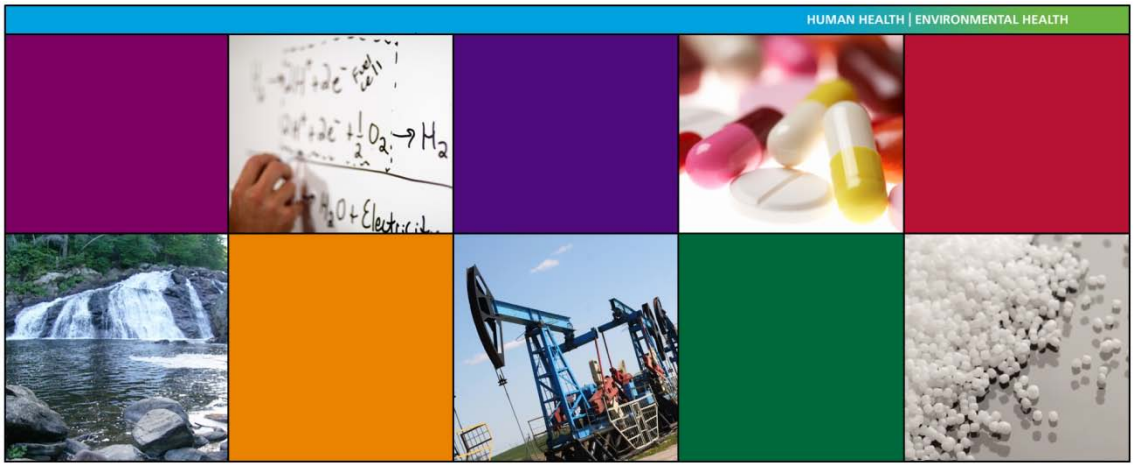
MOLECULAR SPECTROSCOPY

RamanStation 400 Series



Getting Started Guide

PerkinElmer®

Release History

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Introduction

About This Manual

This Getting Started Guide contains all of the information that you will need to set up your RamanStation 400 Series Raman Spectrometer, and then start collecting spectra.

The Getting Started Guide is divided into the following chapters:

Introduction

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

Safety Practices

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

Raman History, Theory and Instrumentation

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

Preparing your Facility

This chapter shows you how to position your RamanStation 400 Series instrument, and what utilities are required; for example, electricity and temperature control.

Installing your RamanStation 400 Series

This chapter contains step-by-step instructions about how to unpack and assemble your RamanStation 400 Series instrument and information on installing your instrument in the software.

Introduction to the RamanStation 400 Series

This chapter describes how to use the instrument with Spectrum software.

Fiber Optic Probe

This chapter contains an introduction to the fiber optic probe.

Using the RamanStation 400

This chapter contains a number of Standard Operating Procedures (SOPs). These are a series of procedures that describe how to perform certain tasks; for example, how to collect a spectrum, or how to calibrate the spectrometer.

Maintenance and Troubleshooting

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

Conventions Used in this Manual

Normal text is used to provide information and instructions.

Bold text refers to text that is displayed on the screen.

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

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

The term RamanStation 400 Series means the RamanStation 400 and the RamanStation 400F Raman Spectrometers.

Notes, Cautions and Warnings

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

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

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

WARNING

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

D

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

DK

Warning (Advarsel)
*Betyder, at brugeren kan blive **kvæstet**, hvis anvisningen ikke overholdes.*

E

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

F

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

I

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

NL

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

P

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

Safety Practices

Overview

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

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

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

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General Operating Conditions

WARNING

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

The RamanStation 400 Series spectrometers have been designed and tested in accordance with PerkinElmer specifications and in accordance with the safety requirements of the International Electrotechnical Commission (IEC). The RamanStation 400 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 RamanStation 400 Series spectrometers indoors and under the following conditions:

Temperature	15 °C to 30 °C
Relative Humidity	80% maximum (non-condensing)

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

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

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

Whenever it is likely that the RamanStation 400 Series instrument is unsafe, make it inoperative. The RamanStation 400 Series may be unsafe if it:

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

Fire Safety

- Ensure that there is at least 15 cm (6”) between the rear of the instrument and the nearest wall.
- Ensure that any power supply unit (PSU) vents or fan do not become obstructed.

Electrical Safety

The spectrometer comes assembled within a protective housing to prevent exposure to electrical hazards. There are also a number of exterior service panels that should only be removed by PerkinElmer Service Engineers.

WARNING

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

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

WARNING

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.

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 RamanStation 400 Series instruments and the stage control box (for motorized XYZ stage only) are each powered by an external PSU. The PSUs used with the spectrometer (Table 1) and stage controller box can adjust automatically to a variety of AC power sources.

Table 1 PSU input voltages and mains fluctuations information

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

NOTE: If you have a deep depletion CCD installed in your RamanStation 400 Series, the instrument will be powered by two external PSUs.

WARNING

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

Connect the PSUs to a three-wire (live, neutral and ground/earth) socket. The ground must be a wire not carrying current that is connected to the earth ground at the main distribution box. The power points (sockets) for the instrument PSUs should be easily accessible for when the instrument is serviced.

WARNING

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

Power Cords (Between PSU and Power Outlet Socket)

The power cords supplied to connect the instrument PSUs to the power points (sockets) should be appropriate for the country listed as the shipping destination.

WARNING

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

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

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

Fuses

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

EMC Compliance

EC directive

The RamanStation 400 Series instruments have been designed and tested to meet the requirements of the EC directive 2004/108/EC. The RamanStation 400 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 RamanStation 400 Series complies with the requirements of IEC 60825-1:2007 and 21CFR 1040.10 FDA performance standards for laser products, except for deviations pursuant to Laser Notice No. 50, dated 26 July 2001.

WARNING

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

The RamanStation 400 is classified as Class 1. The laser shutter is automatically closed if the sample compartment cover is opened. The RamanStation 400F is Class 3B because when the fiber optic probe (FOP) is attached it provides a focused beam of laser radiation at 785 nm which is classified as a Class 3B laser source.

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

NOTE: If the supplied fiber port cover assembly (L1320263) is attached so that it encloses the fiber probe couplers on the rear panel, the instrument may be regarded as a Class 1 laser product. For more information see *Attaching the Fiber Port Cover Assembly* on page 46.

WARNING

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

Class 3B Safety Precautions

When using the fiber optic probe you should follow Class 3B laser safety precautions such as wearing laser safety goggles and using door interlocks. For information on these precautions please refer to an appropriately trained laser safety officer within your own facility. Laser safety is very important. Inappropriate use of laser-emitting devices may result in permanent injury. In the United States the precautions are described in the ANSI Z136.1-2000 Standard. For information on the ANSI Z136 standard please consult your laser safety officer and/or consult http://www.z136.org.

Outside the United States the guidelines are described in PD IEC TR 60825-14 (Safety of laser products–Part 14: A user's guide). You should also be familiar with the guidelines published by the national authorities in your country.

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

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

1. Class 3B lasers may emit visible and/or invisible radiation up to a maximum of 0.5 W continuous wave (CW is longer than 0.25 s). They present a hazard to the eye if the direct beam or spectral reflections are viewed without appropriate protection. You must only operate Class 3B lasers in a designated area.

2. You must fit each Class 3B device with a captive key control switch, such that the key cannot be removed from the lock except in the OFF position. The key should be removed from the laser when it is not in use and kept in a safe place in the custody of a nominated person. Keys must never be issued to unauthorized persons.

3. Each Class 3B device should be assessed by a suitably trained laser safety officer to determine the accessible radiation emitted by the device. The laser safety officer should consider laser power, wavelength and beam divergence when determining if the Class 3B device's remote interlock connector should be connected to an interlock switch at the entrance. For certain laser systems, the laser safety officer may require the shutter to be interlocked with the laboratory entrance in order to prevent inadvertent exposure of persons entering to very hazardous laser radiation.

4. Rooms in which Class 3B devices are used should have notices affixed to the doors so that they conform to the requirements of appropriate Laser Safety Regulations.

If you have a fiber optic probe then please refer to *Attaching the External Interlock* and *Calculation of Nominal Hazard Zones for use of Fiber Optic Probe* for information on safe operating distances when using the fiber optic probe.

General Laser Safety Issues

Laser Keyswitch

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

Laser Warning Labels

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

Fiber Optic Probe Safety

NOTE: This section is only applicable to RamanStation 400F 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 1) 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/cover) to avoid unnecessary exposure. The laser should also be turned off and the probe cap replaced when the system is not in use. **AVOID DIRECT EXPOSURE TO THE BEAM.**

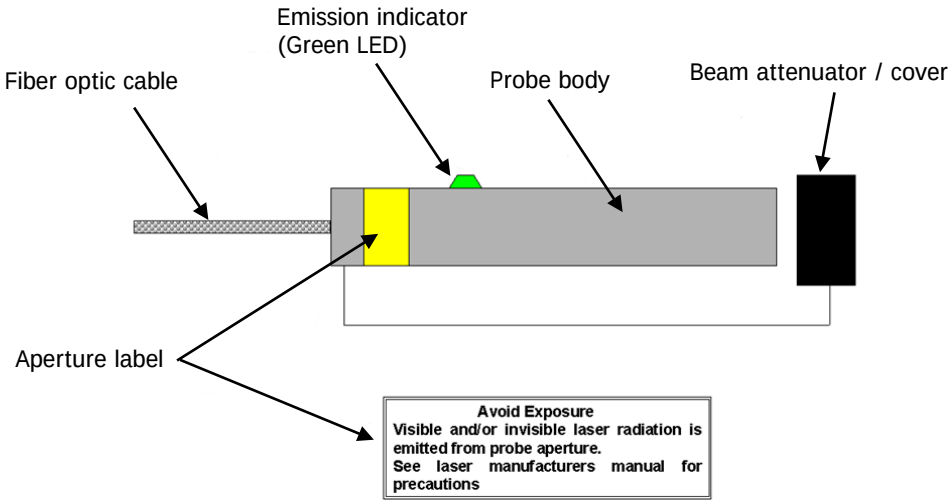


Figure 1 Schematic of the fiber optic probe (not to scale)

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

- Use an interlock switch on the door to the laboratory and/or the reaction vessel when you are using a fiber optic probe.
- 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.
- 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.
- 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 (L1323518).

Triggered Fiber Optic Probe Safety

When attached to the RamanStation 400F, 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 (L1323518).
- Do not use the TFOP if it shows any sign of damage.
- If repair or servicing is needed, call your PerkinElmer Service Representative for advice.

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

Calculation of Nominal Hazard Zones for use of Fiber Optic Probe

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

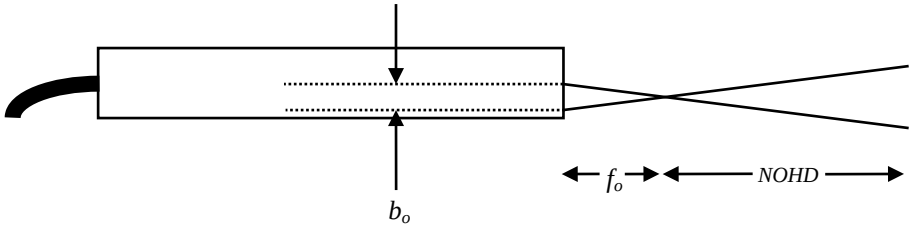


Figure 2 Probe parameters used to calculate the nominal ocular hazard distance

The equation for a focused beam, as given here, is used to calculate the nominal ocular hazard distance (NOHD) for the probe:

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

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 expo sure (J cm^{-2})

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

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

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

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

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 × 10^{-2} J cm^{-2} or 1.49 × 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 (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 an interlock system associated with the sample compartment lid (Figure 3). If the door is open then the laser cannot be emitted from within the instrument without deliberate user intervention.

The interlock switches cannot be over-ridden by using simple magnets. The placing of a specifically coded magnetic actuator (MA) in the vicinity of a magnetic proximity switch (MPS) may disable the interlock and allow laser light to be emitted from the instrument. Placing a specifically coded magnetic actuator (MA) in the vicinity of this magnetic proximity switch (MPS) is not recommended under any circumstances and is considered highly dangerous, compromising the safe operation of the instrument. This type of action is deemed to be a deliberate attempt to bypass the internal instrument safety interlocks and as a consequence of this the instrument will be classified as Class 3B.

