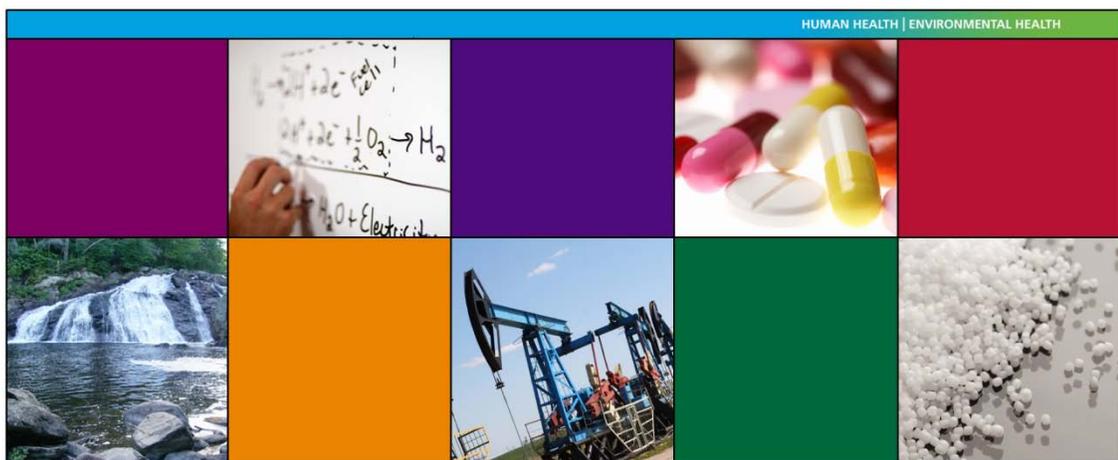


# RamanMicro 300 Accessory



## Getting Started Guide



## Release History

Part Number	Release	Publication Date
L1321910	B	February 2011

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# **Introduction**

## **About This Manual**

---

This Getting Started Guide contains all of the information that you will need to setup your RamanMicro 300 Raman Microscope Accessory and start collecting spectra.

The RamanMicro 300 Raman Microscope Accessory is used in conjunction with the RamanStation 400F Raman spectrometer, or with the RamanFlex 400 Series Raman spectrometer. It cannot be used in isolation.

**NOTE:** For a detailed description of the RamanStation 400F or RamanFlex 400 Series installation procedure, see the *Getting Started Guide* supplied with the instrument.

This Getting Started Guide is divided into the following chapters:

### **Introduction**

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

### **Safety Practices**

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

### **Raman History, Theory and Instrumentation**

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

### **Preparing your Facility**

This chapter shows you how to position your RamanMicro 300 Accessory, and what utilities are required; for example, electricity and temperature control.

### **Installing your RamanMicro 300**

This chapter contains instructions about how to assemble your RamanMicro 300 Accessory.

### **Using the RamanMicro 300**

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

### **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 instrument means the RamanMicro 300 Raman Microscope Accessory used in conjunction with the RamanStation 400F or the RamanFlex 400 Series 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 system must read and follow the precautions before unpacking, installing or using the instrument.

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

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

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## General Operating Conditions



**WARNING**

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

The RamanMicro 300 microscope has been designed and tested in accordance with PerkinElmer specifications and in accordance with the safety requirements of the International Electrotechnical Commission (IEC). The RamanMicro 300 system meets the requirements of EC directive 2006/95/EC (low voltage directive).

Only use the RamanMicro 300 Accessory 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 hazard involved.

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

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

**CAUTION**

*Ensure that there is at least 15 cm (6") between any cooling vents and adjacent equipment or walls.*

Whenever it is likely that the RamanMicro 300 Accessory is unsafe, make it inoperative. The RamanMicro 300 Accessory 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.

## ***Electrical Safety***

---

### ***Power Cords***

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



**WARNING**

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

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

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

### ***Fuses***

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

## Laser Safety

---

The RamanMicro 300 Raman microscope is used in conjunction with the RamanStation 400F Raman spectrometer, or with the RamanFlex 400 Series Raman spectrometer. As part of the system, the RamanMicro 300 complies with the requirements of IEC 60825-1: 2007 and 21CFR 1040.10 FDA performance standards for laser products, except for deviations pursuant to Laser Notice No. 50, dated 26 July 2001.



