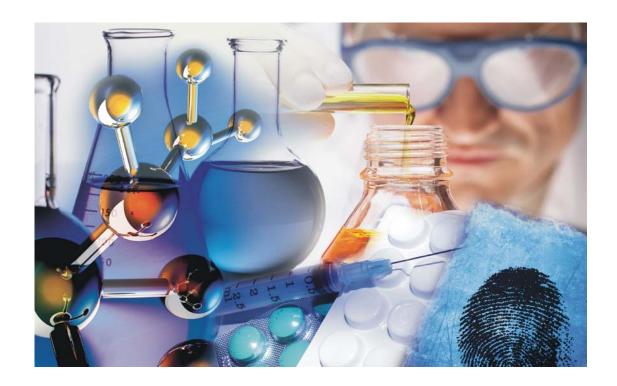
RamanMicro 200 Series



Getting Started Guide



Release History

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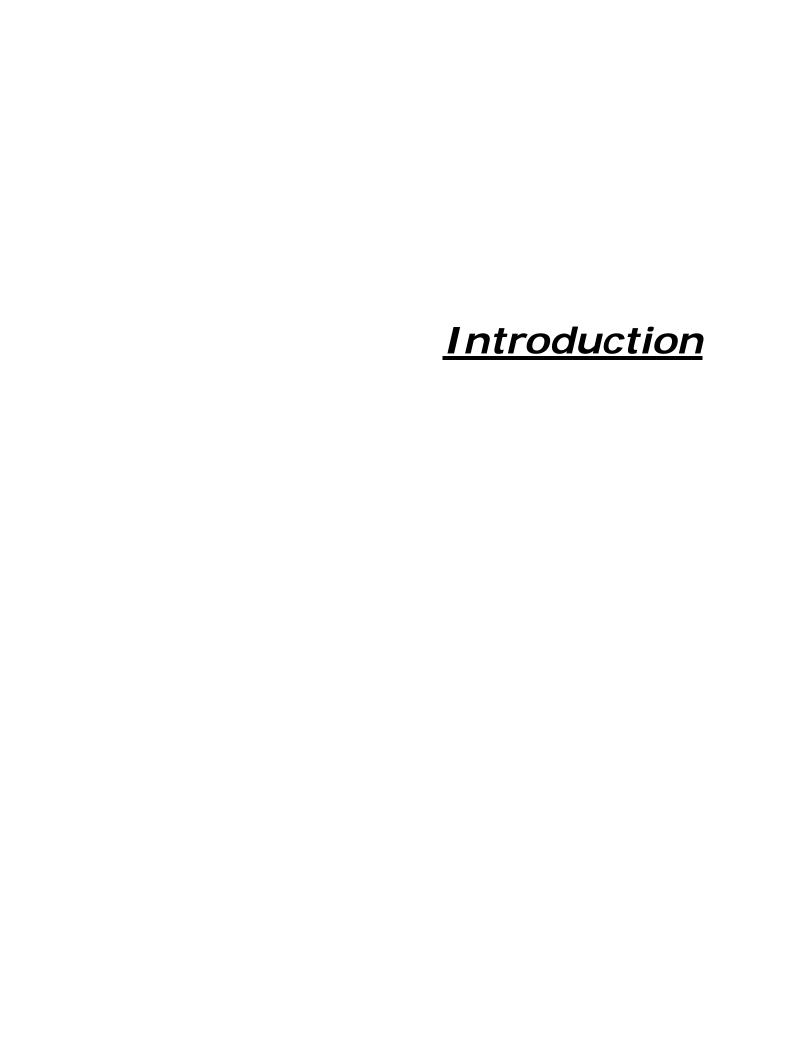
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About This Manual

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 in the manual.

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, and what utilities are required, for example electricity, temperature control.

Installing your RamanMicro 200

This chapter contains instructions about how to assemble your RamanMicro 200 system and how to install the software.

Using the RamanMicro 200

This chapter contains a number of Standard Operating Procedures (SOPs). These are a series of procedures which 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 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 recuadros 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.
	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.

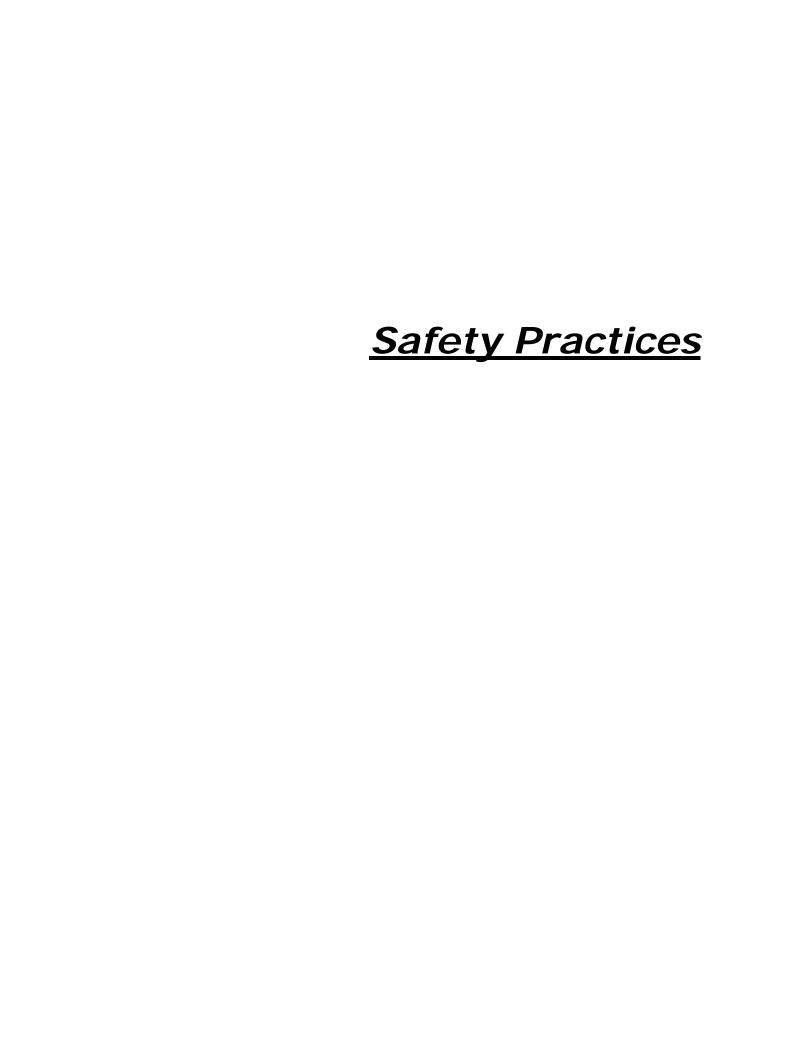


We use the term **WARNING** to inform you about situations that could result in **personal injury** to yourself or other persons. Details about these circumstances are in a box like this one.

D Warning (Warnung)

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

- Warning (Advarsel)
 Betyder, at brugeren kan blive kvæstet, hvis anvisningen ikke overholdes.
- 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.
- 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.
- Warning (Aviso)
 Significa que a não observância da instrução referida poderá causar um ferimento ao usuário.



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

The RamanMicro 200 and RamanMicro 200F 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 microscope indoors and under the following conditions:

Temperature 15 °C to 30 °C

Relative Humidity 75% 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 hazard involved.

Whenever it is likely that the RamanMicro 200 is unsafe, make it inoperative. The RamanMicro 200 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.

Environmental Conditions

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 ±10% of the nominal voltage.



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

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 (Figure 1).

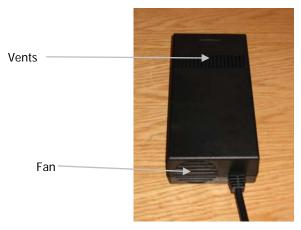


Figure 1 Power supply unit

Electrical Safety

The spectrometer module comes assembled within a protective housing to prevent exposure to electrical hazards. There are 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 105.

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 (Figure 1). The PSU used with the spectrometer module can adjust automatically to a variety of AC power sources as outlined in Table 1. For information on line frequency and current please review the rating plate on the PSU.

Table 1 PSU input voltages and mains fluctuations information

Input Voltage	Mains Fluctuations
100- 240 VAC	±10% of the nominal voltage



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 Engineer. 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.



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.



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.

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

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.



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.



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.



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



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.

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:

- 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 27.

Microscope Safety

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

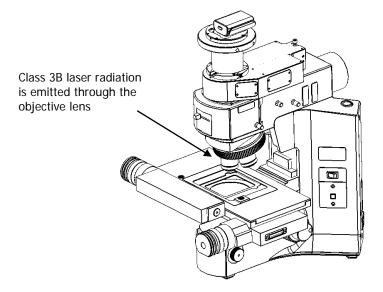


Figure 2 Schematic of microscope

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.