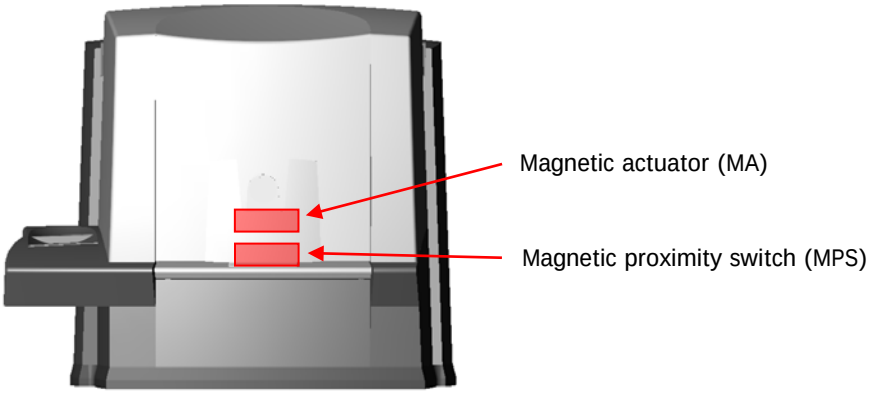


Figure 3 The RamanStation 400 Series safety interlock system, showing the proximity of the MPS and MA when the sample lid is closed. Note that the MPS and MA are located inside the instrument enclosure

WARNING

Do not tamper with the safety interlock mechanism.

Defeating the interlocks by using a specifically coded magnetic actuator (MA) in the vicinity of a magnetic proximity switch (MPS) should only be performed by a PerkinElmer Service Engineer during service/maintenance or repair of the instrument (observing Class 3B laser safety precautions at all times).

External Interlock System

NOTE: This section is only applicable to systems equipped with a fiber optic probe or RamanMicro 300 Accessory, or systems being used in a Class 3B fashion.

Fiber optic probes and the RamanMicro 300 Accessory are Class 3B laser products and have the potential to cause harm or injury to those using them or others in the vicinity of the spectrometer or associated fiber optic probe. For more information refer to *Attaching the External Interlock* on page 45.

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 RamanStation 400F

The following labels are displayed on the exterior of the instrument.

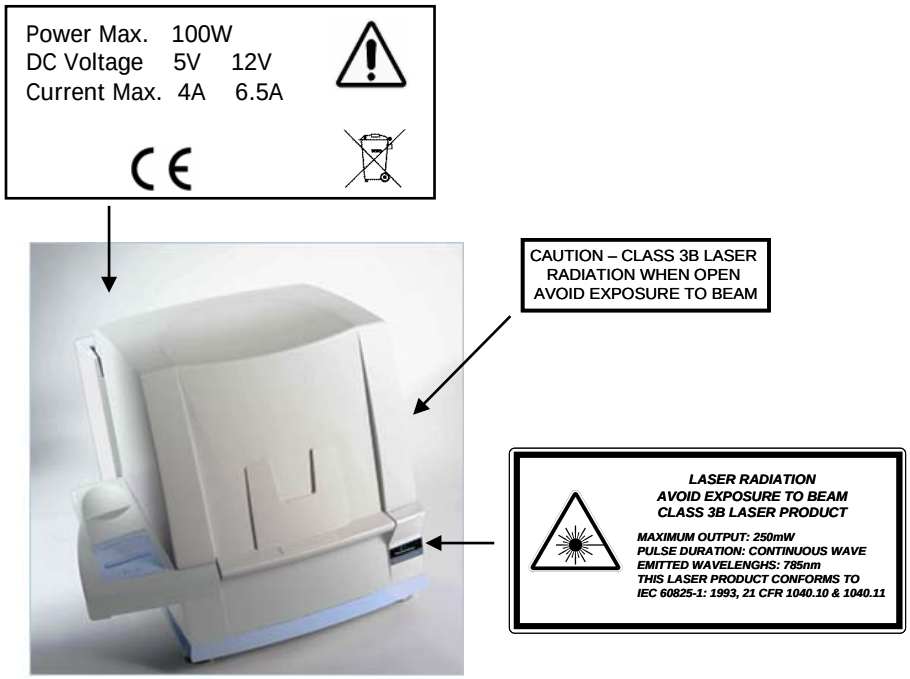


Figure 4 Labels on the RamanStation 400F spectrometer

Labels on the Exterior of the RamanStation 400

The following labels are displayed on the exterior of the instrument.

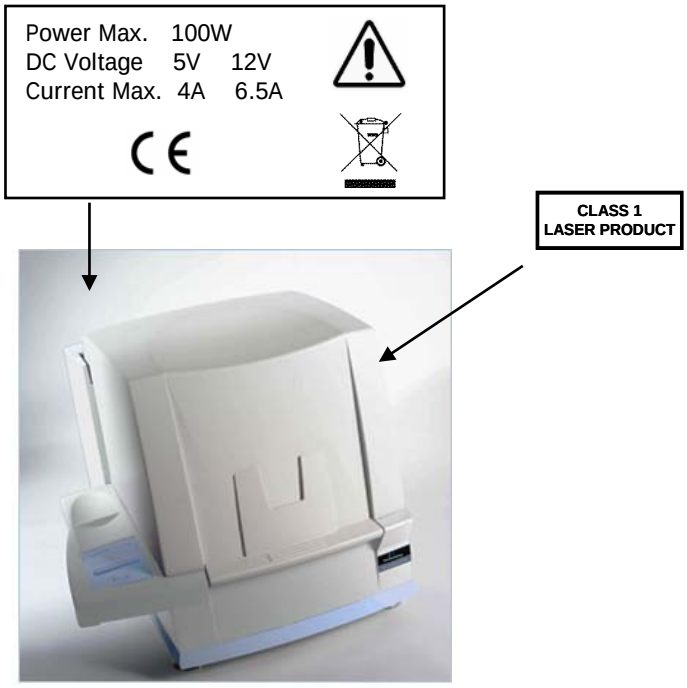


Figure 5 Labels on the RamanStation 400 spectrometer

Raman History, Theory and Instrumentation

Raman Theory

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

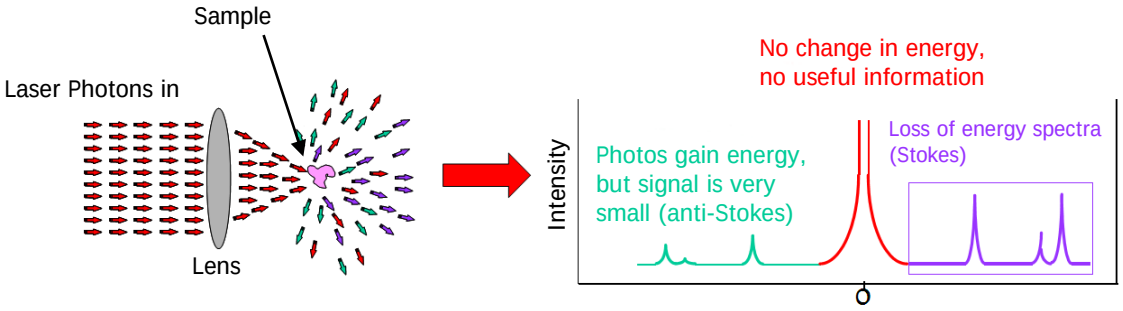


Figure 6 The Raman effect: Irradiation of sample and subsequent scatter

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

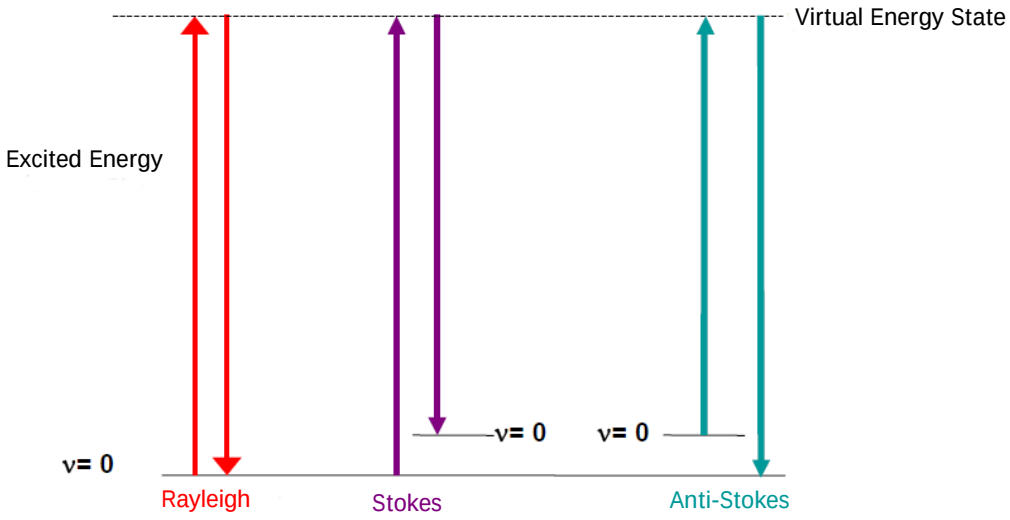


Figure 7 Rayleigh, Stokes and Anti-Stokes scattering

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

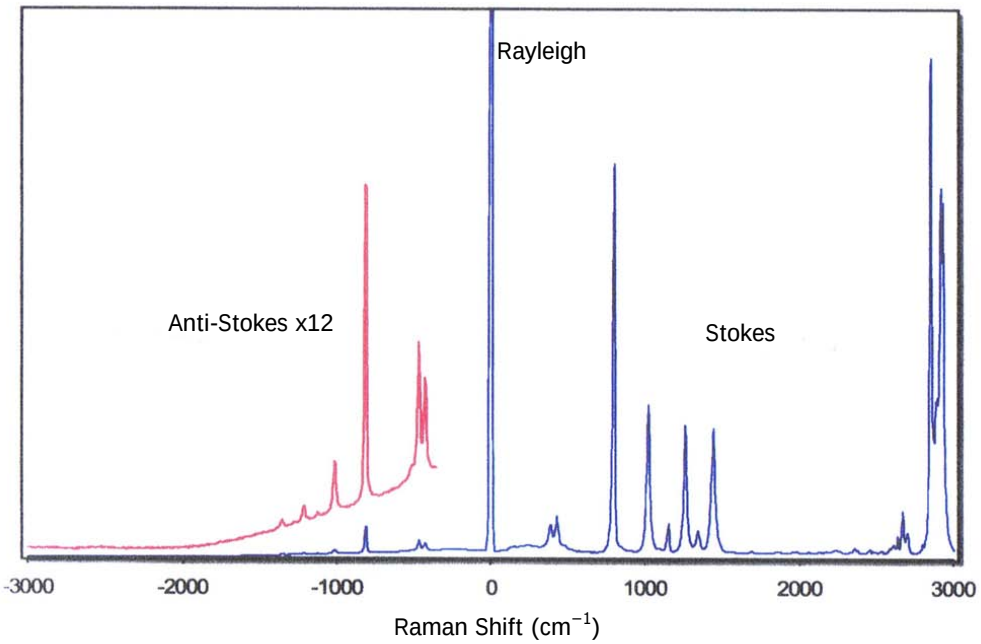


Figure 8 Raman spectrum of cyclohexane, showing Stokes, anti-Stokes and Rayleigh scattering

The RamanStation 400 Series

The RamanStation 400 Series are bench-top spectrometers with laser excitation wavelength at 785 nm. It has a sample compartment with high throughput capabilities. The RamanStation 400F can be used with a fiber optic probe or a RamanMicro 300 Accessory.

Figure 9 The RamanStation 400 Series

The instrument (Figure 9) is composed of a laser, mirrors, filters, lenses, spectrograph and a CCD detector. The laser supplies light through an objective lens onto the sample through the sample compartment snout or through the fiber optic probe. The scattered light from the sample is collected with the same objective lens and transmitted back to the spectrometer. A detailed schematic of the optical configuration is provided in Figure 11.