**WARNING**

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



**WARNING**

*Removal of the protective housing or external panels is dangerous.*

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

The RamanMicro 300 emits a focused beam of laser radiation at 785 nm from the microscope objective lens. This radiation is classified as Class 3B. The maximum laser power accessible at the stage is 100 mW.



**WARNING**

*Avoid direct exposure to the laser beam. Do not look at the laser light or scattered laser light. Laser light at 785 nm is nearly invisible and only appears dim to the eye.*



**WARNING**

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

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

## **Class 3B Safety Precautions**

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

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

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.

**NOTE:** The RamanMicro 300 Accessory can be fitted with an optional light-proof enclosure, which is fitted with interlock sensors to ensure that the microscope cannot be used with the enclosure doors open. The Raman system is NOT reclassified to a Class 1 instrument.

## General Laser Safety Issues

### Laser Keyswitch

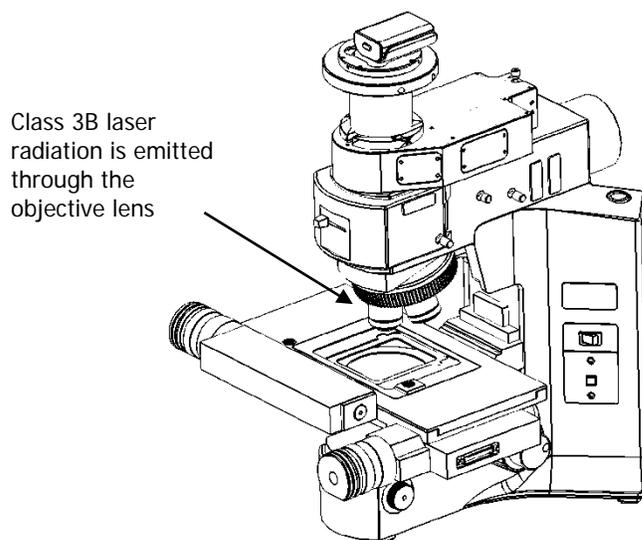
There is a captive laser key control switch on the rear panel of the RamanStation 400F, or the front panel of the RamanFlex. This key is required to operate the laser. A laser safety officer or laboratory manager should control the use of this key.

### 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 19.

### Microscope Safety

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



**Figure 1 Schematic of the microscope**

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

- Never look directly into the laser beam or at the laser light scattered from a reflective surface or sample.
- Avoid direct exposure to the laser light.
- Place warning signs near the laser operating areas.
- Control laser areas and limit access to individuals trained in the safe operation of lasers.
- Use protective laser safety goggles, with an OD (optical density) of at least 3 at 785 nm, as a precaution against accidental exposure to the direct, or reflected, laser light. Suitable goggles are available from PerkinElmer (part number L1323518).
- Use an interlock switch on the door to the laboratory, if required.



**WARNING**

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

*In particular:*

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

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



**WARNING**

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

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

## Warning Labels

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*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 Microscope

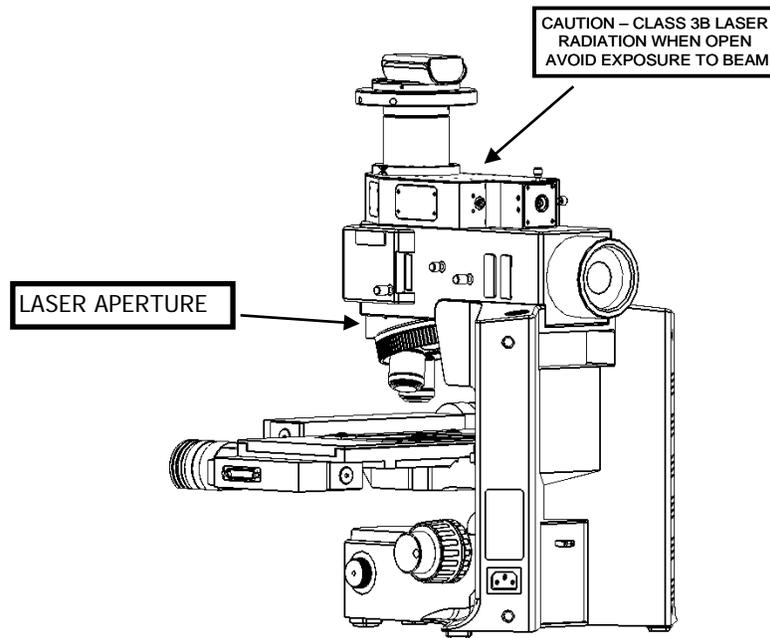
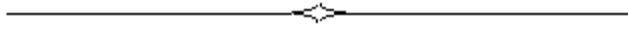


