will invalidate the instrument warranty.

Maintenance and Service of the Microscope

The microscope is designed to be maintenance free and should not be opened or adjusted by anyone other than a PerkinElmer Service Representative. Contact PerkinElmer if you have any questions regarding maintenance or servicing.

The optical surface of all microscope objectives should be left free from debris. To clean the lenses, wipe clean with alcohol soaked cotton swabs or lint-free tissues. For further information refer to the documentation supplied with the microscope.

Damage to the microscope as a result of accident, neglect, misuse, or as a result of service or modification by anyone other than PerkinElmer will invalidate the warranty.

Refer to the Olympus manual provided with your microscope for the replacement of the light bulb.

Maintenance and Service of the Fiber Optic Probe

The FOP is designed to be maintenance free and should not be opened or adjusted by anyone other than a PerkinElmer Service Engineer. Contact PerkinElmer if you have any questions regarding maintenance or servicing of the FOP.

The immersion sleeve (if purchased) is manufactured with 316 stainless steel or Hastelloy C and contains either a quartz or sapphire optical window that is sealed. The quartz window can be removed for cleaning by unscrewing the end of the immersion tube. The sapphire window cannot be removed as it is welded into place. If removing the quartz window, do not lose the PTFE O-rings, as these are required to maintain the immersion seal. Without these PTFE rings the immersion tube will leak and the FOP will be damaged.

The front lens or window on the fiber probe body and the immersion sleeve window should be left free from debris. To clean simply wipe the fiber faces clean with alcohol soaked cotton swabs or lint-free tissues.

Damage to the FOP as a result of accident, neglect, misuse, or as a result of service or modification by anyone other than PerkinElmer will invalidate the FOP warranty.

NOTE: For details of how to maintain and clean a triggered fiber optic probe, see the *Raman Triggered Fiber Optic Probe* leaflet (L1321940).

Replacing the Stage Controller Fuse

NOTE: This section is only applicable if you have a motorized stage.



Electrical hazard

To prevent potential injury to yourself and damage to the instrument, switch OFF all components in the system and disconnect them from the line power supply before you alter, or make any new, electrical connections.

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

The only other fuses that you may have to replace are those in the plugs of the mains power cords for the spectrometer and microscope (UK-type plugs only).

Troubleshooting

This section is a reference guide if you are having any problems with your instrument. Troubleshooting outlines what to do if your instrument or software malfunctions or crashes.

Hardware

Problem:	During the installation procedure the USB devices are not recognized.
Description	This may mean that there is not enough power on the USB sockets on your PC.
Solution	Ensure that your PC has USB 2.0 sockets. If you are not sure, use a USB 2.0 power hub.

Problem:	You are prompted for the CCD drivers.
Description	This may happen if you disconnect the instrument control USB cable and reattach it to a different USB port, or if you switch the instrument off and then switch it on again.
Solution	Refer to the <i>Spectrum Administrator's Guide</i> (part number L1050095) for details of how to reinstall the drivers.

Problem:	Cannot collect data because an interlock is breached.
Description	This means that, for some reason, the interlock system is detecting an open circuit.
Solution 1	Ensure all interlocks, including any remotely interlocked doors, are closed properly.
Solution 2	Ensure your external interlock is wired up correctly and that any interlocked room entrances or sampling enclosures are closed.

Software

Problem	Spectrum software stops responding.
Description	When you are using the Software, for some reason it stops working, that is, mouse clicks have no effect.
Solution	Hold down the CTRL+ALT+DELETE keyboard buttons to display the Windows Security dialog. Click Task Manager and then click the Processes tab. In the list under Image Name look for IRWinLab.exe (to find this you may have scroll through the list using the scroll bar on the right side) click on this, then click End Process . Then select Raman.exe and then click End Process . If a Task Manager Warning dialog is displayed click OK . Re-start the software. If you are unsure how to do this please refer to SOP-1. If you do not intend to restart the software, you must switch the power to the instrument off. Otherwise the laser may not shut down.

Problem:	The motorized stage does not move to the expected position on a sample holder when requested.
Solution 1	Go to the Setup Instrument Basic tab and ensure that the selected sample holder is correct. If it is not, then select the appropriate sample holder. Check the Graphic view on the Setup Microscope XYZ Stage tab – it may be that you were directing the stage to a prohibited region of the sample holder (hashed area on the Graphic view).
Solution 2	Go to the Setup Instrument Basic tab and ensure that the selected microscope objective is correct. If the wrong microscope objective is selected then the wrong scale will be displayed on the Setup Microscope XYZ Stage tab
Solution 3	If the Accessory options are correct, it may be that the stage needs to be reset. On the Setup Microscope XYZ Stage tab, click  Toolbox and then select Reset Stage . The motorized accessory will now reset itself.
Solution 4	If the above solutions do not solve your problem, you should contact your PerkinElmer Service Representative.

Problem: **The message “Video Image not available” is displayed on the Live Video image on the Setup Microscope XYZ Stage tab.**

Description This means that the software searched for a video camera on the system and could not locate one.

Solution Is the USB cable from the microscope to the PC properly attached? If not attach the USB cable correctly at both ends and restart the software.

Problem: **Unable to communicate with stage.**

Description When you open the Spectrum software the error message **Unable to communicate with Stage. The stage controls will not operate.** is displayed.

Solution Disconnect the stage controller box power supply from the mains power socket, wait a few seconds, and then reconnect the PSU to the mains power socket. You will then be prompted to reset the stage.

Decontamination

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

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

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

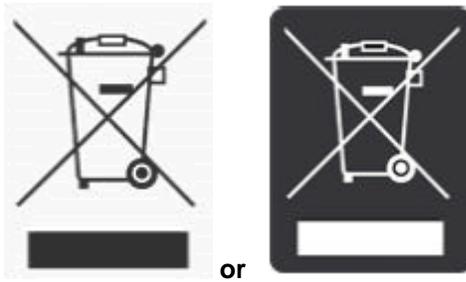
<http://las.perkinelmer.com/OneSource/decontamination.htm>

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

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

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

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.