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.



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 3) 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.**

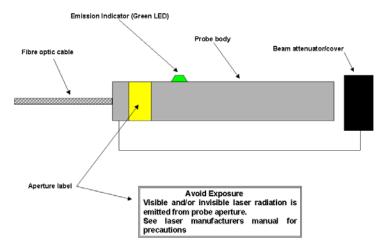


Figure 3 Schematic of the fiber optic probe (not to scale)

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 (L1321887)

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.

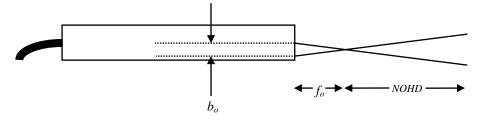


Figure 4 Probe parameters used to calculate the nominal ocular hazard distance

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:

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

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 x 10⁻² J cm⁻² or 1.49 x 10⁻³ W cm⁻² for a 10 second exposure or longer. Table 2 shows the calculated nominal ocular hazard distance for 785 nm Raman systems.

Table 2 Nominal Ocular Hazard Distance (NOHD) for 785 nm

Probe focal length / mm	NOHD for 100 mW exiting fiber optic probe / cm
7.5 (± 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 44.

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

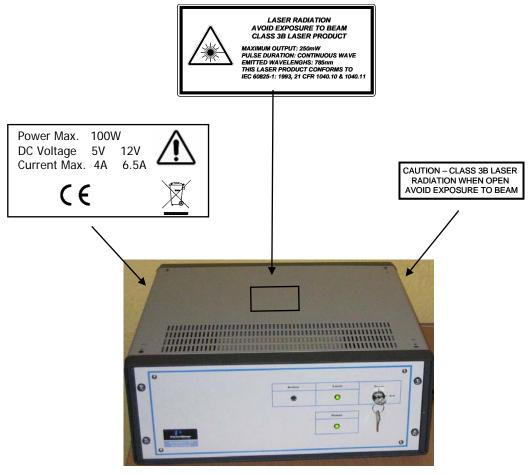


Figure 5 Labels on the spectrometer module

Labels on the Exterior of the Microscope

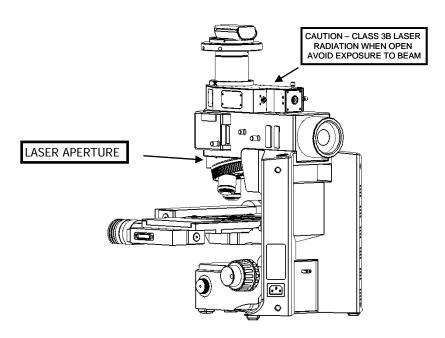


Figure 6 Labels on the microscope

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 7) 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 7).

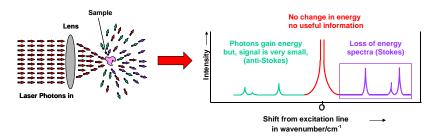


Figure 7 The Raman effect: irradiation of sample and subsequent scatter

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 8).

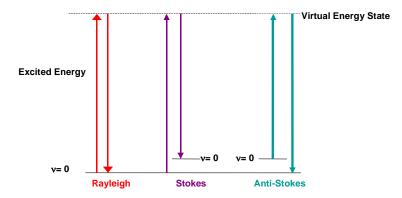


Figure 8 Rayleigh, Stokes and Anti-Stokes scattering

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.

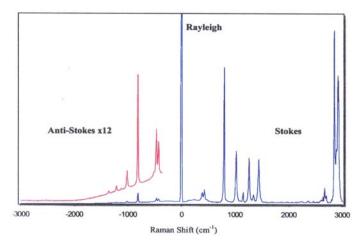


Figure 9 Raman spectrum of cyclohexane, showing Stokes, anti-Stokes and Rayleigh scattering

The RamanMicro 200

The RamanMicro 200 is a fiber-optic Raman microscope with laser excitation wavelength at 785 nm.

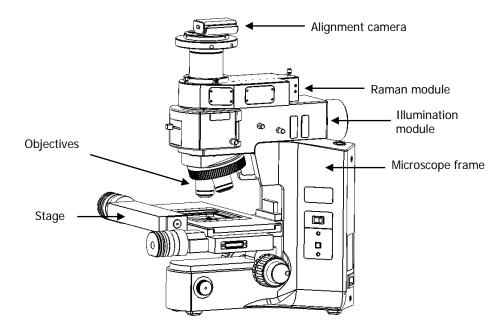


Figure 10 The RamanMicro 200 microscope

The microscope (Figure 10) 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 11.

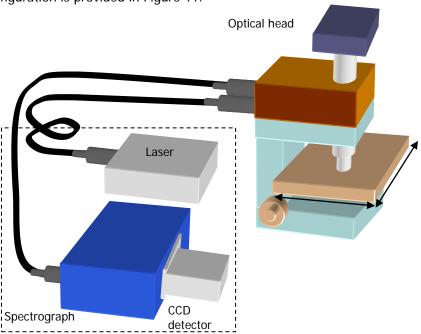


Figure 11 Schematic of the RamanMicro 200 system

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.

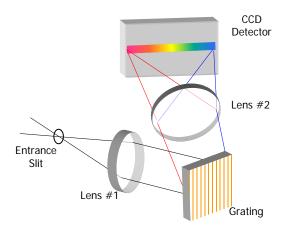


Figure 12 Schematic of the Czerny-Turner spectrograph

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 140–3200 cm⁻¹ at a resolution of about 8 cm⁻¹ or 140–1800 cm⁻¹ at about 5 cm⁻¹ resolution.

Preparing Your Facility

Specifications

	Spectrometer	Microscope	Stage Controller
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
Operational Temperature	15 °C (59 °F) – 30 °C (86 °F)		
Recommended Temperature	20 °C (68 °F) – 25 °C (77 °F)		
Power Supply	100–240 V ±10%, 50–60 Hz		
Primary Fuse	-	See Olympus manual	1.6 A T (time-lag)
Laser Class	Class 3B		-

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.

PC Requirements

The following pages provide details of the requirements for the PC that will run the Spectrum software and communicate with the instrument. To ensure successful installation of the software, please check these requirements before starting the installation.

Hardware Requirements

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

- Intel[®] Pentium 4 400 or 533 MHz processor;
- At least 256 MB of Random Access Memory (RAM);
- Video card and monitor capable of displaying at least high color (16 bit) at 1024×768 SVGA, but ideally a screen resolution of 1280×1024 pixels;
- 10 GB hard disk with at least 1 GB free space as an NTFS drive;

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

- CD-ROM drive;
- Ethernet network connection;
- A keyboard and USB or PS/2[®]-style mouse;
- Hi-Speed USB 2.0 port (x2);
- Serial (RS232) port;
- Parallel port.

Software Requirements

Spectrum requires that Windows XP Professional Service Pack 2 operating system is installed on the PC before you install the software.

We have specified Windows XP Professional because it is a robust, industry strength operating system that provides inbuilt security and auditing.

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

Windows Administrator Level

It is important to note that you must be logged on at Administrator level on Windows before installing the software. Logging on as an Administrator ensures that installation of the software can be undertaken and that the necessary system registry updates that form part of the installation process are successfully completed. Administrators have the capability to assign privileges and logon rights and therefore have the ability to make system-wide changes. Users do not have this ability, or may have restricted abilities depending on the rights and privileges assigned by the Administrator.

Finding a Suitable Location for the Instrument

The following conditions should be observed:

- The environment must be suitable for a Class 3B laser system.
- If your system has a manual stage 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 there must be a minimum of 5 power points (1 for the microscope, 1 for the spectrometer, 1 for the stage control box and 2 for the PC).
- 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.

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 instrument and any ancillary equipment without warping or wobbling. The bench must be at least 610 mm (24") in depth.

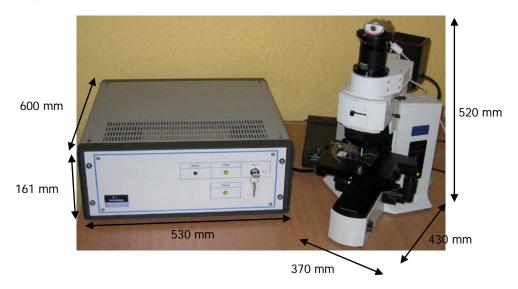


Figure 13 Dimensions of the RamanMicro 200 system

Installing Your RamanMicro 200

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 the RamanMicro 200 microscope system 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 (LX100884), the Raman Instrument Configuration Disk (LX108869)), the Raman Instrument Installation Disk (LX108925) and the RamanMicro 200 Series Getting Started Guide (L1321844)
OR	
LX108871	Spectrum ES for Raman Software Kit Contains the Spectrum ES Software CD (LX100881), the Raman Instrument Configuration Disk (LX108869)), the Raman Instrument Installation Disk (LX108925) and the RamanMicro 200 Series Getting Started Guide (L1321844)
L1323503	Laser key (x2)
L1320821	USB Cable
L1321802	Mains PSU
L1320264	External Interlock Override
L1323055 OR	Manual Stage
L1323011	Motorized Stage
L1865749	RS232 Cable (systems with motorized stages only)
L1860289	Stage Controller (systems with motorized stages only)
L1865723	Joystick (systems with motorized stages only)

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

Lifting the Instrument

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



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



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.