Figure 2 Labels on the microscope



**Raman History, Theory**  
**and Instrumentation**

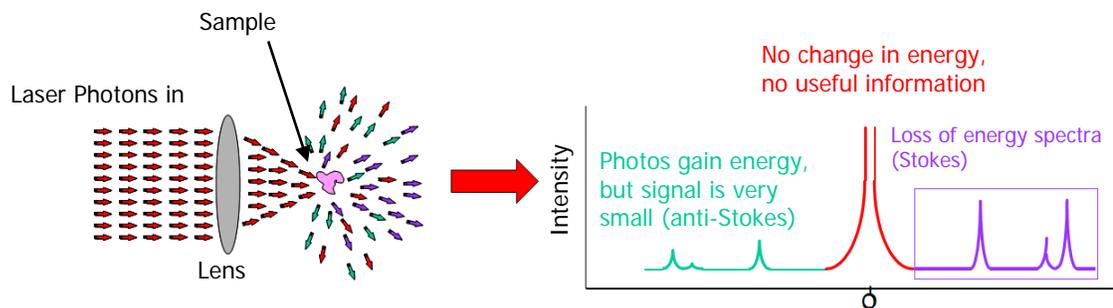
## ***The History of Raman Spectroscopy***

---

Inelastic light scattering was first predicted in 1923 by A. G. Smekal in Germany. Research carried out by A. H. Compton discovered inelastic scattering of X-rays, which led the Indian scientist C. V. Raman to investigate this phenomenon. Raman and his assistant Krishnan observed what came to be known as the Raman effect (Figure 3) for the first time in 1928. They separated the blue light from sunlight and observed the scattering from water and alcohol visually. In addition to the blue incident light, a faint green light also scattered, later named Stokes scattering. By the 1930s much of the theory underlying Raman scattering was understood, but due to the complexity of the instrumentation the initial interest in Raman spectroscopy was not maintained. Improvements in charge-coupled device (CCD) detectors, filters and lasers over the past 25 years have radically simplified Raman instrumentation, and interest in Raman spectroscopy has been renewed. Lasers are ideal excitation sources for Raman because they give highly monochromatic radiation and provide high irradiances at the sample.

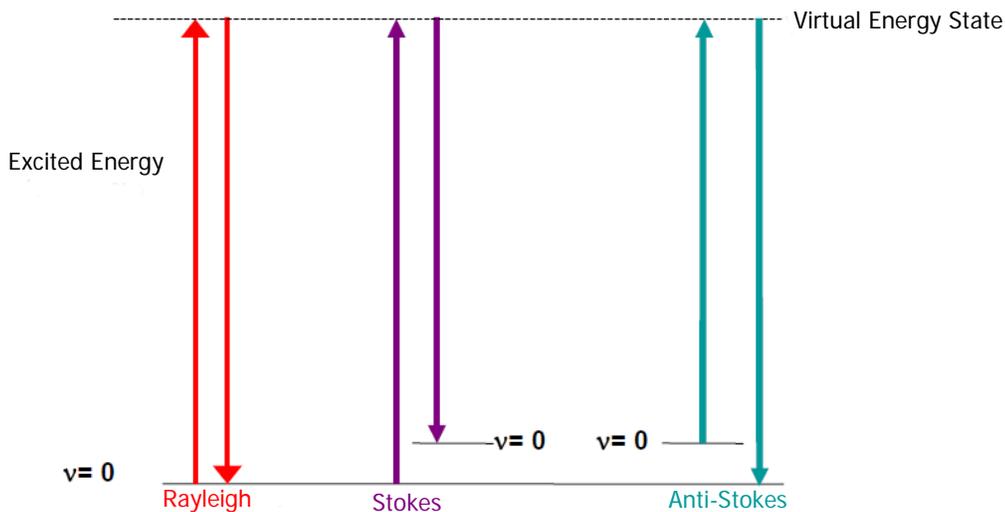
## Raman Theory

When photons from a monochromatic light source impinge on a sample, a small fraction are scattered in all directions. Some of the scattered photons are of the same energy as those of the incident beam, and hence the same frequency, giving rise to Rayleigh scattering (Figure 3).