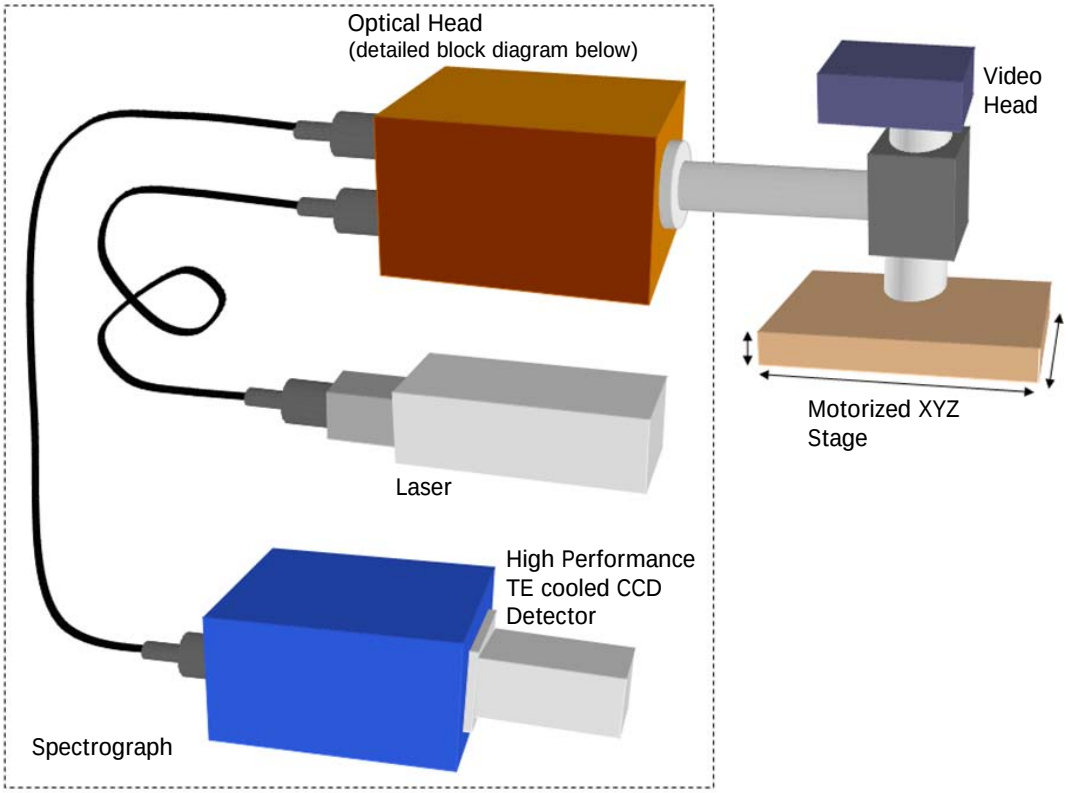


Figure 10 Schematic of the RamanStation 400 Series

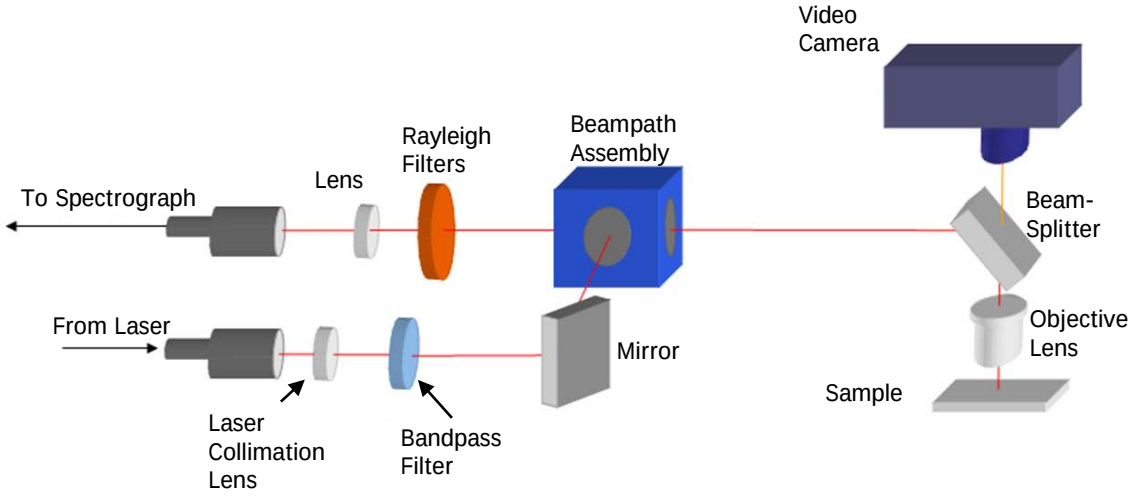


Figure 11 Schematic of the optical head optical layout

Spectrograph Detector Systems

The RamanStation 400 Series is equipped with an Echelle spectrograph.

The Echelle Spectrograph

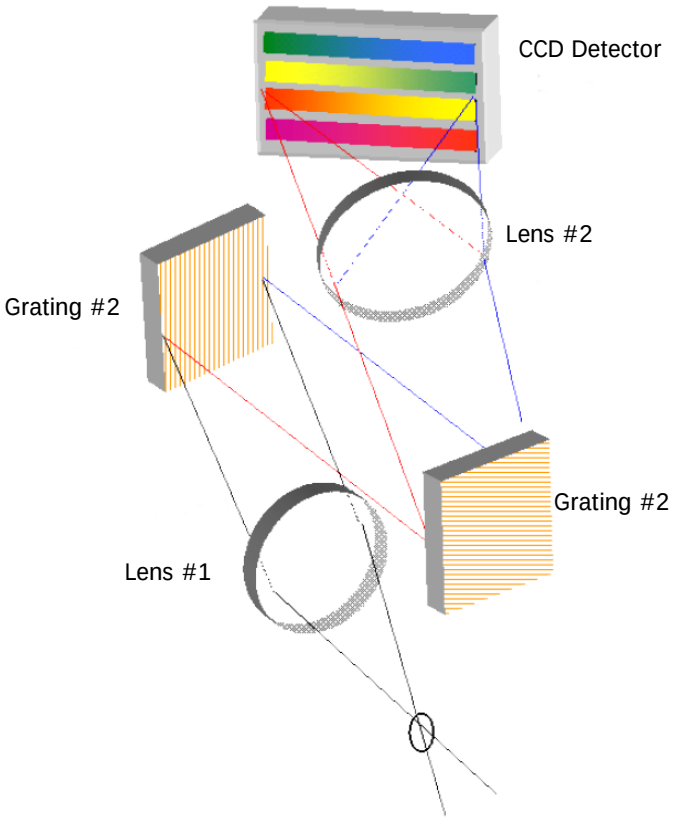


Figure 12 Schematic of the Echelle spectrograph

PerkinElmer's Echelle detector is shipped in the RamanStation 400 and RamanStation 400F spectrometers. The system contains two components; the Echelle spectrograph and a two-dimensional CCD detector (Figure 13). The system works by dispersing the light in two dimensions by using two gratings. The first grating disperses the light horizontally onto the second grating, where the light is further dispersed vertically onto the CCD detector. The light is spread over a number of strips on the CCD that are read simultaneously; therefore a full spectral range (at high resolution) can be achieved with one acquisition.

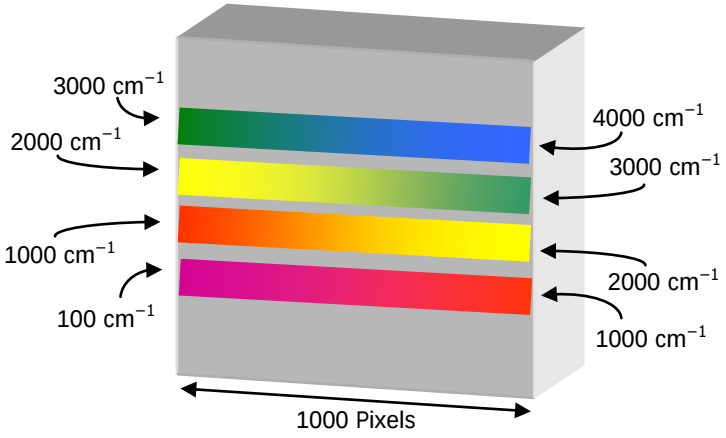


Figure 13 Strips of light on the CCD detector using the Echelle spectrograph

Range 1 + Range 2 + Range 3 + Range 4 = Complete Spectrum (all ranges acquired simultaneously).

Figure 13 shows a schematic diagram of light falling on the CCD detector. Here 4 strips of light are shown for clarity, but in practice up to 10 strips are acquired and resolution of up to 4 cm^{-1} (peak FWHM) can be achieved.

The advantages of PerkinElmer's Echelle spectrograph are:

- Full spectral range at high resolution in a single acquisition.
- It takes less than a second to acquire a high resolution spectrum with a full spectral range.
- There are no moving parts in the spectrograph, which improves instrument reliability, ruggedness and the quality of calibration.
- The absence of moving parts also means that as you acquire the spectrum, there is no change in the spectrograph's stray light performance, meaning that there are no stitch marks in the final spectrum.

No light is lost, all the light goes onto the detector, and all the strips are read out simultaneously. The concept of an Echelle detector is not unique to PerkinElmer. Echelle detectors are commonly used in applications where high resolution and wide spectral range are required. Examples include plasma spectroscopy (ICP and LIBS) and Echelle detectors find very widespread use in astronomy.

Preparing Your Facility

Specifications

Spectrometer

	RamanStation 400	RamanStation 400F
Weight	45 kg	45 kg
Height	490 mm	490 mm
Width	590 mm	590 mm
Depth	640 mm	640 mm
Laser Class	Class 1	Class 3B
Operational Temperature	15 °C – 30 °C	
Recommended Temperature	20 °C – 25 °C	
Power Supply	100–230 V AC ±10%, 50–60 Hz	

Stage Controller

	Stage Controller (for motorized XYZ stage only)
Weight	2.8 kg
Height	90 mm
Width	220 mm
Depth	184 mm
Operational Temperature	15 °C – 30 °C
Recommended Temperature	20 °C – 25 °C
Power Supply	100–240 V AC, 50–60 Hz
Primary Fuse	1.6 A T (time-lag)

Electricity supply

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

Finding a Suitable Location for the Instrument

The following conditions should be observed:

- There must be a minimum of 3 power points (1 for the spectrometer; 2 for the PC). If your system has a deep depletion CCD you will also need 1 power point for the recirculating water cooler. If your system has a motorized stage you will also require 1 power point for the stage control box.
- 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.

If the instrument is equipped with a fiber optic probe or a RamanMicro 300 Accessory, it is advisable to install the instrument in a room with no windows which meets the appropriate safety requirements for a Class 3B laser product.

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

Instrument Dimensions

The RamanStation 400 Series spectrometer weighs approximately 45 kg. The stage can accept loads up to 1.0 kg. Laboratory benching must be strong enough to support the combined weight of the instrument and samples, typically 46 kg, without warping or wobbling. The bench must be at least 640 mm (25”) in depth. There must be at least 400 mm (16”) above the instrument to accommodate the open sample compartment lid.

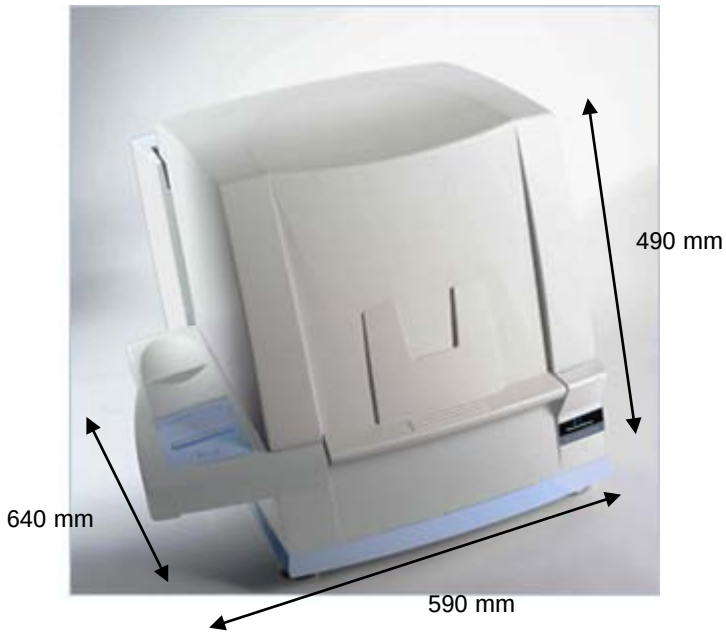


Figure 14 Dimensions of the RamanStation 400 Series

Installing Your RamanStation 400 Series

Unpacking the Instrument

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

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

Part Number	Description
–	RamanStation 400 Series Spectrometer
LX108870	Spectrum Std for Raman Software Kit Contains the Spectrum Std Software CD (LX100877), the Raman Instrument Configuration Disk (LX108869), the Raman Spectral Libraries CD (L1321877), and the Spectrum Insight Software CD (LX108872)
L1323503	Laser key (x2)
L1321802	RamanStation Mains PSU
L1320821	USB Cable
L1320264	External Interlock Override (RamanStation 400F only: L1320005/L1320009/L1320033/L1320035)
L1320263	Cover for Fiber Probe Couplers (RamanStation 400F only: L1320005/L1320009/L1320033/L1320035)
L1181228	Polystyrene Sample
L1320227	Versatile Sample Holder

If you have ordered a motorized XYZ stage, you will also receive:

L1320295 OR L1320296	XYZ Stage with standard lens snout OR XYZ Stage with Polarization Accessory Both are supplied with an XYZ 3-way controller connector cable.
L1860289	Stage Controller Box
L1865723	Joystick
L1865749	RS-232 cable
LX108897	SpectrumIMAGE Software Kit (systems with motorized stages only)

If you have a deep depletion CCD installed in your RamanStation 400 (L1320032/L1320033/ L1320034/L1320035) you will also receive:

L1323525	Deep Depletion PSU
L1323010	Recirculating Cooler
L1321674	Tygon Tubing

NOTE: Your instrument will be assembled and tested by a PerkinElmer Service Engineer. There are no user-serviceable parts in the RamanStation 400 Series. The following sections are provided for your information only.

Lifting the Instrument

The RamanStation 400 Series instrument weighs 45 kg. To avoid injury or damage to the instrument, please use proper lifting techniques.

WARNING

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

WARNING

Do not lift the RamanStation 400 Series using the instrument console located on the left hand side of the instrument.