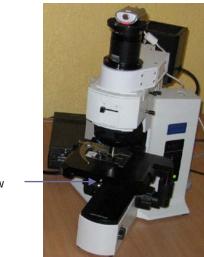


To prevent potential injury to yourself and damage to the instrument, switch OFF all components in the system and disconnect them from the line power supply before you perform any of the procedures described in this section.

Fitting the Stage on the Microscope

To fit the stage:

- 5. Unpack the stage from the shipping material.
- 6. Remove the objectives from the microscope.
- 7. Carefully locate the stage above the stage support ring and then lower the stage into position.
- 8. Ensure that the stage is level, and then fasten the stage in position by tightening the screw at the front of the stage (Figure 14).



Fixing screw

Figure 14 Fitting the stage

9. Replace the objectives.

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 is powered by the stage controller box (L1860289) and joystick (L1865723).



Figure 15 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 16).



Figure 16 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 laser-safe enclosure, you may find it easier to attach the z-axis focus motor and sleeve before fitting the enclosure. If you install the enclosure first, you will need to pass the cable through the rear port of the enclosure.

1. Slide the focus motor sleeve over the z-axis drive control on the left side of the microscope, and then tighten the connections using an allen key.

OR

Push the focus motor into the sleeve until it touches the drive control, and then tighten the screw to finger tight.

 Connect the 9 pin connector from the focus motor into the 9 pin connector end of the z-axis adaptor cable (L1321689), and then connect the other end into the MOTOR Z connector on the rear of the stage controller box (Figure 16).



Figure 17 Z-axis focus motor and sleeve attached to the microscope inside the optional laser-safe enclosure

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 18), fasten the two screws so that the source is supported.
- 3. Connect the black cable from the illumination source to the microscope frame.

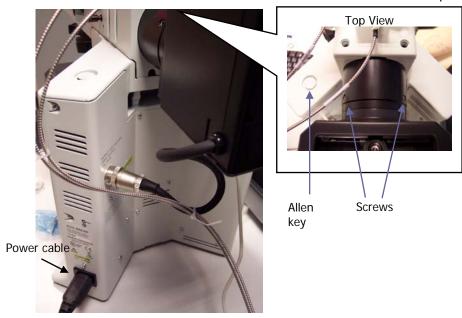


Figure 18 Attaching the illumination source to the microscope

Connecting the Raman Module to the Microscope

The Raman module is positioned on top of the illuminator module so that the connecter 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 19 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.

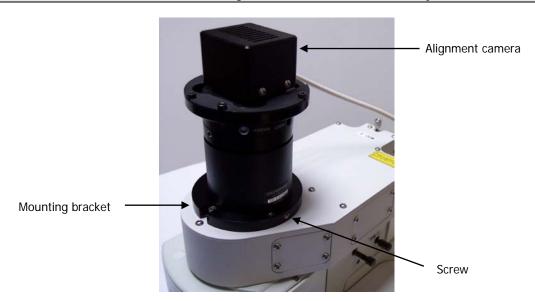


Figure 20 Connecting the alignment camera

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 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 this can damage the fiber.

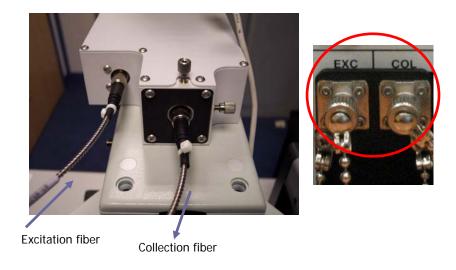


Figure 21 Fiber optic cables connecting the Raman module to the spectrometer

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.

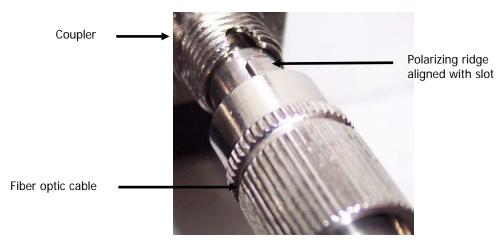


Figure 22 Connecting the fiber optic cable

2. Connect the collection cable (L1323332) from the Raman module to the **COL** coupler 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 FOP cable is aligned with the slot in the coupler casing before carefully pushing the cable into the coupler (Figure 22).

CAUTION

Try to bend the fiber as little as possible. If you bend the fiber through a tight angle this can damage the fiber.

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.

Unpacking and Assembling the PC

Please refer to the manufacturer's instructions for unpacking and assembling the PC system.

NOTE: Do not attach any cables between the instrument and the PC at this time.

- 1. Connect the mains power cords from the PC and the monitor into the mains electrical supply, and then switch on.
- 2. If you are prompted to login to Windows when you start the PC, log in.

If your PC was provided by PerkinElmer your username and password will be:

Username: Administrator Password: Administrator

3. Click OK.

You can now start using the PC.

For PC security, we recommend that the Administrator Password is changed after installation. For more information, see your Microsoft Windows documentation.

Switching on the Instrument

- 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 18), and then switch on the microscope.
- 4. If you have a motorized stage, switch on the stage controller box (Figure 16).

Installing Spectrum Software

Before you Start

NOTE: We strongly suggest you read the *PC Requirements* starting on page 37 before attempting to install your software.

NOTE: Before installing the software, we recommend that you read and print the Release Notes which can be found as a .rtf file and a .pdf file on the root of the CD, because they contain important information that may not be in this manual or the Spectrum Help.

To read .pdf files you will need Adobe Reader 5.0 or later. An installation of this software is available on the *Software Utilities CD*.

Before starting the installation procedure, please ensure that:

- All programs have been closed, including any anti-virus software running on the PC.
- The USB cables between the PC and the RamanMicro 200 and, if appropriate, between the PC and triggered fiber optic probe, are NOT connected.
- The USB cable from your alignment camera, if fitted, is not connected into the USB socket in the sample compartment of the RamanMicro 200.

If the PC you intend to use already has a copy of Spectrum installed and is being used to control IR instruments, you should follow the steps described below before you carry out the installation required to enable the PC to control both the Raman and IR instruments.

- 1. Launch the Spectrum software.
- 2. When you are prompted to select an instrument, choose the **Work Offline** option from the drop-down list and then click **OK**.
- 3. Use the menu option Administration > Instrument and Accessory Configuration > Remove Instrument to remove each IR instrument, in turn.
- 4. When you have removed all of the IR instruments, close down Spectrum.
- 5. Uninstall the Spectrum software from your PC.

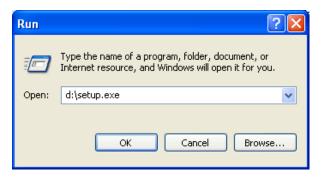
You are now ready to begin the installation procedure. Once you have installed the software, you can reinstall the IR instruments.

Installation Procedure

NOTE: It is important to note that you must be logged on at Administrator level on Windows before installing the software.

The Installation Wizard helps you install the software on your PC.

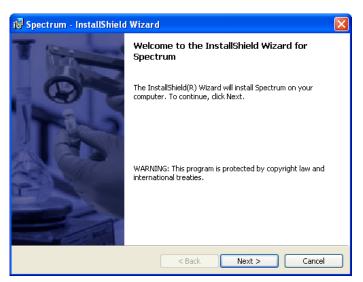
- 1. Place your *Spectrum Software CD* into your CD drive. The Spectrum software must be version 6.3.2 or later.
- 2. If the installation program does not start automatically, from the Start Menu select **Run**. The Run dialog is displayed.



3. Enter d:\Setup.exe and then click OK.

Replace d:\ with the drive letter for your CD.

The InstallShield Wizard dialog appears while the system is preparing to install the software. When the installer is ready, the Welcome dialog is displayed.



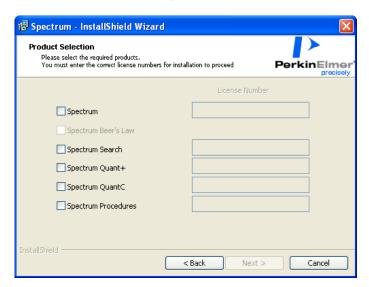
If your PC does not meet the software requirements given in *PC Requirements* starting on page 37, an appropriate error message will inform you of the problem. You will need to correct this problem before the installation can be performed.