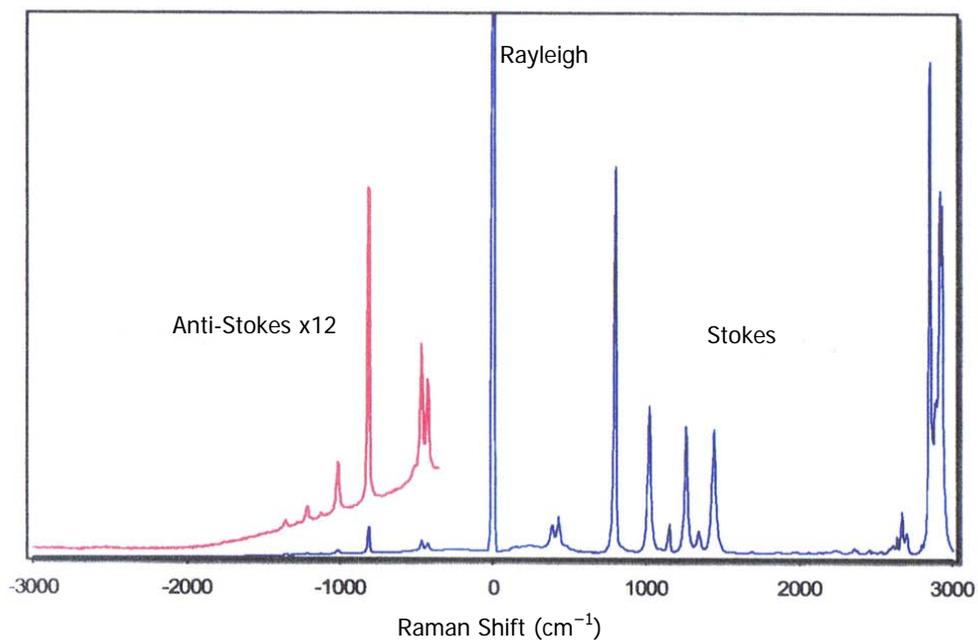
**Figure 3 The Raman effect: irradiation of sample and subsequent scatter**

Some photons will be inelastically scattered and will be detected at either longer wavelengths (lower frequencies,  $\nu'$ , Stokes scattering) or shorter wavelengths (higher frequencies,  $\nu''$ , 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 4).



**Figure 4 Rayleigh, Stokes and Anti-Stokes scattering**

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



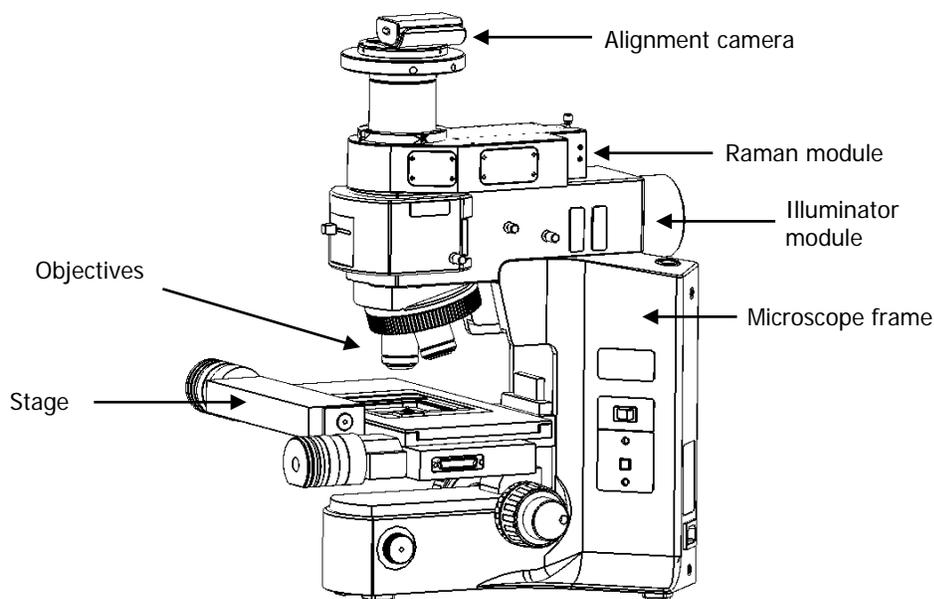
**Figure 5 Raman spectrum of cyclohexane, showing Stokes, anti-Stokes and Rayleigh scattering**