Always lift the instrument using the positions under the instrument base as shown in Figure 15.

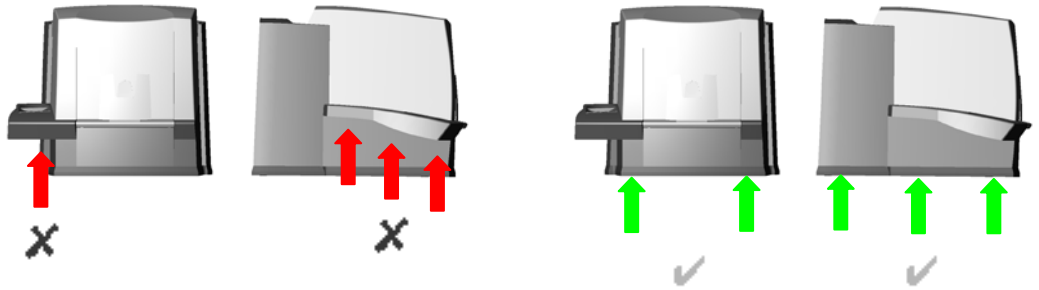


Figure 15 Appropriate lifting points on the RamanStation 400 Series

Preparing the Motorized XYZ Stage

Before you use your motorized XYZ stage, follow the procedures described below.

Removing the clamping assembly

Before you use your instrument you must remove the fixing bolt that is fitted to the stage during shipping to prevent damage to the stage.

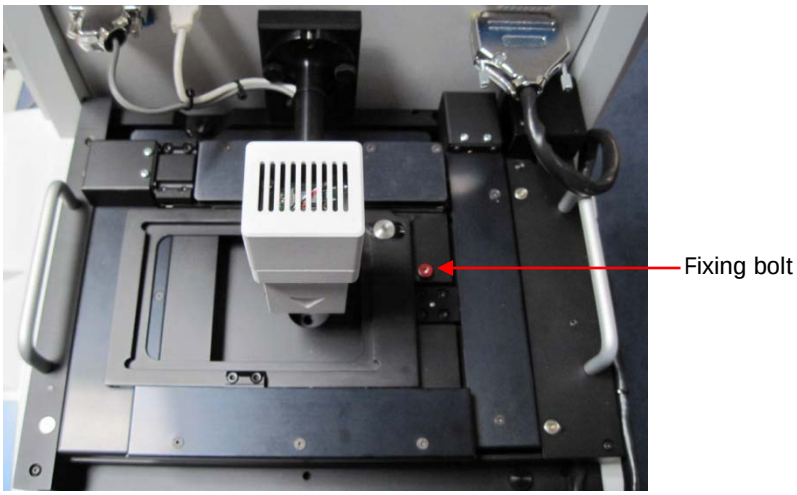


Figure 16 RamanStation 400 Series Stage Fixing Bolt

Use a 5 mm hexagonal key to loosen the bolt and retain it for future use.

Installing the motorized XYZ stage

Open the sample compartment of your RamanStation. Place the stage on the base of the sample compartment. The stage slots into two locator pins protruding from the base. The stage is then secured to the base of the sample compartment using two thumbscrews.

Connect the serial cable coming from the stage to the **Sample Accessory** port at the back of the sample compartment.

Connecting the stage controller box

To connect the stage controller box:

1. Connect the cable from the joystick to the socket labeled **JOYSTICK** on the rear panel of the stage controller box (Figure 17).

Figure 17 Rear panel of the Stage Controller Box

2. Connect the three ends of the connector cable to the sockets labeled **MOTOR X**, **MOTOR Y** and **MOTOR Z** on the rear panel of the stage controller box.

   The three ends of the connector cable are labeled X, Y and Z, as appropriate (Figure 18).

Figure 18 XYZ connector cable

3. Connect the single end of the same cable to the **Sample Accessory** port on the back of the spectrometer.

4. Connect the RS232 cable (L1865749) from the COM1 port on the PC to the socket labeled **RS232** on the rear panel of the stage controller.

5. Connect the power cable from the stage controller box to the mains supply.

   Do not switch on the stage controller box until the rest of the system has been assembled correctly.

Attaching the External Interlock Override

The RamanStation 400F has two interlock switches, an internal interlock located on the inside of the sample compartment door, and an external interlock located on the rear panel. To operate the instrument both interlocks must be satisfied; that is, the sample compartment door must be closed to collect data, even if data is being collected using the fiber optic probe.

You must attach the External Interlock Override (L1320264) into the 7 way DIN connector labeled **EXT INTERLOCK** on the rear panel of 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.

NOTE: If you have purchased a RamanStation 400, then your instrument is a Class 1 laser product and you do not need to attach an external interlock override.

Attaching the Fiber Port Cover Assembly

NOTE: This section is only applicable if you have a RamanStation 400F.

The RamanStation 400F is classified as a Class 3B laser product according to the label attached to the instrument. However if required, the RamanStation 400F can be classified as a Class 1 laser product provided the following conditions are met:

- No fiber optic probe is connected to the instrument
- The fiber optic probe cover assembly (L1320263) is attached to the instrument and completely encloses the EXC and COL couplers at the rear of the instrument. The EXC and COL screw-in caps are fully screwed into their ports before the fiber optic probe cover assembly is secured in place.

A Class 1 laser product is safe under all reasonably foreseeable conditions of use and may be used in an open laboratory environment.

To attach the fiber port cover to the rear panel, fasten the supplied screw through the cover into the hole near the COL coupler as shown in Figure 19.

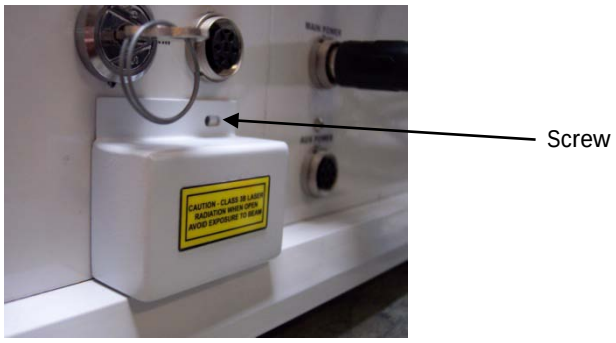


Figure 19 Attaching the fiber port cover

Installing the Instrument in the Software

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

NOTE: A PerkinElmer Service Representative will install the Spectrum software on the PC and set up your instrument in the software. However, details of the software installation can be found in the *Spectrum Administrator's Guide* (L1050095) which is supplied on the *IR & Raman Manuals CD* (L1050002).

After the software is installed, you should switch on your instrument. See *Switching on the Instrument* on page 48. Wait a few moments for your instrument to initialize and then connect the instrument USB cable to the PC.

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

You should now continue to connect the fiber optic cables to the RamanStation 400F instrument for any fiber optic probe or triggered fiber optic probe, as described in *Attaching and Removing the Fiber Optic Probe* on page 62.

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

Switching on the Instrument

NOTE: Do not connect the USB from the instrument to the PC until after the software is installed. See *Installing the Instrument in the Software* on page 47.

1. Insert the key into the laser power key switch on the rear panel of the RamanStation 400 and ensure that it is turned to the ARM position.

   The RamanStation 400 is supplied with two keys. One should be placed in the instrument 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. If your instrument is fitted with a deep depletion CCD, connect the 7.5 V PSU (L1323525) from the **DD POWER** socket on the rear panel to a mains power socket.

3. If your instrument is fitted with a deep depletion CCD, connect the Tygon tubing between the water recirculator (L1323010) and the WATER IN/OUT connectors on the rear panel, fill the storage tank of the cooler with deionized water and then plug in and switch on the recirculating cooler.

   For more information about the recirculating cooler, see the operating manual supplied with the system.

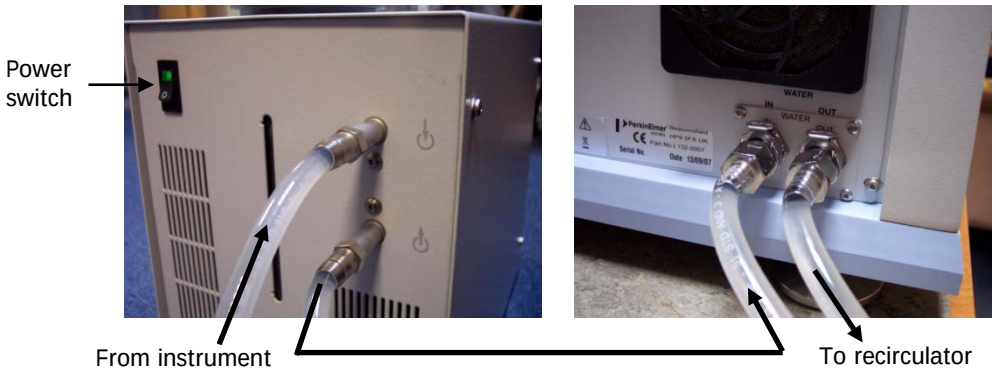


Figure 20 Connecting the water recirculator to the RamanStation 400 Series

4. Connect the instrument power supply (large PSU with 8 pin connector) to the RamanStation 400 **MAIN POWER** socket and then switch on (Figure 21).

   The instrument power cable has a red band around the end. The Main Power socket is indicated by a red dot. Do not connect to the **AUX POWER** connector.

Figure 21 Connecting the main power supply

5. If appropriate, connect the motorized XYZ stage controller box to a mains power socket and switch it on.

   The green LED on the controller box will light up.

Moving the Instrument

WARNING

The RamanStation 400 weighs about 45 kg. Two people are needed to lift the instrument.

If you need to ship your instrument you must use the original supplied packing materials to prevent damage to the system and follow the procedures described in this section.

NOTE: When the instrument has reached its destination and has been reassembled, you should perform the appropriate calibration/verification procedures as described in the *Calibration and Performance Verification SOPs* on page 79.

Motorized XYZ Stage

Before moving your instrument you must fix the stage in position to prevent damage to the stage.

To prepare the stage:

1. Remove your sample holder from the stage.
2. Using the Joystick, or using the Stage Position controls on the Setup Instrument XYZ Stage tab, move the stage to its lowest position in the Z axis and then roughly to the center of possible travel in the X and Y axes until the hole for the packing bolt is clearly visible (see Figure 16 on page 43).

   The front and right sides of the stage will be aligned.
3. Place the packing bolt in the hole in the center of the stage.
4. Close Spectrum software.
5. Remove the alignment camera assembly from the sample compartment by removing the USB cable from the socket labeled  and then unscrewing the lens snout from the stage.
6. Tighten the packing bolt using a 5 mm hexagonal key.

Triggered Probe Holster

NOTE: This section is only applicable if you have a RamanStation 400F and have the optional triggered fiber optic probe holster (L1320297) fitted to your instrument.

Before packing your instrument for shipping, you must remove the triggered fiber optic probe holster.

Figure 22 Triggered Fiber Optic Probe Holster

1. Make sure that the instrument is switched off and disconnected from the mains supply.

2. Carefully move the instrument so that the right-hand side is positioned over the edge of your workbench.

WARNING

While the instrument is not stood flat on the bench, you must keep it supported at all times.

3. Locate and remove the two screws securing the holster bracket to the base of the instrument (located behind the front foot).

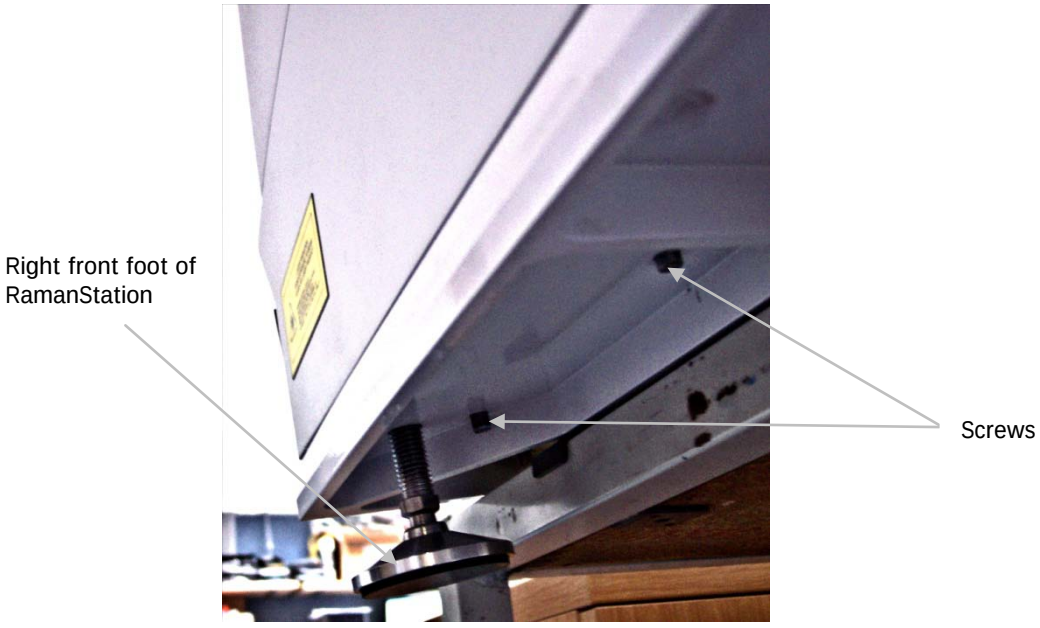


Figure 23 Base of the RamanStation 400 Series instrument

4. Return the instrument to its correct position on your workbench.

Introduction to the RamanStation 400 Series

Spectrometer Front Panel

The front panel of the spectrometer module contains 3 LED indicators and 1 control button (Figure 24).