4. Click Next.

The License Agreement page is displayed.



5. Read the license and if you accept the terms, select that option and then click **Next**. You will then be asked which products you want to install.



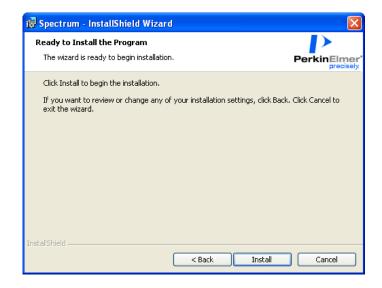
6. Select the required product(s) and then enter the **License Number(s)** as found on the Software License Certificate.

You must enter the correct license number(s) to proceed with the installation. If you enter an incorrect license number the **Next** button is disabled.

The Spectrum Beer's Law check box is automatically selected when you select the Spectrum check box. You do not need to enter a license number for Spectrum Beer's Law.

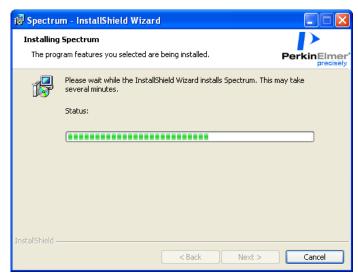
7. Click Next.

The Ready to Install the Program page is displayed.

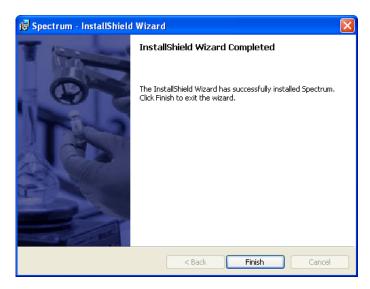


8. Click **Install** to begin installing Spectrum.

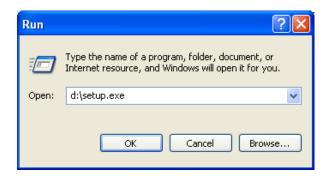
The Installing Spectrum page is displayed, which informs you of the status of the installation.



When the installation is complete, the InstallShield Wizard Completed page is displayed.



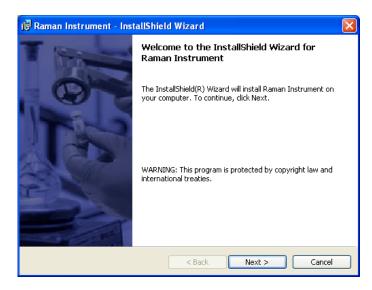
- 9. Click Finish.
 - Before you can collect spectra you must configure your Raman instrument.
- 10. Place your Raman Instrument Installation Disk (LX108925) into your CD drive.
- 11. If the installation program does not start automatically, from the Start Menu select **Run**. The Run dialog is displayed.



12. Enter d:\setup.exe and then click OK.

Replace d:\ with the drive letter for your CD.

The InstallShield Wizard dialog appears while the system is preparing to install the software. When the installer is ready, the Welcome to the InstallShield Wizard for Raman Instrument page is displayed.

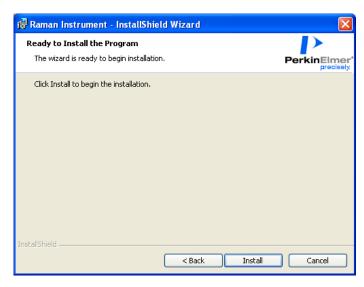


13. Click Next.

The License Agreement page is displayed.



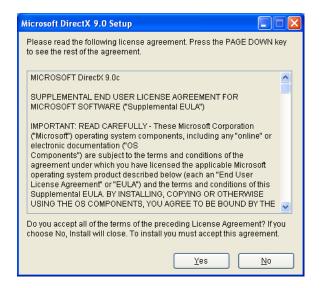
Read the license and if you accept the terms, select that option and then click Next.
 The Ready to Install the Program page is displayed.



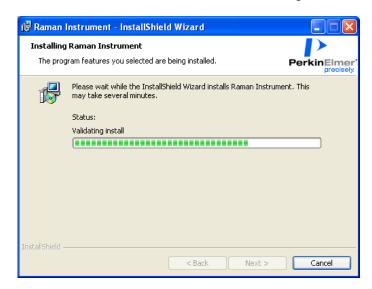
Click Install to begin installing the Raman instrument.
 The Installing Raman Instrument page is briefly displayed.



During this installation, the DirectX Setup page is displayed.



Read the license and if you accept the terms, click Yes.
 The Installing Raman Instrument page is displayed again, which informs you of the status of the installation.



17. Click Next.

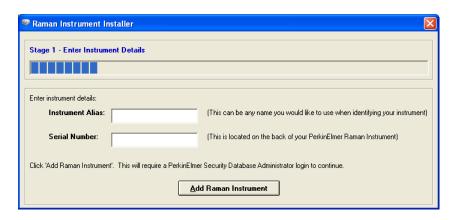
The Raman Instrument Install Wizard opens at the Welcome page.

NOTE: Ensure that the USB cable from your alignment camera (on the instrument and/or the microscope) is not connected into the USB socket in the sample compartment of the instrument.



18. Click Start.

The Enter Instrument Details page is displayed.



19. In the **Instrument Alias** field, enter a name for your Raman instrument. This should be a unique name to identify this instrument.

- 62 . RamanMicro 200 Series Getting Started Guide
- 20. In the **Serial Number** field, enter the serial number of your instrument. This is displayed on the rear panel of the instrument.
- 21. Click Add Raman Instrument.

If you have already installed PerkinElmer software on the PC, the PerkinElmer Login dialog is displayed.



Login as a PerkinElmer Software Administrator.

Use the Administrator **User name** that you use for the PerkinElmer software that is already installed on the PC.

The default **User name** and **Password** are **administrator**.

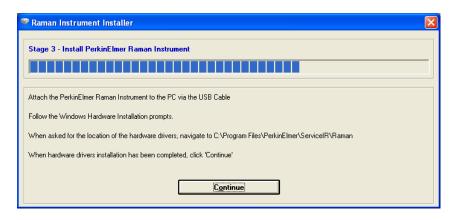
The Locate Instrument Configuration Files page is displayed.



22. Place your *Raman Instrument Configuration Disk* into your CD drive, wait a few seconds, and then click **Copy Configuration Files**.

If your configuration files have already been installed on your PC, click **Browse** and navigate to their location before you click **Copy Configuration Files**.

The Install PerkinElmer Raman Instrument page is displayed.



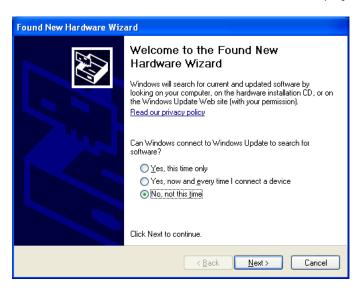
23. Attach your instrument to the PC using the supplied USB 2.0 cable (L1320821).



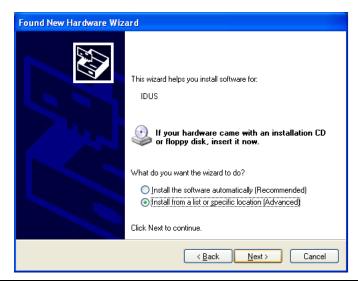
Figure 23 USB 2.0 cable

NOTE: If you disconnect the instrument control USB cable and reattach it to a different USB port, then you will be prompted for the drivers. By default this driver is stored in C:\Program Files\PerkinElmer\ServiceIR\Raman.

24. Wait until the Welcome to the Found New Hardware Wizard is displayed.



Select No, not this time and then click Next.
 The Found New Hardware Wizard page is displayed.



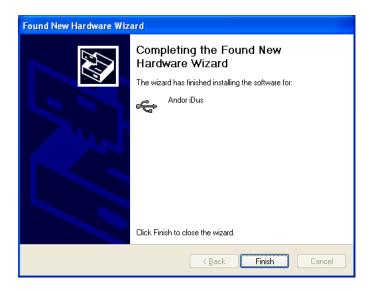
NOTE: The Found New Hardware Wizard page may inform you that it is helping you install software for **USB Device**. In this case continue with the installation in the same way as for **IDUS**.

26. Select **Install from a list or specific location (Advanced)** and then click **Next**. The Please choose your search and installation options page is displayed.



- 27. Select **Include this location in the search**, click **Browse**, and then navigate to C:\Program Files\PerkinElmer\ServiceIR\Raman.
- 28. Click Next.

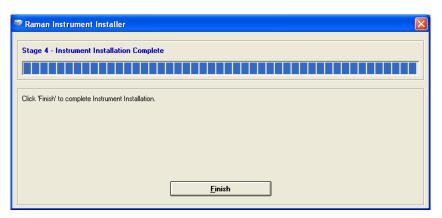
The Completing the Found New Hardware Wizard is displayed.