## ***The RamanMicro 300***

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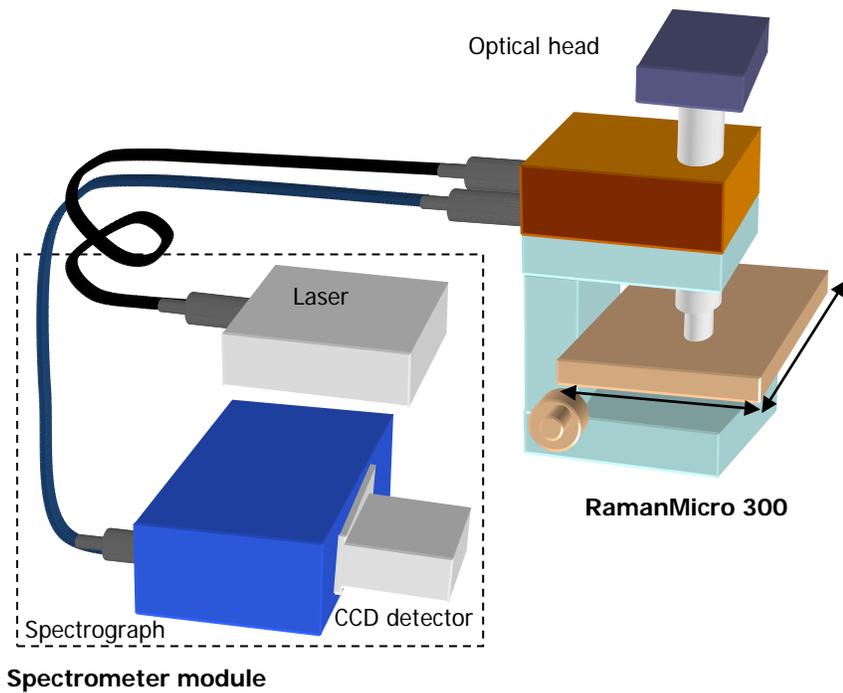
The RamanMicro 300 Raman microscope is used in conjunction with either the RamanStation 400F Raman spectrometer, or the RamanFlex 400 Series Raman spectrometers.

When used with the RamanStation 400F, use the sample compartment in the RamanStation 400F to measure your macroscopic samples, and to measure samples on the microscope use the RamanMicro 300. You can also attach an optional fiber optic probe to the RamanStation 400F to further extend the range of sample types that you can analyze.



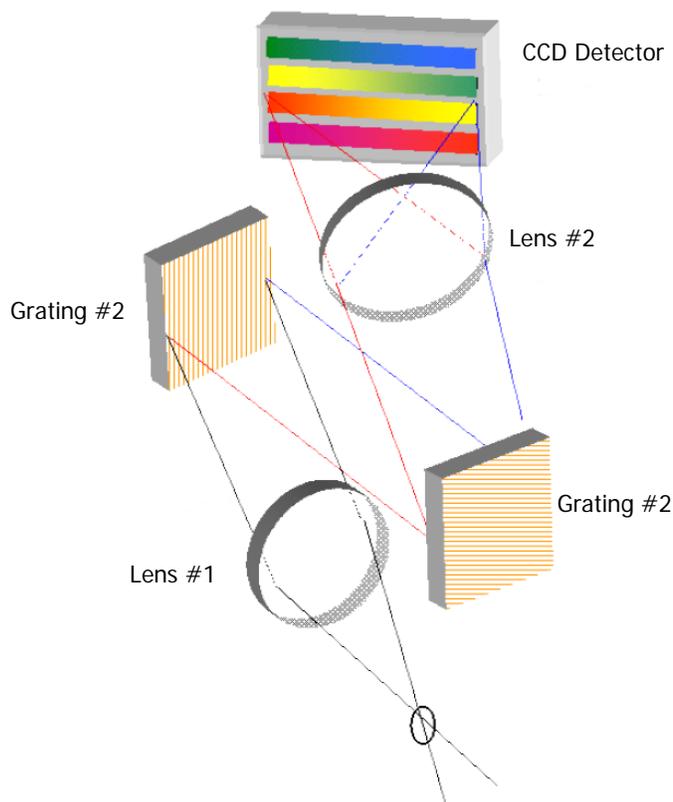
**Figure 6 The RamanMicro 300 microscope**

The microscope (Figure 6) is composed of a frame, illumination module, Raman module and alignment camera module. This is connected to the spectrometer module in the RamanStation 400F or RamanFlex 400 by two fiber optic cables. The spectrometer module contains the laser, spectrograph and CCD detector. The laser supplies light through an objective lens onto the sample. The scattered light from the sample is collected with the same objective lens and transmitted back to the spectrometer. A schematic of the system configuration is provided in Figure 7.