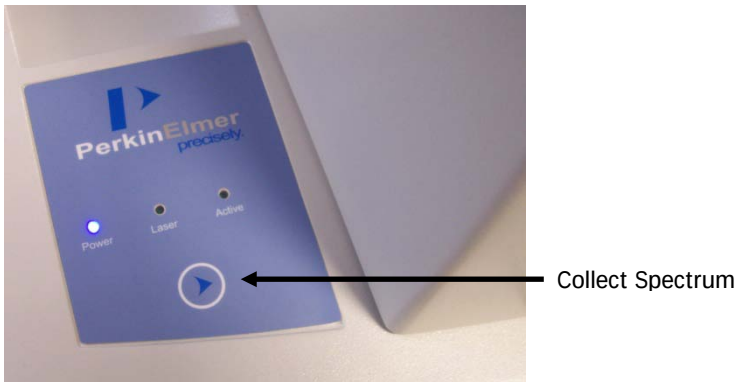


Figure 24 Spectrometer module front panel

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 laser radiation present throughout the optical path of the instrument.

NOTE: If you open the sample compartment lid when the Active LED is illuminated, the safety interlocks will close the shutters.

The control button shown in Figure 24 can be used to collect a spectrum and display the spectrum on screen.

NOTE: The control button is deactivated if you are using a triggered fiber optic probe. For details of how to use a triggered fiber optic probe, see the *Raman Triggered Fiber Optic Probe* leaflet (L1321940).

Sample Compartment

To open the sample compartment, lift the cover using the handle at the front of the RamanStation 400 Series as shown in Figure 25.

Figure 25 Opening the sample compartment of the RamanStation 400 Series

Motorized XYZ Stage Controller and Joystick

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

Figure 26 Stage controller and joystick

NOTE: When the joystick is used to move the stage, care should be taken that excessive movement does not bring the lens snout into forceful contact with the sample or stage, as this may cause damage to both.

Sample Accessories (for Motorized XYZ Stage)

To use your sample accessories to collect data, place them in the motorized XYZ stage in the RamanStation 400 Series sample compartment (Figure 27).

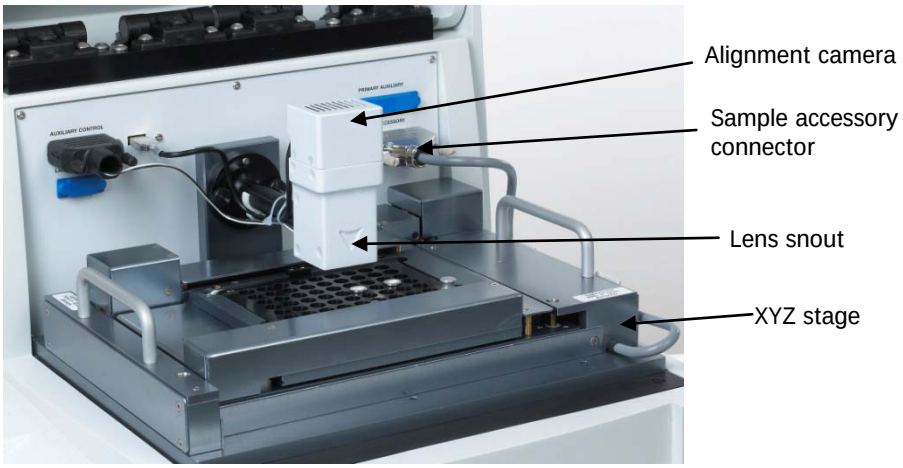


Figure 27 RamanStation 400 sample compartment

The Versatile Sample Holder (L1320227) is supplied with your RamanStation 400 Series (Figure 28). You can use this holder to collect data from microscope slides, cuvettes, sample tubes and capillary tubes.

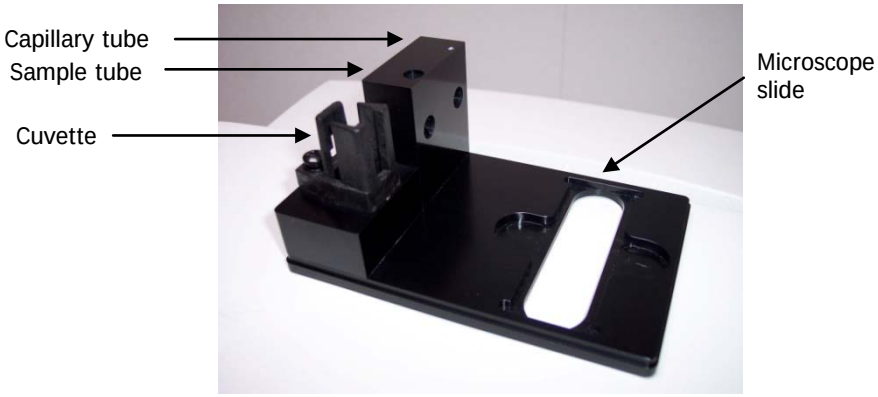


Figure 28 Versatile Sample Holder

NOTE: When the joystick is used to move the stage, care should be taken that excessive movement does not bring the camera into forceful contact with the sample, as this may cause damage to both. Particular care should be taken when using the Versatile Sample Holder.

The Raman Polarization Accessory (L1320284) that can be fitted to the RamanStation 400 Series spectrometers enables you to collect polarized Raman data in the RamanStation 400 sample compartment.

Figure 29 Raman Polarization Accessory

If you have a RamanStation 400F instrument, you can collect data using a fiber optic probe (see *Fiber Optic Probe* on page 59 for more details), which enables the remote measurement of samples.

The RamanStation 400F instrument can also be used in conjunction with the RamanMicro 300 Raman microscope. The RamanMicro 300 Raman microscope system is designed to produce high-quality Raman data from either physically small samples, such as fibers and single crystals, or from small areas of larger samples, such as contamination on a surface.

Fiber Optic Probe

Important: Read This First

This section is only applicable if you have a RamanStation 400F that is fitted with a fiber optic probe, or is being utilized in a Class 3B mode of operation.

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

Please read and understand the following general safety precautions before proceeding.

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

Your instrument should have been installed in accordance with *Installing Your RamanStation 400* starting on page 39.

Fiber Optic Probe (FOP)

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

General Overview of the Fiber Optic Probe

Your RamanStation 400F spectrometer fitted with a Fiber Optic Probe is a Class 3B laser system.

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

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

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

Attaching and Removing the Fiber Optic Probe

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

Attaching the FOP

1. Ensure the attenuator cap is attached to the FOP.

2. Turn off the instrument and disconnect the main power cable and, if appropriate, the stage controller power cable.

3. Carefully move the instrument to permit easier access to the rear panel.

4. Remove the fiber port cover assembly if fitted (see *Attaching the Fiber Port Cover Assembly* on page 46).

5. Remove the two retained screw-in caps from the coupler ports at the bottom left of the panel.

6. 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 30).

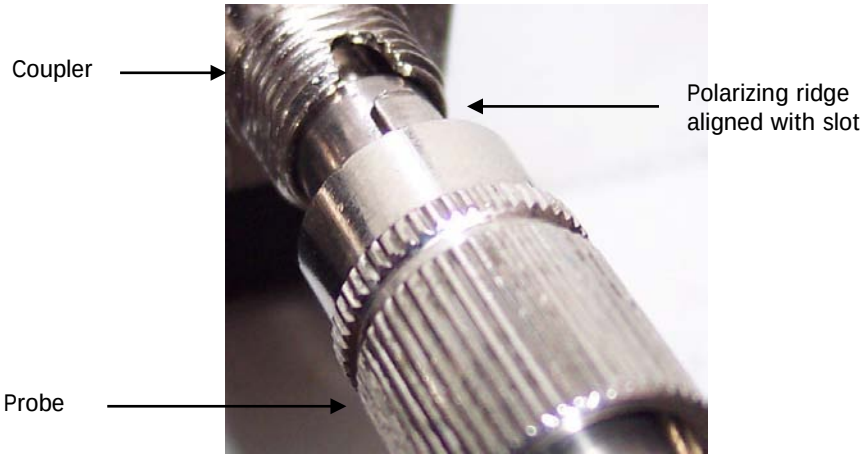


Figure 30 Connecting the fiber optic probe

7. 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.

8. Screw the screw-in caps from the fiber optic cables onto the coupler casings.

9. If you have a standard probe, connect the DIN connector to the nearby socket marked **LED FIBER #1**.

   OR

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

10. 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 and, if appropriate, the stage controller power cable.

3. Carefully move the instrument to permit easier access to the rear panel.

4. If you have a standard probe, remove the DIN connector from the socket marked **LED FIBER #1**.

   OR

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

5. Carefully remove the retained ends of the FOP by unscrewing and pulling the fiber optic cables away from the EXC and COL couplers perpendicularly.

   Be extremely careful when removing the FOP as you can easily damage the instrument and the fiber optic cables.

6. Replace the protective plastic caps on the EXC and COL 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 of the instrument.

8. Replace the fiber port cover assembly if required (see *Attaching the Fiber Port Cover Assembly* on page 46).

9. Reconnect the power cables.

Acquiring Spectra with the Fiber Optic Probe

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

Sampling Solids

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

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

If using a RamanStation 400F, and a fiber optic probe or RamanMicro 300 Accessory is connected, ensure that the probe or microscope objectives are covered (or, if fitted, that the microscope enclosure is closed) and that the microscope illumination is off and the switch is set to Dark Field (DF) when using the sample compartment to prevent contamination.

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.

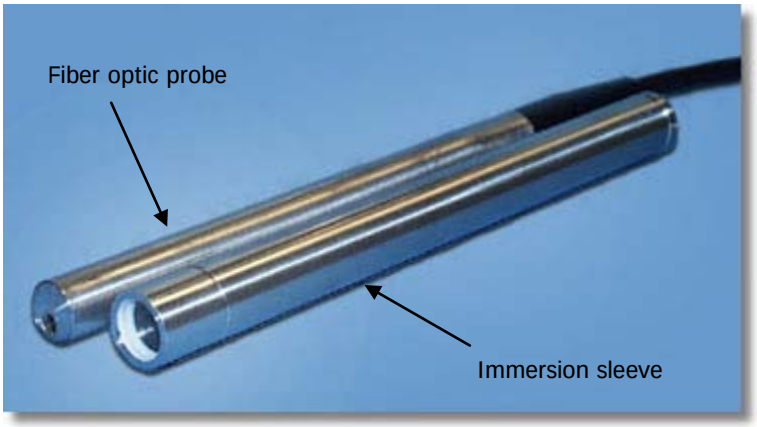


Figure 31 Fiber Optic Probe with the immersion sleeve detached

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.

Gas Sampling

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

WARNING

The standard FOP is not designed for immersion use*. The FOP is sealed against minor splashes. However, immersion in solvents will cause serious damage. Ensure an immersion sleeve is in place before immersing your FOP, see Figure 31. Please contact PerkinElmer if you have any questions in relation to sampling using the FOP.*

Using the RamanStation 400

Introduction

The RamanStation 400 and RamanStation 400F combined with the Spectrum software are very flexible instruments. The Standard Operating Procedures (SOPs) provided in this chapter demonstrate how to perform a number of tasks, which you can use as a starting point to develop your own SOPs that better suit your specific mode of use. This chapter also acts as a "learn by doing" section, allowing you to explore the functionality of the software and the spectrometer.

The General SOPs demonstrate how to setup your instrument and software. For SOPs that demonstrate how to collect data using a fiber optic probe, please refer to *Fiber Optic Probe SOPs*. For step-by-step instructions for calibrating the spectrometer, see *Calibration and Performance Verification SOPs*.

General SOPs

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 rear panel of the instrument.
4. Ensure the main power supply is connected to a mains outlet and that the outlet is switched on.
5. To turn on the spectrometer, press the PSU power switch ON (Figure 32):

| - ON O - OFF

Figure 32 The PSU power switch

6. Check that the Power LED on the instrument keypad 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. If appropriate, connect the stage controller box to a mains outlet and ensure that the outlet and the controller are switched on.

8. From the Start menu select **Programs**; the **PerkinElmer Applications** group; the **Spectrum** sub-group and then the **Spectrum** application.

   The Spectrum start-up screen is displayed, followed by a dialog that may require your login details.