29. Click Finish.

The Found New Hardware Wizard closes.

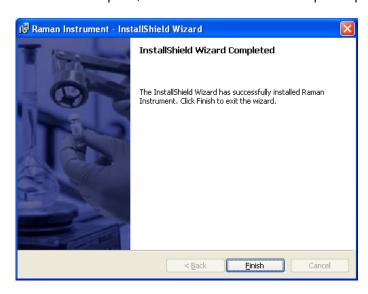
30. On the Install PerkinElmer Raman Instrument page, click **Continue**. The Instrument Installation Complete page is displayed.



31. Click Finish.

The Raman Instrument Installer closes.

When the installation is complete, the InstallShield Wizard Completed page is displayed.



32. Click Finish.

The Raman Instrument Installer Information dialog is displayed.



- 33. Click No.
- 34. Plug the USB cable from your alignment camera into the USB socket in the sample compartment of the instrument.

The Found New Hardware Wizard is displayed.

- 35. Repeat steps 25 to 29 to install the drivers for the alignment camera.
- 36. If you have a Videology alignment camera, you will need to install the Videology USB2.0 Viewer. Go to Step 37.

OR

If not, go to Step 45.

37. Navigate to C:\Program Files\PerkinElmer\ServiceIR\Raman\Videology and double-click on **Autorun.exe**.

The Welcome to Videology USB2.0 Camera Installation Wizard page is displayed.



38. Click Next.

The Please Plug in Your Camera Now page is displayed.



39. Click Next.

The Your Camera is Successfully Installed page is displayed.



40. Click Finish.

The Videology USB2.0 Camera Software Setup page is displayed.



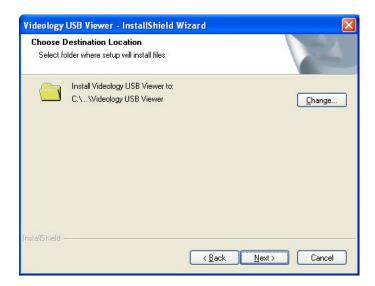
41. Click Install Viewer.

The Welcome to the InstallShield Wizard for Videology USB Viewer is displayed.



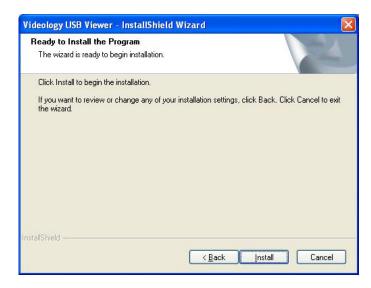
42. Click Next.

The Choose Destination Location page is displayed.



43. If required, click **Change** and navigate to your chosen destination folder and then click **Next**.

The Ready to Install the Program page is displayed.



44. Click Install.

The Setup Status page is displayed.



When the installation is complete, the InstallShield Wizard Complete page is displayed.



- 45. Click Finish.
- 46. Restart your PC.

This completes the installation of the Spectrum software.

If appropriate, you should now install the triggered fiber optic probe drivers, as described in the *Raman Triggered Fiber Optic Probe* leaflet (L1321887) and then connect the fiber optic cables from the triggered fiber optic probe to the RamanMicro 200, as described for a standard fiber probe in *Attaching and Removing the Fiber Optic Probe* on page 79.

NOTE: You should not allow the PC to enter Standby mode. On the Screen Saver page of the Display Properties dialog, click **Power** and then set **System standby** and **Turn off hard disks** to **Never**.

Alignment of the Microscope



Do not disconnect the microscope from the spectrometer. Only trained PerkinElmer Service Engineers 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 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

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).
- 2. Select the Bright Field (BF) switch position on the Olympus illuminator module.
- 3. Place a slide with an easily recognizable feature on it (for example, a graticule slide).
- 4. In the Spectrum software, select **Instrument** from the Setup menu or click The Scan and Instrument Setup dialog is displayed.
- On the Hardware tab select the beam path of the objective from the Beam path dropdown list.
- 6. Click Video Preview.

The Alignment Camera window opens.

- 7. Move the stage until the feature is in focus and in the center of the crosshairs in the Alignment Camera window.
- 8. 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.
- 9. Repeat Steps 7–8 until all the objectives are aligned.

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 an appropriately trained PerkinElmer Service Engineer, and in a Class 3B environment.

Aligning the Video Camera

The crosshairs displayed on the Alignment Camera window in the Spectrum software represent the position of the laser. If the microscope is knocked this crosshair may not correspond to the true position of the laser. Minor corrections of the crosshair position can be made in the software. If the laser spot is very far from the crosshair a complete realignment of the video camera is required:

- 1. Select the 50x objective lens on the microscope.
- Place a flat sample on a microscope slide.
 We recommend that you do not use a colored polymer sample.
- 3. In the Spectrum software, select **Instrument** from the Setup menu or click The Scan and Instrument Setup dialog is displayed.
- 4. On the Hardware tab select Microscope 50x from the Beam path drop-down list.
- 5. On the Instrument page, reduce the laser power to approximately 5–10%.
- 6. Click Video Preview.

The Alignment Camera window opens.

- 7. From the Calibrate menu in the Alignment Camera window, select **Show crosshair controls**.
- 8. Click Spectral Preview.
- 9. Set the **Preview Scan time** to 10 s.

The laser should be observable as a definite circle in the Alignment Camera window. After 10 s the laser spot will disappear temporarily, but it will appear again after approximately 0.5 s. This is normal.

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

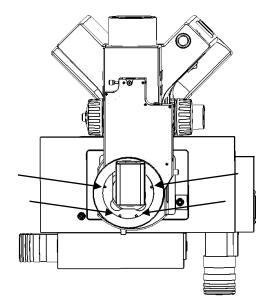


Figure 24 Top view of the microscope showing the screws used to adjust the position of the camera module

- 11. Move the camera module until the crosshairs line up as closely as possible with the center of the laser spot.
- 12. Re-tighten the 4 screws.
- 13. Use the arrows in the Alignment Camera window to fine-tune the position of the crosshairs to be in the center of the laser spot.

NOTE: If the video camera image is inverted, select **Invert Image** from the Camera menu.

14. If required, select **Save Calibration** from the Calibrate menu to save the new settings.

Aligning the Raman Coupler

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. The procedure for alignment is as follows:

- 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. Using Video Preview in Spectrum, ensure that the sample is in focus and centered.
- 4. Using Spectral Preview note the maximum number of counts in the window.
- 5. Very slowly move one of the coupler knobs clockwise and counter-clockwise until the counts level is maximized.

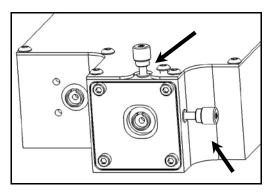


Figure 25 Adjustment of the XY Raman coupler

- 6. Move the other knob in a similar manner until the counts level has maximized.
- 7. Return to the first knob and move again to maximize the counts (the movement should be much less than used the first time).
- 8. Return to second knob and do likewise.
- 9. Tighten each retaining screw a little and move the xy coupler knobs to compensate each time.
- 10. Tighten screws until the plate at the rear coupler is secure do not over tighten.

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).

If the system is misaligned, we recommended that you contact a PerkinElmer Service Engineer.

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 the sample is flat. PerkinElmer recommends the use of a flat silicon surface.

Moving the Instrument



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 you should perform the calibration routines described in *Calibration and Performance Verification SOPs* on page 94.

<u>Using the</u> <u>RamanMicro 200</u>

Spectrometer Front Panel (Console)

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 are activated (open) and the laser radiation will be present throughout the optical path of the instrument.

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 (L1321887).

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. There is also a plug attached to the end of two wires which connects the fiber optic LED to the instrument.

Attaching the FOP

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



Figure 27 Attaching the FOP

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

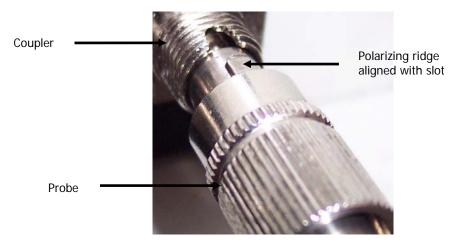


Figure 28 Connecting the fiber optic probe

- Connect the collection cable of the FOP to the coupler marked COL.
 Make sure that the polarizing ridge on the FOP cable is aligned with the slot in the coupler casing before carefully pushing the cable into the coupler.
- 7. Connect the plug to the nearby socket marked **LED FIBER 2**.
- 8. 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. Remove the plug in the socket marked **LED FIBER 2**.
- 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.
- 6. Replace the plastic caps on the 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 (L1320002) 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.

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.

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.