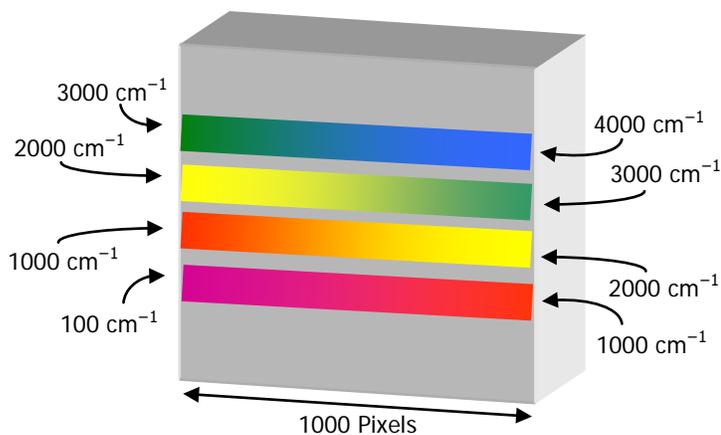
**Figure 7 Schematic of the RamanMicro 300 connected to the RamanStation 400F or RamanFlex 400 Series**

## The Echelle Spectrograph



**Figure 8 Schematic of the Echelle Spectrograph**

The RamanMicro 300 is connected to the Echelle spectrometer in the RamanStation 400F or RamanFlex 400 Series. The spectrometer contains two components; the Echelle spectrograph and a two-dimensional CCD detector. The spectrograph works by dispersing the light in two dimensions by using two gratings (Figure 8). 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 9 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 9 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 12 strips are acquired and resolution of up to  $2 \text{ cm}^{-1}$  (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

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

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

## ***Finding a Suitable Location for the RamanMicro 300***

---

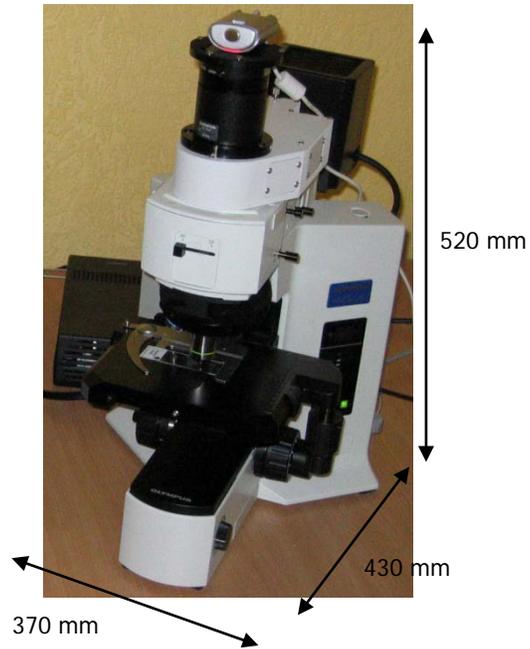
The following conditions should be observed:

- The environment must be suitable for a Class 3B laser system.
- If your RamanMicro 300 has a manual stage there must be 1 power point for the microscope in addition to the power points required for the spectrometer module. If your system has a motorized stage you will also require 1 power point for the stage controller box.
- The workbench must be flat and level.
- The workbench where the microscope is 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.

## ***Accessory Dimensions***

---

The RamanMicro 300 microscope weighs approximately 20 kg. Laboratory benching must be strong enough to support the combined weight of the microscope, spectrometer and any ancillary equipment without warping or wobbling.



**Figure 10 Dimensions of the RamanMicro 300**

**Installing Your  
RamanMicro 300**

## ***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.

<b>Part Number</b>	<b>Description</b>
L1320102	RamanMicro 300 Accessory Contains the microscope, the Raman module and the alignment camera module
L1323055 OR L1323011	Manual Stage Motorized Stage
L1323532	Two Port PCI Serial Card (systems with motorized stages only)
L1865749	RS232 Cable (systems with motorized stages only)
L1860289	Stage Controller (systems with motorized stages only)
L1865723	Joystick and cable assembly (systems with motorized stages only)
L1321678	Z-axis Focus Motor and Sleeve (systems with motorized stages only)
LX108897	SpectrumIMAGE Software Kit (systems with motorized stages only)
L1181228	Polystyrene Sample
N1870151	Microscope Tools Kit

**NOTE:** The accessory is intended for installation by a PerkinElmer Service Representative. There are no user-serviceable parts in the RamanMicro 300. The following sections are provided for your information only.