9. If required, enter your **User name** and **Password**, and then click **OK**.

10. Select the **Instrument** you want to use and click **Connect**.

    OR

    If you want to work with data that has been collected previously, without connecting to an instrument, select **Work Offline**.

    Spectrum starts.

    If you selected to connect to the instrument, it will prepare for use by turning on the laser and cooling the detector to its operating temperature (usually −50 °C).

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

Messages will be displayed in the Status bar at the bottom of the workspace, and on the Scan icons on the Measurement bar.

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

SOP-2: Turning the Instrument Off

1. Shut down Spectrum software by selecting **Exit** from the File menu to return to the Windows operating environment.

2. Switch off the PC and monitor

3. Switch off the spectrometer by pressing the power switch on the PSU to OFF:

   | - ON O - OFF

4. If appropriate, switch off the stage control box.

   However, we recommend that you leave the stage controller box switched on, even if you switch off the instrument. If the stage control box is switched off, you will need to reset the stage upon restarting the software.

5. If appropriate, ensure that all sample holders have been removed from the sample compartment in preparation for the next time the instrument is used.

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

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

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

You can shut down your PC with the Raman instrument icon displayed. The system will shut down correctly.

NOTE: If Spectrum software stops responding, refer to *Troubleshooting* starting on page 95 for details of how to shut down the instrument and restart the software.

SOP-3: Installing and Changing Sampling Holders

This SOP describes changing sample holders on the motorized XYZ stage. The standard XYZ sample accessory holds a variety of sample holders. This procedure outlines installing a 96 multi-well plate and then changing to the sample bottle on the Versatile Sample Holder.

NOTE: For information on the use of a polarization accessory, see the *Raman Polarization Accessory* leaflet (L1321939).

Before you start, please ensure that the XYZ sample accessory stage is connected to the correct port on the back plate inside the sample compartment. The sample accessory stage should be connected to the **Sample Accessory** port and not to the **Auxiliary Control** port.

Install the 96 well plate

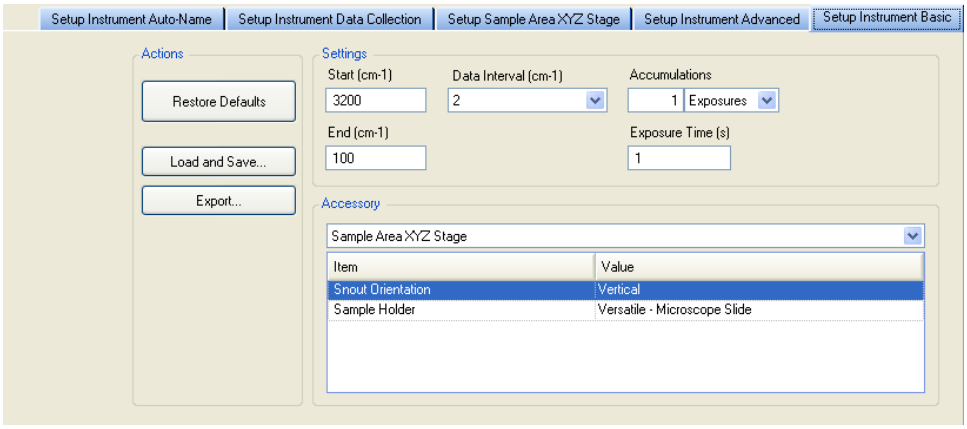
1. Ensure the RamanStation 400 Series is switched on.
   If not, follow SOP-1.

2. If you did not connect to your instrument on starting Spectrum software, select **Instruments** from the Setup menu and then **Select Instrument** from the sub-menu.
   The Instrument Connection dialog is displayed.

3. Select your Raman instrument and then click **Connect**.

4. Select **Raman Instrument** from the Setup menu in Spectrum.
   The Setup Instrument tabs are displayed.

5. Select the Setup Instrument Basic tab.



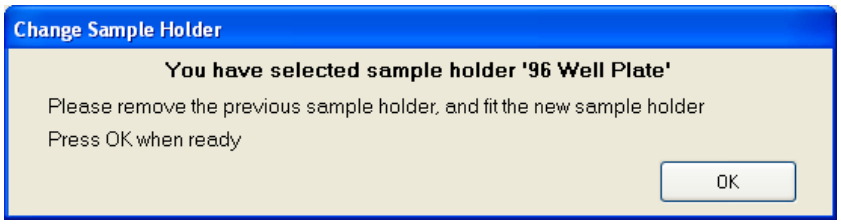
6. In the Accessory section, ensure that **Sample Area XYZ Stage** is selected as the Accessory.

7. Ensure the lens snout is pointing towards the sample (the sample holder and snout should point down).

8. Ensure that **Vertical** is selected as the Snout Orientation.

9. Select **96 Well Plate** from the Sample Holder drop-down list.

   Follow the prompts onscreen. The stage will move to a safe position for unloading a sample holder. You will then be prompted to remove any sample holder from the sampling accessory and to load the new sample holder. The sample accessory stage then drives to a preset position.



10. Install the 96 well plate.

    Slide the plate into the motorized stage, being careful to avoid the snout. The A1 position for all multi-well plates is the left rear corner as you look at the sample accessory stage.

11. Close the sample compartment lid.

12. Click **OK**.

    The stage will then move to the A1 position.

The instrument can now be used to position the sample (SOP-5), and set up the experimental parameters and collect data (SOP-4).

NOTE: You can move the stage to the A1 position by clicking **Move to first well** on the toolbar on the Setup Sample Area XYZ Stage tab.

If there is an offset between the stage position and the position of A1 in the software then you may need to reset your well positions; refer to the Spectrum on-screen help.

Install the Versatile Sample Holder (7 mm Sample Bottle) for use with a sample bottle

1. Click **Move to remove sample holder** on the Setup Sample Area XYZ Stage tab to move the stage to a safe position for you to unload your current sample holder.

2. Remove the 96 well plate.

3. Unscrew the lens snout from the sample compartment as shown in Figure 31.

4. Rotate the lens snout through 90° and resecure.

   The laser aperture should now point towards point to the left side of the instrument as shown in Figure 33 (which will be toward the sample bottle mount when the versatile sample holder is installed).

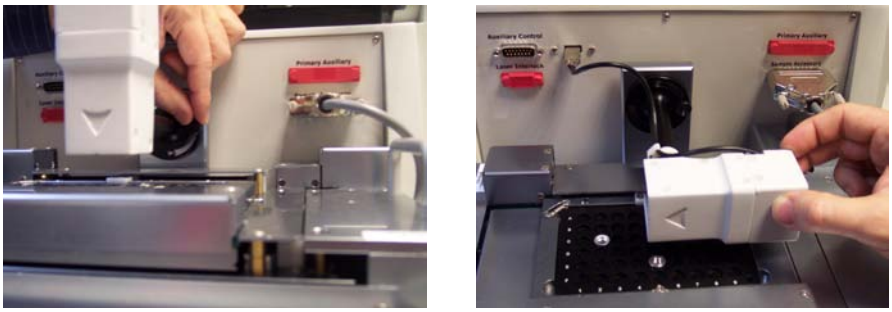


Figure 33 Unscrew the lens snout and rotate

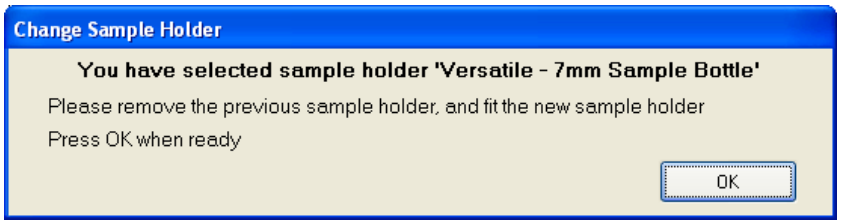
5. Select the Setup Instrument Basic tab.

6. In the Accessory section, select **Horizontal** as the Snout Orientation.

   The Sample Holder drop-down list will update to include sample holders suitable for this snout orientation; the stage will not move.

7. Select **Versatile – 7 mm Sample Bottle** from the Sample Holder drop-down list.

   You will be prompted to remove any sample holders from the sampling accessory and fit the new sample holder.



8. Load the versatile sample holder onto the motorized XYZ stage, ensuring that the cuvette block is located to the *left side of the lens snout at all times*.

9. Click **OK**.

   The stage then moves to a suitable position on the new sample holder.

10. Load the sample bottle into the versatile sample holder.

11. Close the sample compartment lid.

The instrument can now be used to position the sample (SOP-5), and set up the experimental parameters and collect data (SOP-4).

SOP-4: Setting-Up Experimental Parameters and Collecting Data

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.

NOTE: If you are using a Triggered Fiber Optic Probe, refer to the *Raman Triggered Fiber Optic Probe* leaflet (L1321940) supplied with the probe for configuration and usage details.

1. Ensure the RamanStation 400 is switched on.
   If not, follow SOP-1.

2. If you did not choose to connect to your instrument on starting Spectrum software, select **Instruments** from the Setup menu and then **Select Instrument** from the sub-menu.

3. Select your Raman instrument and then click **Connect**.

4. Select **Raman Instrument** from the Setup menu.
   The Setup Instrument tabs are displayed in the dialog pane.

5. If required, select the appropriate **Accessory** from the drop-down list on the Setup Instrument Basic tab.
   The accessories available to you will depend on your instrument configuration. Depending on the Accessory you have selected, further options may become available. See *SOP-3: Installing and Changing Sampling Holders* and *SOP-5: Selecting the Fiber Optic Probe* for further information.

6. Using the Instrument Settings toolbar, check and set the instrument parameters, such as the **Start** and **End** points of the scan range (in Raman Shift, for example, 3200 cm^{-1} to 100 cm^{-1}), and enter a unique **Sample ID** and **Description**.

7. Set the **Exposure Time** and number of **Accumulations**.
   You can define the Accumulations as a number of Exposures, or as a number of minutes or seconds.
   Typical values for Exposure Time and Accumulations are 5 seconds and 5 exposures, respectively. Spectra may be optimized by increasing or decreasing the Exposure Time and number of exposures. To obtain a good signal-to-noise ratio, you should co-add a number of spectra by choosing a suitable number of exposures. A minimum of two exposures is recommended for cosmic ray removal.

8. If required, adjust the **Data Interval** in Raman Shift (cm^{-1}).

   The default data interval is 2 cm^{-1}. The Data Interval can be set on the Setup Instrument Basic tab.

9. If you wish to edit the data collection parameters, such as how background scans are collected, select the Setup Instrument Data Collection tab.

10. In the Background Exposures section, we recommend that you select **As samples**.

    This sets the number of background scans to the same value as the number of sample scans. You may also choose one of the Background Collection options to define when a background will be taken.

11. If required, select any Auto Processing options.

    These options, if selected, will be applied to all spectra collected, and cannot be undone.

12. If you select **Save after each measurement**, your spectra will automatically be saved using the Sample ID.

13. If you have a Sample Area XYZ Motorized stage, select the Setup Sample Area XYZ Stage tab, and select the **Sampling Pattern**.

    Select the sampling pattern type from **Single Point**, **Super Macro Point**, **Custom Grid** and **Custom Line**. The spectra collected using SuperMacro, Custom Grid or Custom Line sampling patterns are co added to produce one spectrum.

    For more information, see the Spectrum on-screen Help.

14. If you have a Sample Area XYZ Motorized stage, select any **Focus** options.

    You can select to perform a **Raman AutoFocus** or **Video AutoFocus** before each sample measurement.

15. When you have finished defining your experimental parameters, position, and optimize conditions for, your sample as described in SOP-5 and SOP-7.

16. Click  .

NOTE: The Scan options on the Measurement menu and toolbar are disabled if you are using a Triggered Fiber Optic Probe. If you have selected the triggered fiber optic probe and the accessory is ready to scan the appearance of the scan icons will change to indicate this:  . For details of how to acquire data using a triggered fiber optic probe, see the *Raman Triggered Fiber Optic Probe* leaflet (L1321940).

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

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

If you would like to export the current instrument settings as a *.REX for use with AssureID, click **Export**.

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

NOTE: For information on how use the Setup Sample Area XYZ Stage tab to set up Markers, Cell Markers, Maps and Line Scans, and to collect a Visible Image Survey, refer to the Spectrum on-screen help.

SOP-5: Sample Positioning using the Motorized XYZ Stage

You can use Monitor to help position your sample for optimum spectral collection efficiency.

Sample positioning and alignment in Monitor 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 monitoring cannot be saved. To save data you must collect spectra (SOP-4).

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

1. Ensure the RamanStation 400 is switched on.
   If not, follow SOP-1.

2. Load the appropriate sample holder onto the motorized XYZ stage and ensure that the lens snout is correctly aligned as described in SOP-3.

3. Close the sample compartment lid.
   In normal use, if the sample compartment cover is opened, then the laser interlock is breached and laser radiation cannot be emitted from the instrument. Spectrum software will display the safety interlock is breached Laser Status icon  .