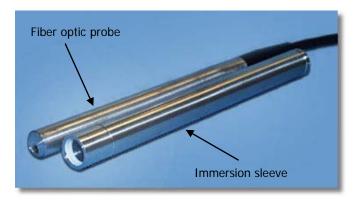


Figure 29 Fiber optic probe with the immersion sleeve detached

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.



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 92. For step-by-step instructions for calibrating the spectrometer, see *Calibration and Performance Verification SOPs* on page 94.

SOP-1: Turning the Instrument On

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

- ON

O-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, any power socket switches and then switch the PSU on again.

7. Start the Spectrum software.

On the Windows start menu select Programs/PerkinElmer Applications/Spectrum. When you start the software the instrument will prepare for use by turning on the laser and cooling the detector to its operating temperature (usually -50 °C).

SOP-2: Turning the Instrument Off

NOTE: We recommend that you always leave the spectrometer module switched on, but switch the PC off overnight if required.

- 1. Shut down Spectrum software by selecting **Exit** from the File menu to return to the Windows operating environment.
- 2. Switch off the spectrometer by pressing the power switch on the PSU to OFF:

- ON O - OFF

SOP-3: Setting the Limits of the Motorized Stage

You may need to adjust the height of the stage, for example, when you want to measure thicker samples. You should adjust the stage height using the longest objective mounted in the nosepiece.

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

CAUTION

- 1. Remove the objective nosepiece from the microscope.
- 2. Place your sample on the stage.
- 3. Using the joystick, move the stage to the highest position.
- 4. Using an allen key, loosen the hexagonal fixing screw on the side of the microscope (Figure 31).

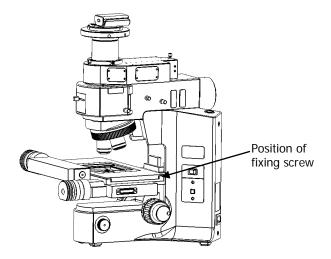


Figure 31 Adjusting the height of the motorized stage

- 5. Manually move the stage down so that you can replace the objective nosepiece.
- 6. Replace the objective nosepiece, and then select the longest objective.
- 7. Carefully, move the stage up manually until it is at the highest position possible without the sample touching the objective.

Be very careful not to touch the objective.

CAUTION

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). Set the highest position of the stage to approximately 2 mm below the objective.

- 8. Re-secure the hexagonal fixing screw to fix the height of the stage.
- 9. Ensure the instrument is switched on by following SOP-1.
- 10. Open the Scan and Instrument Setup dialog, and select the Set-up tab.
- 11. Click Stage Control.
- 12. Click **Reset Stage**, and then follow the prompts on-screen.

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

SOP-4: Positioning the Sample on the Microscope

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

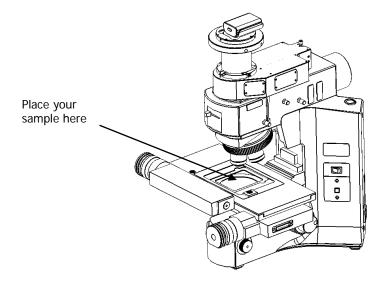


Figure 32 Positioning the sample under the objective lens

- 3. Select the 50x objective lens on the microscope.
- 4. Move the sample so that it is approximately lined up below the objective lens.
- 5. Select the Bright Field (BF) switch position on the Olympus illuminator module.
- 6. Switch on the illumination source.
- 7. Open the Scan and Instrument Setup dialog, and on the Hardware tab select **Microscope 50x** from the Beam path drop-down list.
- Focus on the sample using the video camera.
 Use Video Preview on the Scan and Instrument Setup dialog. Take care not to crush the sample.
- 9. Use the stage to accurately position the sample in the x and y directions.
- 10. 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.

- 11. On the Hardware tab select the appropriate objective lens from the Beam path dropdown list.
- 12. Focus the sample and again move the sample in x and y directions. The crosshair indicates the spot where analysis takes place.
- 13. When the sample is correctly positioned, switch off the illumination source.

14. Select the Dark Field (DF) switch position on the Olympus illuminator module.

You are now ready to acquire data. For more information see SOP-6 and SOP-7.

Remember, there is no "best" objective. High-magnification objective lenses give the most efficient Raman collection from thin samples, like 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 lights and sunlight will contaminate your spectra. Take care not to misinterpret room lights or sunlight features as Raman features.

SOP-5: Sample Positioning using a Motorized Sample Stage

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



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.

You can use the **Spectral Preview** button in the Scan and Instrument Setup dialog to help you position the sample for optimum spectral collection efficiency.

Sample positioning and alignment in preview mode allows you to position and focus on a specific area and to collect spectral data from that location. It can also be used to enhance the signal-to-noise ratio of spectral data. The data displayed on screen during a spectral preview cannot be saved. To save data you must collect spectra (SOP-7).

- 1. Follow SOP-1 to turn the instrument on.
- 2. Load the appropriate sample holder onto the sample stage.
- Select **Setup** from the Instrument menu in Spectrum.The Scan and Instrument Setup dialog is displayed.
- 4. Click Spectral Preview.

The RamanMicro 200 will start collecting data in the Spectral Preview window.

- 5. Use the joystick to move the stage, or click **Stage Control** and then use the appropriate fields in the Sample Position section to move the stage.
 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.
- Set the Preview Scan time to 1 second and then press Enter.
 This will automatically acquire another background. You can enter a Preview Scan time in the range 0.1–10 seconds.

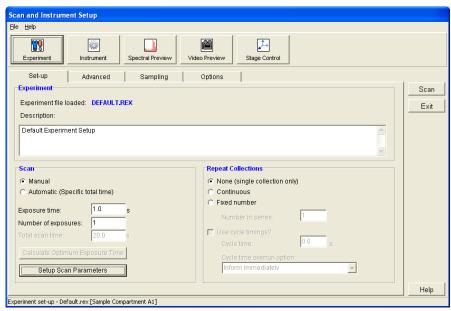
The sample may not be positioned and aligned correctly for optimized data collection. The spectral data can be optimized by manipulation of the sampling accessory motors.

You can now collect and save spectral data.

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. Ensure that the instrument is switched on by following SOP-1.
- 2. Start the Spectrum software and then select **Instrument** from the Setup menu. The Scan and Instrument Setup dialog is displayed.



The Scan and Instrument Setup dialog has a number of pages that enable you to setup and save the experimental parameters, choose the beam path and align the samples.

3. On the Experiment page, select the Set-up tab and then set the **Scan time** and **Number of scans**.

Typical values for Scan time and Number of scans are 5 seconds and 5 scans, respectively. Spectra may be optimized by increasing or decreasing the Scan time and Number of scans. To obtain a good signal-to-noise ratio, you should co-add a number of spectra. This can be carried out by choosing a suitable number of scans.

4. On the Advanced tab, in the Background Handling section, we recommend that you select **#Backgrounds=#Scans**. This sets the number of background scans to the same value as the number of sample scans.

You may also choose one of the Renew Background options to define when a background will be taken. For extended kinetic experiments, for example, you may find it useful for the background to be renewed during the course of your experiment.

- 5. On the Advanced tab, in the Spectral Information section, set the required values.
- 6. On the Sampling tab, select the appropriate options.
- 7. On the Hardware tab, select the appropriate objective lens.
- 8. If you wish these parameters to be stored as a new set of experimental setup default parameters, select **Save As** from the File menu.

Experimental parameters are stored as *.REX files. This is the "Raman Experiment" file. If you would like to save this set of parameters as the default set, that loads when the software is opened, then save your experiment settings as Default.rex.

If you select **Save** from the File menu, the current experimental parameters are stored as the default parameters in the software.

NOTE: The Spectrum software automatically saves to the currently loaded .REX file, without prompting, any configuration changes made that affect the safety of the instrument, such as the beampath selection. You should be aware of this when creating your own .REX file and, as soon as you have saved the configuration you want to be retained, you should reload DEFAULT.REX (or some other .REX file), using the **Load Experiment File** option from the File menu.

NOTE: In Spectrum ES, the File menu options **Save** and **Save As** are not available to users logged on as an Analysts. The role of an Analyst is to use .REX files prepared by Administrators.

Table 3 Recommended experimental setup

Parameter	Recommended Set Up
Scan time (Experiment page/Set-up tab)	5 seconds (sample dependant)
Number of scans (Experiment page/Set-up tab)	5 (sample dependant)
Spectral Information Range (Experiment page/Advanced tab)	140-3200 cm ⁻¹
Spectral Information Data Interval (Experiment page/Advanced tab)	2 cm ⁻¹ (default)
Background Handling - Number of Background Scans (Experiment page/Advanced tab)	5 (the recommendation is that this should be same as Number of scans , and a minimum of 1)
Background Handling - Renew background at start of each sample (Experiment page/Advanced tab)	Yes
Sampling Area (Experiment page/Sampling tab)	Select the number and style of sampling points
Sampling Type (Experiment page/Sampling tab)	If applicable, select the spacing and number of points
Accessory Configuration – Sample accessory and Sample holder (Instrument page/Hardware tab)	Select the type Sample accessory and Sample holder
Accessory Configuration - Beam path (Instrument page/Hardware tab)	Designated sample output
Video Preview (Video Preview page)	You can set the sample position using the cross hair alignment.
Spectral Preview (Spectral Preview page)	You can monitor the spectrum of your sample.

SOP-7: Defining Scan Parameters and Starting Data Collection

NOTE: If you are using a Triggered Fiber Optic Probe, refer to the *Raman Triggered Fiber Optic Probe* leaflet (L1321887) supplied with the probe for configuration and usage details.

- 1. Ensure the instrument is switched on, if not follow SOP-1.
- 2. Setup the experimental parameters by following SOP-3.
- If appropriate, position your sample.
 For more information see SOP-4 or SOP-5.
- 4. On the Scan and Instrument Setup dialog, click **Scan**.

NOTE: The **Scan** button is not available if you are using a Triggered Fiber Optic Probe. To define your probe scan parameters, click on the **Setup Scan Parameters** button, on the Set-up tab, to go to the Scan Parameters dialog.

The Scan Parameters dialog is displayed.

This dialog displays a summary of the experimental configuration. It also allows you to add a comment; specify a file prefix to be used on files containing spectrum data; and specify the folder in which the scan data will be stored.

NOTE: If you choose to save spectra to a folder other than the default one specified for your instrument, the data will not be displayed automatically. To view the spectrum you will need to open the data file manually, using the **Open** option from the File menu, after the scan has completed.

5. Click Scan.

NOTE: If you have come to this screen via the **Setup Scan Parameters** button on the Scan and Instrument Setup screen Experiment page, the **Scan** button is replaced with an **OK** button, which you should use to save your scan parameters.

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 within the message with, for example, *Collecting Background x of y*, where *x* is the current scan and *y* is the total number of backgrounds. You can enter the number of backgrounds on the Scan and Instrument Setup dialog (SOP-6). When the collection of backgrounds is complete, the software will automatically begin collecting spectra. The *Collecting data x of y*, progress bar is displayed, where *x* is the current scan and *y* is the total number of scans.

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 (L1321887).

SOP-8: Selecting the Fiber Optic Probe

- 1. Ensure the instrument is switched on, if not follow SOP-1.
- 2. Select **Setup** from the Instrument menu in Spectrum. The Scan and Instrument Setup dialog is displayed.
- 3. Click
- 4. In the Accessory Configuration section, select **Fiber Optic Probe** from the Beam Path drop-down list.
- 5. To setup an experiment and collect data using the FOP please refer SOP-6 and SOP-7.

The probe can now be positioned so as to achieve the best spectrum. Laser light will be emitted from the fiber optic during data collection and when the software is running in Spectral Preview mode.

SOP-9: Sample Positioning when using a Fiber Optic Probe

You can use the Spectral Preview button to position your sample for optimum spectral collection efficiency. Sample positioning and alignment in preview mode allows you to position and focus on a specific area and to collect spectral data from that location. It can also be used to enhance the signal-to-noise ratio of spectral data. The data that is displayed on screen during a spectral preview cannot be saved.

- 1. Ensure that the instrument is switched on. If not, follow SOP-1.
- 2. Select **Setup** from the Instrument menu in Spectrum. The Scan and Instrument Setup dialog is displayed.
- 3. Click Spectral Preview.

Real time spectral acquisition will commence and spectra are displayed in the spectral preview tab.

4. Set the **Preview Scan time** to 1 second, and then press **Enter**.

This will automatically acquire another background. You can enter a Preview Scan time in the range 0.1–10 seconds.

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.

5. Use the Pause and Play buttons to examine the spectrum in the Spectral Preview panel. When you click Play the preview acquisition resumes.

You can now collect and save spectral data.

Calibration and Performance Verification SOPs

Several different automated calibration tests are included in the software, these are:

- 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 specially doped 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 validation 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/validation 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-10: 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.

On completion of the wavelength calibration verification, a calibration verification report will provide details on the outcome of the verification procedure.

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

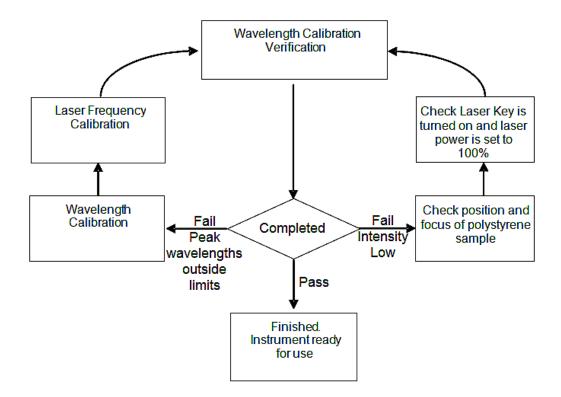


Figure 33 System verification / calibration flow diagram

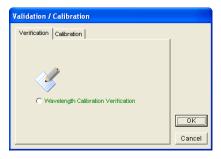
If wavelength calibration verification fails and there is an error message stating that peak wavelengths are outside limits, then wavelength and laser frequency calibrations should be performed. When these calibrations have finished, repeat the wavelength verification to ensure that the instrument has 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. 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.

SOP-11: Wavelength Calibration Verification of the RamanMicro 200

NOTE: Wavelength Calibration Verification can be carried out using any Beam path. In this example, the Microscope 50x Beam path is used.

- 1. Ensure the instrument is switched on by following SOP-1.
- 2. Select the 50x objective lens on the microscope.
- 3. Open the Scan and Instrument Setup dialog.
- 4. On the Hardware tab select **Microscope 50x** from the Beam path drop-down list.
- 5. Click Spectral Preview.
- 6. Focus on a sample of polystyrene (L1181228).
- 7. When the polystyrene sample is optimally focused, close the Scan and Instrument Setup dialog by clicking **Exit**.
- 8. Ensure that Dark Field (DF) is selected as the switch position on the Olympus illuminator module.
- 9. Cover the microscope with dark cloth to minimize stray light from interfering with the calibration and ensure that caps are placed on any external fiber optic probes or accessories.
- 10. Select **Validate** and then **Instrument** from the Instrument menu in Spectrum. The Validation / Calibration dialog is displayed.



11. Select the Verification tab.

12. Select Wavelength Calibration Verification, and then click OK.

The wavelength calibration verification routine begins. This will take a few minutes. After completing the wavelength calibration verification routine, an audit log is generated.

13. 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 33.

Further Details on the wavelength calibration verification are provided as a spectral file (*.sp), which is saved in *C:\pel_data\config\Raman\Calibration Files\Logs* folder.

SOP-12: Wavelength Calibration of the RamanMicro 200

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

- 1. Ensure 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 34 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.

NOTE: During the wavelength calibration, make sure that no light is going into the system.

3. Select **Validate** and then **Instrument** from the Instrument menu in Spectrum. The Validation / Calibration dialog is displayed.



- 4. Select the Calibration tab.
- Select Wavelength (X-axis), and then click OK.
 The instrument will now perform the wavelength calibration routine. This will take some time.

After completing the wavelength calibration routine, a report is generated on-screen which describes the result of the wavelength calibration. The instrument is now wavelength calibrated.

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

SOP-13: Laser Frequency Calibration of the RamanMicro 200

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

- 1. Ensure the RamanMicro 200 is switched on by following SOP-1.
- 2. Select the 50x objective lens on the microscope.
- 3. Open the Scan and Instrument Setup dialog.
- 4. On the Hardware tab select **Microscope 50x** from the Beam path drop-down list.
- 5. Ensure that the laser power is set to 100%.
- Click Spectral Preview in the Scan and Instrument Setup dialog, and focus on a sample of polystyrene (L1181228).
 When the polystyrene sample is optimally focused, close the Scan and Instrument Setup dialog by clicking Exit.
- 7. Ensure that Dark Field (DF) is selected as the switch position on the Olympus illuminator module.
- 8. Select **Validate** and then **Instrument** from the Instrument menu in Spectrum. The Validation / Calibration dialog is displayed.
- 9. Select the Calibration tab.

10. Select Laser Frequency, and then click OK.

The instrument will now perform the laser frequency calibration routine. This will take a few minutes.

After completing the laser frequency calibration routine, a report is generated on-screen that gives details on the outcomes of the laser frequency calibration.

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

The instrument in now laser frequency calibrated.

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

SOP-14: Intensity Calibration of the RamanMicro 200

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.

Intensity calibration should typically be performed on an annual basis, that is during an annual instrument service, but also whenever the instrument is moved.

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.