4. Select the Setup Sample Area XYZ Stage tab, and display the **Live Video** view.
   Live Video shows the live image from the video camera.

5. Use the joystick to position 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.

   OR

   Use the **Stage Position** controls on the Setup Sample Area XYZ tab to position the stage.

NOTE: When using the joystick with the snout in the horizontal orientation, although the movement of the stage will not change, the direction the relative to the on-screen image will be different.

6. Focus on the sample.

   You can use the joystick or Stage Position controls. Alternatively, click  **Video AutoFocus** to perform an automatic video focus or  **Raman AutoFocus** to focus using the strongest Raman signal. Refer to the Spectrum on-screen help for more information.

7. Click  .

   The RamanStation 400 will start collecting data in the Live display.

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

9. Adjust the **Exposure time (s)**.

   This will automatically acquire another background.

   The sample may now be positioned and aligned correctly for optimized data collection using the joystick.

10. If required, adjust the Laser power and Exposure time further to optimize the conditions for your sample.

11. When you have optimized your experimental parameters, click  to exit the Live display.

You can now collect and save spectral data (SOP-4).

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

Fiber Optic Probe SOPs

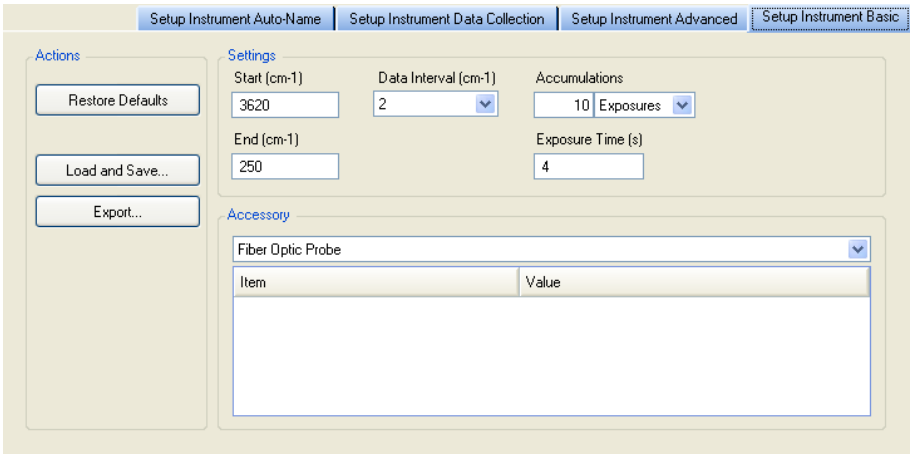
NOTE: The SOPs included on the following pages are only appropriate for a standard fiber optic probe. For details of how to acquire data using a triggered fiber optic probe, see the *Raman Triggered Fiber Optic Probe* leaflet (L1321940).

SOP-6: Selecting the Fiber Optic Probe

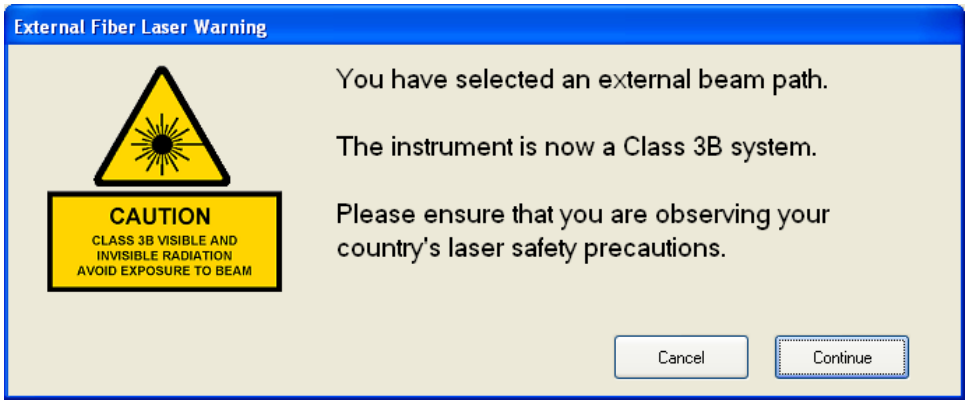
1. Ensure the RamanStation 400F is switched on and the fiber optic probe connected to the instrument.
   If not, follow SOP-1.

2. Select **Raman Instrument** from the Setup menu in Spectrum.
   The Setup Instrument tabs are displayed in the Dialog pane.

3. Select the Setup Instrument Basic tab.



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



5. Ensure that laser safety precautions are being observed, and then click **Continue**.
   If you have any doubts about the required safety precautions click **Cancel**.

Laser light will be emitted from the probe during data collection and when the software is in Monitor or Preview modes. When the laser is on and the shutter is open the Laser Status on the Measurement bar will be updated to Class 3B,  . The probe can now be positioned so as to achieve the best spectrum. See SOP-7.

SOP-7: Sample Positioning when using a Fiber Optic Probe

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

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

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

1. Ensure that the RamanStation 400F is switched on.
   If not, follow SOP-1.

2. Select **Monitor** from the measurement menu.

   OR

   Click  on the Measurement toolbar.
   The Live tab will be displayed and real-time spectral acquisition will commence.

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

4. Adjust the **Exposure time (s)**.
   This will automatically acquire another background.
   The sample may now be positioned and aligned correctly for optimized data collection by adjusting the distance between the Fiber Optic Probe and the sample.

5. If required, adjust the Laser power, Exposure time and probe position further to optimize the conditions for your sample.

6. When you have optimized your experimental parameters, click  to exit the Live display.

You can now collect and save spectral data. To set up an experiment and collect data using the FOP please refer to SOP-4.

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

Calibration and Performance Verification SOPs

Several different automated calibration tests are included in the software:

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

An additional wavelength calibration verification routine has been included in the software, which compares a series of peaks for polystyrene (ASTM E 1840) with values collected using a polystyrene sample. These calibration/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 if there have been appreciable temperature variations in the local environment, or following a service repair of the instrument or accessories.

NOTE: The sample compartment lid must be kept closed during all stages of calibration.

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 SOP.

SOP-8: Calibration / Performance Verification

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

When carrying out the calibration/verification procedures, the instrument laser power should be set to 100% and the Data Interval to 2 cm^{-1} or smaller. You can perform these procedures with any accessory. The examples given in these SOPs assume that the RamanStation 400 sample compartment is used.

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

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

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

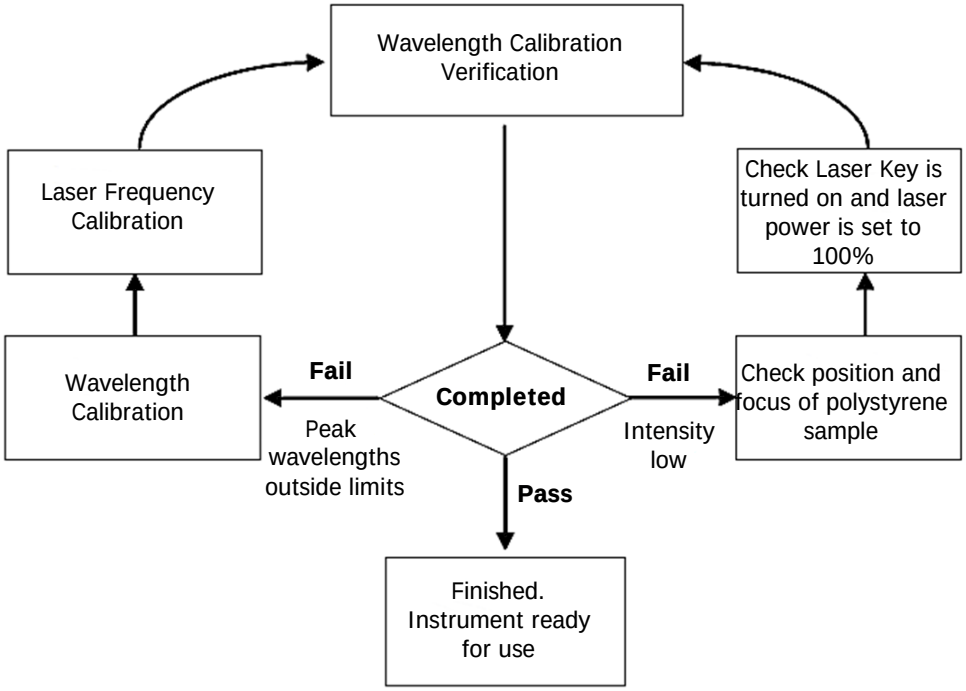


Figure 34 System verification / calibration flow diagram

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

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

SOP-9: Wavelength Calibration Verification

1. Ensure the RamanStation 400 Series is switched on.
   If not, follow SOP-1.

2. Select **Raman Instrument** from the Setup menu.
   The Setup Instrument tabs are displayed.

3. Select the Setup Instrument Advanced tab and ensure that the laser power is set to 100%.

4. Ensure that **Sample Area XYZ Stage** or **Sample Area**, as applicable, is selected as the Accessory on the Setup Instrument Basic tab.

5. Ensure that the lens snout is set to vertical physically, and that the **Vertical** orientation is selected in the software.

6. Load a polystyrene sample (L1181228) into a suitable sample holder.

7. Select the appropriate **Sample Holder** in the drop down list and load the sample holder onto the sample stage, following the instructions on-screen if you have a motorized stage.

8. Ensure that the RamanStation 400 Series sample compartment is closed and, if you have a RamanStation 400F, ensure that any external accessory is capped or covered to prevent stray light interfering with the measurement.

   If you have a RamanMicro 300 Accessory connected to your RamanStation 400F, ensure that the microscope illumination is switched off.

9. Focus on the sample of polystyrene.

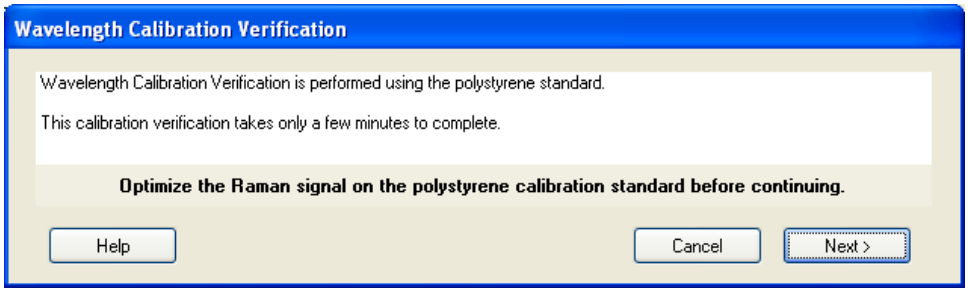
   If you have a motorized stage, you can use the joystick or Stage Position controls on the Setup Sample Area XYZ Stage tab. Alternatively, click **Raman AutoFocus** to focus using the strongest Raman signal. The Focus Limits should be set to **Full Range**.

10. Select **Toolbox** on the Setup Instrument Advanced tab.

    The Adjustments Toolbox dialog is displayed.

11. Select .

    The Wavelength Calibration Verification dialog is displayed.



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

    The wavelength calibration verification routine begins. This will take a few minutes. When the verification is complete, the result is displayed.

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 34. If the RamanStation 400F wavelength calibration verification fails for the sample compartment, this does not necessarily mean that the performance of any other accessories is also affected.

SOP-10: Wavelength Calibration

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

1. Ensure the RamanStation 400 is switched on.

   If not, follow SOP-1.

2. Attach the external neon calibration accessory (L1320220) to the rear panel of the RamanStation 400 Series.

   Push the large connector into the socket labeled **CAL ASSY** and carefully connect the small cable to the **COL** coupler, ensuring that the cable is fully inserted.

NOTE: As wavelength calibration is not required on a regular basis, the external neon calibration accessory is not included in the basic RamanStation 400 Series package. The external neon calibration accessory can be purchased separately.

3. Ensure that the RamanStation 400 Series sample compartment is closed and, if you have a RamanStation 400F, ensure that any external accessory is capped or covered, to prevent stray light interfering with the measurement.

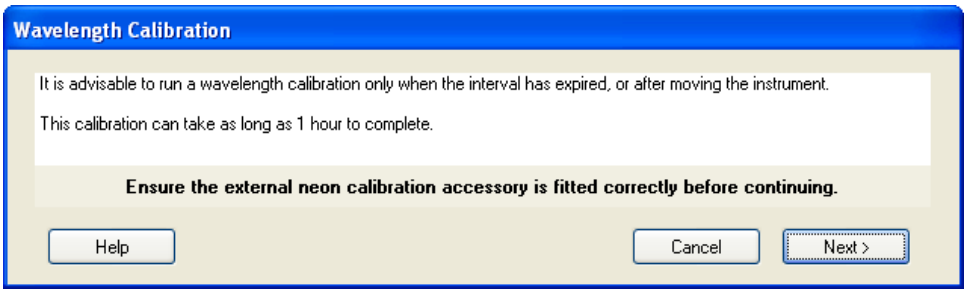
   If you have a RamanMicro 300 Accessory connected to your RamanStation 400F, ensure that the microscope illumination is switched off.