- 1. Ensure the RamanMicro 200 is switched on, if not follow SOP-1.
- 2. Select the 50x objective lens on the microscope.
- 3. Ensure that sample NIST 2241 is on the test plate, with the dull surface facing upwards.
- 4. Open the Scan and Instrument Setup dialog, and on the Hardware tab select **Microscope 50x** from the Beam path drop-down list.
- 5. Ensure that the laser power is set to 100%.
- Click Spectral Preview and then focus on the NIST standard.
 When the NIST standard is optimally focused, close the dialog by clicking Exit.
- 7. Select the Dark Field (DF) switch position on the Olympus illuminator module.
- 8. Select **Validate** and then **Instrument** from the Instrument menu in Spectrum. The Validation / Calibration dialog is displayed.
- 9. Select the Calibration tab.
- 10. Select Intensity Calibration Y-axis, and then click **OK**.

The instrument will now perform the intensity calibration routine.

After completing the intensity calibration routine, a report is generated on-screen, which gives details of the outcome of the intensity calibration.

In the unlikely event that the intensity calibration routine should fail, please ensure the NIST sample is correctly positioned, and that the laser key switch is set to on (ARM). If this is the case shut down the PC, switch the instrument off, restart the PC and switch the instrument back on and repeat the calibration.

- 11. Repeat steps 2–10 for each objective lens.
- 12. Remove the intensity correction standard and store safely.

Any data acquired will now be intensity corrected.

Remember that if you have a fiber optic probe, you must intensity calibrate the fiber optic probe as well as the microscope objective lenses. Ensure that the fiber optic probe has a lens cap in place during intensity calibration of the objectives.

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.
- The intensity calibration should only be performed on a yearly basis or if the environment (temperature and / or humidity) changes significantly, or if 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, 20x, 50x and 100x long working distance objective lenses

Sampling Accessories

Part Number	Description
L1323514	Quartz microscope slide, $25 \times 75 \times 1 \text{ mm}$
L1860335	Tablet holder kit for motorized stage
L1860336	Powder holder kit for motorized stage
L1321675	Multiwell plate holder for motorized stage
L1321676	Multi microscope slide holder Holds 4 standard (25 \times 75 \times 1 mm) microscope slides
L1321677	Universal sample holder Holds standard (25 \times 75 \times 1 mm) microscope slides, 50 \times 75 mm glass slides, and Petri dishes up to 60 mm

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) – for use with L1320030 and L1320031
L1320325	Raman probe adjustable spacer kit – for use with L1320030 and L1320031
L1320070	Short immersion sleeve for TFOP – for use with L1320030 and L1320031
L1320071	Ultra-long working distance lens adaptor for FOP

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

<u>Maintenance and</u> <u>Troubleshooting</u>

Maintenance

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

Cleaning the Spectrometer and Power Supply Units



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 Engineer.



Do not directly clean electrical contacts.



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. Only use a soft, dry cloth to clean the spectrometer 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 35 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 105). There are no other user-serviceable parts in the instrument. If you require an instrument service, please contact PerkinElmer. Only PerkinElmer Service Engineers should attempt to service the spectrometer.



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.

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 (L1321887).

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.
- 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).

then switch it on again.

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

Description

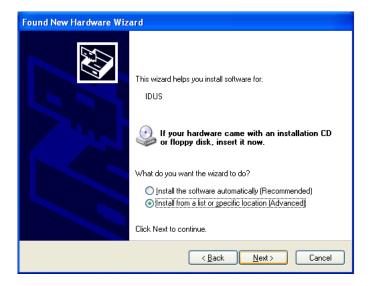
Problem:	During the installation procedure the USB devices are not recognized.
Description	This may mean that there is not enough power on the USB sockets on your PC.
Solution	Ensure that your PC has USB 2.0 sockets. If you are not sure, use a USB 2.0 power hub.
Problem:	The coftware installation stone during the Philips VCheck ston
Problem:	The software installation stops during the Philips VCheck step.
Description	In the VCheck dialog that is displayed while the software performs a number of checks on your system, the Progress bar halts before the checks have been completed.
Solution	Click Cancel and then repeat the installation using the procedure described in <i>Installing Spectrum Software</i> on page 54 to install the alignment camera software.
Problem:	You are prompted for the camera drivers more than once.

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

Solution When the Welcome to the Found New Hardware Wizard is displayed:

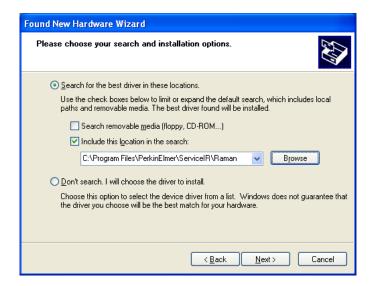


Select No, not this time and then click Next.
 The Found New Hardware Wizard page is displayed.



NOTE: The Found New Hardware Wizard page may inform you that it is helping you install software for **USB Device**. In this case continue with the installation in the same way as for **IDUS**.

2. Select **Install from a list or specific location (Advanced)** and then click **Next**. The Please choose your search and installation options page is displayed.

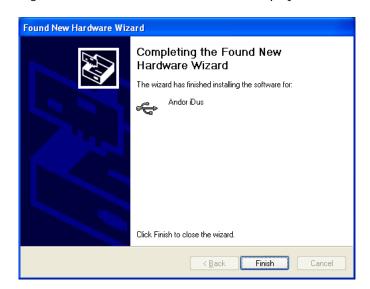


3. Select **Include this location in the search**, click **Browse**, and then navigate to C:\Program Files\PerkinElmer\ServiceIR\Raman.

These options may be displayed automatically as you work through the wizard.

4. Click Next.

The Completing the Found New Hardware Wizard is displayed.



5. Click Finish.

The Found New Hardware Wizard closes.

Problem: A motorized sample accessory does not go to the correct position when asked.

Description This means that for some reason the motorized stage is jammed. This typically happens if the sample holder on the stage is different to that selected on the Sampling tab in the Scan and Instrument Setup dialog.

Solution 1 Go to the Sampling tab and ensure that the selected sample holder is correct. If it is not then select the appropriate sample holder.

Solution 2 Go to the Stage Control section and then click **Reset Stage**. The motorized accessory will now reset itself.

Solution 3 If solution 2 is not successful, go to the Spectral Preview tab in the Scan and

Instrument Setup dialog, for all motor directions/positions in turn type 1, then press enter, once all positions have been set to 1 and all accessory movements are complete, disconnect the accessory from its electrical connector in the sample compartment (there is no need to physically remove the accessory). Perform Solution 2 again, and wait for the software routine to complete, now reconnect the accessory and perform Solution 2 yet again. The accessory should be in the correct position for the selected sample holder.

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: Scan and Instrument Setup dialog stops responding.

Description When you are using the Scan and Instrument Setup dialog, 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. Open **Task Manager** and then click the **Processes** tab. In the list under Image Name look for *Raman.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**. 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.

Problem: You get any of the following messages 'An unexpected error has

occurred', 'EAccess Violation' or 'Divide by Zero'

Description While working in the software, a dialog is displayed with one of these

messages. The software may appear to operate correctly but you must

perform the following solution.

Solution Close and open the Spectrum software.

Problem: There is no Video Preview tab in the Scan and Instrument Setup

dialog.

Description This means that the software searched for a video camera on the system

and could not locate one.

Solution 1 Is the accessory with the video camera loaded into the sample compartment

and USB cable connected? If not, connect this cable and restart the

software.

Solution 2 Is the USB cable from the instrument to the PC properly attached? If not

attach the USB cable correctly at both ends and restart the software.

Problem: During system initialization the laser does not stabilize. Description The laser initialization form displayed during system initialization runs for over 60 seconds. During this time the laser should have stabilized. Solution Ensure that the laser key on the front panel of the spectrometer is set to the ARM position. Problem: Detector cannot achieve operational temperature. Description During system initialization a message box stating, 'Detector temperature could not be achieved' is displayed. Solution Close and open the software. Problem: Need to upgrade to a new version of Spectrum software. Description You must uninstall the current version of Spectrum before installing a new version. Solution To uninstall Spectrum software, open the **Control Panel** and then select Add or Remove Programs. Then remove the Philips ToUcom Pro Camera, the Raman Instrument and then Spectrum Software (in that order). You should also delete files C:\pel_apps\, C:\pel_data, C:\Documents and Settings\All Users\Application Data\PerkinElmer\SecuritySystem, C:\Windows\pel_apps.ini and C:\Windows\pe_sopb.ini using Windows Explorer. To install the new version of Spectrum software follow the instructions in Installing Spectrum Software on page 54.

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)

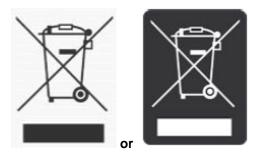
Customer Care Canada: 800-561-4646

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.