4. Select the Setup Instrument Advanced tab and click **Toolbox**.

   The Adjustments Toolbox dialog is displayed.

5. Select  .

   The Wavelength Calibration dialog is displayed.



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

   The instrument will now perform the wavelength calibration routine. This will take some time. When the calibration is complete, the result is displayed.

7. Disconnect the neon calibration accessory and refit the screw-in cap to the **COL** port.

The instrument is now wavelength calibrated.

SOP-11: Laser Frequency Calibration

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

1. Ensure the RamanStation 400 is switched on.
   If not, follow SOP-1.

2. Select **Raman Instrument** from the Setup menu.
   The Setup Instrument tabs are displayed

3. Ensure that **Sample Area XYZ Stage** or **Sample Area**, as applicable, is selected as the Accessory on the Setup Instrument Basic tab.

4. Ensure that the lens snout is set to vertical physically, and that the **Vertical** orientation is selected in the software.

5. Load a polystyrene sample (L1181228) into a suitable sample holder.

6. Select the appropriate **Sample Holder** in the drop down list and load the sample holder onto the sample stage, following the instructions on-screen if you have a motorized stage.

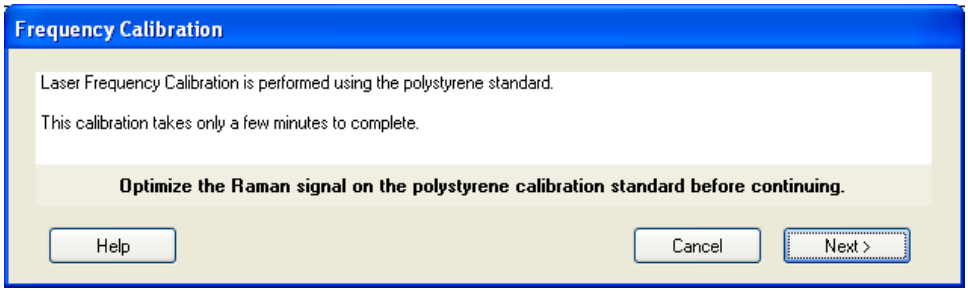
7. Ensure that the RamanStation 400 Series sample compartment is closed and, if you have a RamanStation 400F, ensure that any external accessory is capped or covered, to prevent stray light interfering with the measurement.
   If you have a RamanMicro 300 Accessory connected to your RamanStation 400F, ensure that the microscope illumination is switched off.

8. Select the Setup Instrument Advanced tab and ensure that the laser power is set to 100%.

9. Focus on the polystyrene sample.
   If you have a motorized stage, click  **Raman AutoFocus** on the Setup Sample Area XYZ Stage tab to focus using the strongest Raman signal. The Focus Limits should be set to **Full Range**.

10. When the polystyrene sample is optimally focused for Raman signal, select the Setup Instrument Advanced tab and click **Toolbox**.
    The Adjustments Toolbox dialog is displayed.

11. Select  .
    The Frequency Calibration dialog is displayed.



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

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

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

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

SOP-12: Intensity Calibration

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

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

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

Contact your PerkinElmer Raman Business Unit if you wish to set up a NIST intensity correction for a different temperature range.

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

NOTE: As intensity calibration is not required on a regular basis, NIST Standards are not included in the basic RamanStation 400 package. Standard Reference Materials can be purchased from PerkinElmer (L1321831). Your instrument will be intensity calibrated prior to shipping or during installation.

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

To calibrate using the sample compartment:

1. Ensure the RamanStation 400 is switched on.
   If not, follow SOP-1.

2. Select **Raman Instrument** from the Setup menu.
   The Setup Instrument tabs are displayed.

3. Select the Setup Instrument Basic tab and ensure that the **Sample Area XYZ Stage** or **Sample Area** is selected as the Accessory.

4. Ensure that the lens snout is vertical, and that the Snout Orientation is set to **Vertical** in the software.

5. Load the NIST sample into a suitable sample holder (with the dull surface facing up).

6. Select the appropriate **Sample Holder** in the drop down list and load the sample holder onto the sample stage, following the instructions on-screen if you have a motorized stage.

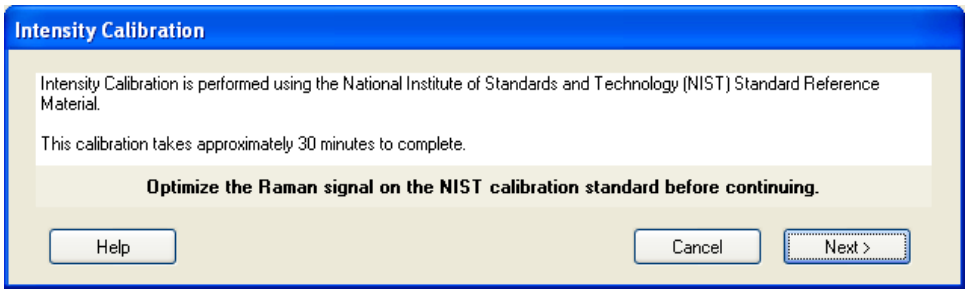
7. Ensure that the RamanStation 400 Series sample compartment is closed and, if you have a RamanStation 400F, ensure that any external accessory is capped or covered, to prevent stray light interfering with the measurement.
   If you have a RamanMicro 300 Accessory connected to your RamanStation 400F, ensure that the microscope illumination is switched off.

8. Select the Setup Instrument Advanced tab and ensure that the laser power is set to 100%.

9. Focus on the NIST standard.
   If you have a motorized stage, click  **Raman AutoFocus** on the Setup Sample Area XYZ Stage tab to focus using the strongest Raman signal. The Focus Limits should be set to **Full Range**.

10. Select the Setup Instrument Advanced tab and click **Toolbox**.
    The Adjustments Toolbox dialog is displayed.

11. Select  .
    The Intensity Calibration dialog is displayed.



12. Ensure that you have followed the instructions on the dialog, and then click **Next**.
    The instrument will now perform the intensity calibration routine. When the calibration is completed, the results are displayed.

13. Remove the intensity correction standard and store safely.

Any data acquired will now be intensity corrected.

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 (armed), and the laser power is 100%.

Summary

- The only calibration procedure that needs to be performed on a regular basis is the wavelength calibration verification procedure.

- The wavelength and laser frequency calibrations should only be performed if the instrument fails the wavelength calibration verification procedure.

- We recommend that the intensity calibration is performed on a yearly basis, unless the environment (temperature and/or humidity) changes significantly, or the instrument is moved or repaired.

Additional Parts and Upgrades

Sampling Accessories

Part Number	Description
L1323514	Quartz microscope slide, 25 × 75 × 1 mm
L1323515	Quartz capillary tubes, 1.8 mm OD (pack of 25)
B0631015	NIR rectangular cell with lid, Quartz SUPRASIL 300, light path 10 mm
B0631033	NIR rectangular cell with PTFE stopper, Quartz SUPRASIL 300, light path 10 mm
L1320284	Raman Polarization Accessory
L1320280	Powder Holder Kit for Motorized Stage Contains powder holder baseplate, powder cup loading base and tray, powder holder cups, 10 cm micro spatula, brush and tweezers
L1320278	Tablet Holder Kit for Motorized Stage Contains tablet holder baseplate, sample discs, tablet holder cups and tweezers
L1245025	Custom Tablet Molding Kit
L1321675	Multi-well Plate Holder for Motorized Stage

SERS Consumables

Part Number	Description
L1329300	SERS gold colloid – 50 nm particles (20 ml)
L1329307	SERS gold colloid – 100 nm particles (20 ml)
L1329308	SERS gold colloid – 200 nm particles (20 ml)
L1329301	Aggregating agent – $MgSO_4$ 0.16 M in milli-Q water (20 ml)
L1329303	Standard crystal violet pigment (0.25 ml)
L1329304	Disposable micropipettes – 200 µl (100 pack)
L1329305	Disposable micropipettes – 50 µl (100 pack)
L1329306	Disposable micropipettes – 2 µl (100 pack)

Safety and Calibration

Part Number	Description
L1320262	Raman Laser Safety Kit (785 nm) Contains external interlock cable, laser glasses, laser detection card, and door warning label
L1320237	Raman Calibration Kit (785 nm) Contains neon lamp and fiber optic connector (for wavelength calibration), calibration plate containing polystyrene (for wavelength calibration verification and laser wavelength calibration), calcite (for resolution verification), and NIST 2241 Intensity Correction Standard
L1321831	NIST 2241 Relative Intensity Correction 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	Raman triggered probe, 7.5 mm working distance, maximum working temperature 80 °C, 5 m cabling
L1320031	Raman triggered probe, 7.5 mm working distance, maximum working temperature 80 °C, 20 m cabling
L1320070	Short immersion sleeve for TFOP – for use with L1320030 and L1320031
L1320324	Raman probe fixed spacer kit (7.5 mm)
L1320325	Raman probe adjustable spacer kit
L1320071	Ultra-long working distance lens adaptor for Raman fiber optic probes
L1320095	Fiber probe sample holder (for measurement of samples in small vials and cuvettes)

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

Maintenance and Troubleshooting

Maintenance

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

Cleaning the Spectrometer and Power Supply Units

WARNING

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

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

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

WARNING

Do not directly clean electrical contacts.

WARNING

To avoid any electrical shock hazards, do not allow liquids to run down into the external PSU. Also, do not allow liquids to run down into the sample compartment of the spectrometer, the rear service panel (with electrical connections, vents and fans) or the instrument control keypad. Only use a soft, dry cloth to clean the inside of the sample compartment, the sample compartment door, the rear panel, the control keypad or the PSU.

To stop the air filter from becoming clogged up, the filter must be cleaned regularly. Unclip the fan filter cover, remove the filter and wash thoroughly with warm soapy water. When the filter is totally dry reassemble the filter cover.

Servicing the Spectrometer

With the exception of the fan filter (mentioned above) there are no user serviceable parts on the instrument. Removal of the protective housing or external panels is dangerous and is not recommended for users under any circumstances. If you require an instrument service please contact PerkinElmer. Only PerkinElmer Service Engineers should attempt to service the spectrometer.

WARNING

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

Servicing the Fiber Optic Probe

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

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

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

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

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

Replacing the Stage Controller Fuse

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

WARNING

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.

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.

1. Switch off the stage controller, disconnect it from the power supply and remove the mains cord.

2. Insert a screwdriver into the slot at the side of the fuse drawer, and pull out and flip to one side over the mains inlet.

   The fuse may now be removed.

3. Fit the replacement fuse into the fuse drawer.

   Make sure that you fit the fuse in the top slot. You require a 1.6 A time-lag fuse (09991641). Always use the recommended replacement fuse.

4. Refit the fuse drawer.

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

Troubleshooting

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

Hardware

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

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

Problem:	**Cannot collect data because an interlock is breached.**
Description	This means that, for some reason, the interlock system is detecting an open circuit.
Solution 1	Ensure all interlocked doors, including sample compartment lid and any other remotely interlocked doors, are closed properly.
Solution 2	If you have a fiber optic probe fitted to your system; ensure your external interlock is wired up correctly and that any interlocked room entrances or sampling enclosures are closed. If you do not have a fiber optic probe, ensure that the Magnetic actuator (a red block positioned on the inside of the sample compartment door) is securely in place.

Software

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

Problem:	**The motorized stage does not move to the expected position on a sample holder when requested.**
Solution 1	Go to the Setup Instrument Basic tab and ensure that the selected sample holder is correct. If it is not, then select the appropriate sample holder. Check the Graphic view on the Setup Sample Area XYZ Stage tab – it may be that you were directing the stage to a prohibited region of the sample holder (hashed area on the Graphic view).
Solution 2	If the Accessory options are correct, it may be that the stage needs to be reset. On the Setup Sample Area XYZ Stage tab, click **Toolbox** and then select **Reset Stage**. The motorized accessory will now reset itself. NEVER attempt to reset the stage with the RamanStation 400 Series lens snout in the Horizontal orientation.
Solution 3	If the above solutions do not solve your problem, you should contact your PerkinElmer Service Representative.

Problem:	**The message "Video Image not available" is displayed on the Live Video image on the Setup Sample Area or Setup Sample Area XYZ Stage tab.**
Description	This means that the software searched for the alignment camera on the system and could not locate one.
Solution	Is the USB cable connected from the alignment camera to the sample compartment? If not, exit the software, connect this cable and then restart Spectrum software.

Problem:	**Unable to communicate with stage.**
Description	When you open the Spectrum software the error message **Unable to communicate with Stage. The stage controls will not operate.** is displayed.
Solution	Disconnect the stage controller box power supply from the mains power socket, wait a few seconds, and then reconnect the PSU to the mains power socket. You will then be prompted to reset the stage

Decontamination

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

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

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

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

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

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

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

WEEE Instructions for PerkinElmer Products

 **or** 

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.