## ***Lifting the Instrument***

---

The RamanMicro 300 microscope weighs 20 kg. To avoid injury or damage to the accessory, please use proper lifting techniques.



**WARNING**

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



**WARNING**

*Always hold the instrument under the base when you lift it.*

## ***Assembly of the RamanMicro 300***

---

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



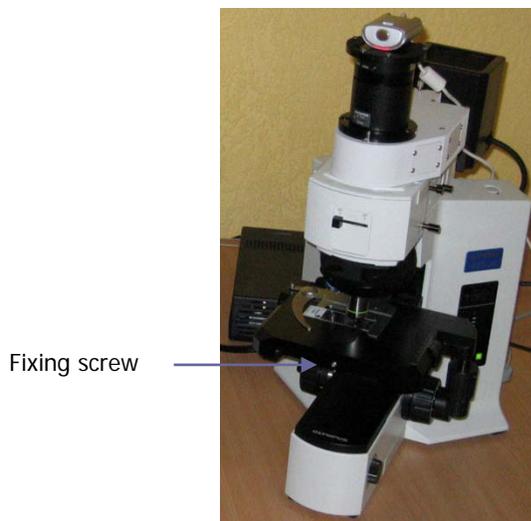
**WARNING**

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

### ***Fitting the Stage on the Microscope***

To fit the stage:

1. Unpack the stage from the shipping material.
2. Remove the objective nosepiece from the microscope by unfastening the grub screw on the right-hand side of the nosepiece.
3. Carefully locate the stage above the stage support ring and then lower the stage into position.
4. Ensure that the stage is level, and then fasten the stage in position by tightening the screw at the front of the stage (Figure 11).



**Figure 11 Fitting the stage (manual stage shown)**

5. Replace the objective nosepiece and tighten the securing grub screw.

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

## Connecting the Motorized Stage

### CAUTION

*If you have a RamanStation 400F spectrometer module you may have more than one joystick and stage controller box. To prevent damage to the stage or spectrometer modules we recommend that you clearly label them.*

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



**Figure 12 Stage controller and joystick**

To connect the motorized stage:

1. Connect the cable from the joystick to the socket labeled **JOYSTICK** on the rear panel of the stage controller box (Figure 13).



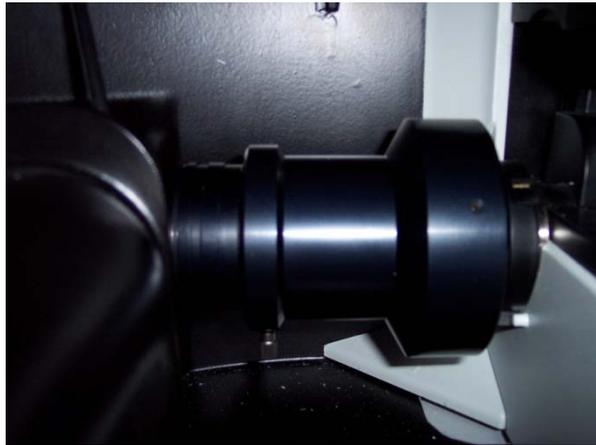
**Figure 13 Rear panel of the Stage Controller Box**

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

### **Attaching the Z-axis Focus Motor and Sleeve**

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

1. Slide the focus motor sleeve over the z-axis drive fine control knob on the left side of the microscope, and then tighten the connections using a hexagonal key.
2. Push the focus motor into the sleeve until it touches the drive control, and then tighten the screw to finger tight.
3. Connect the serial connector from the focus motor into the connection at the rear of the stage controller box labeled **MOTOR Z** (Figure 13).



**Figure 14 Z-axis focus motor and sleeve attached to the microscope inside the optional light-proof enclosure**

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

## Attaching the Illumination Source

To attach the illumination source to the illuminator module:

1. Hold the illumination source in position against the rear of the illuminator module.
2. Using the hexagonal key supplied (Figure 15), fasten the two screws so that the source is supported.
3. Connect the black cable from the illumination source to the microscope frame.

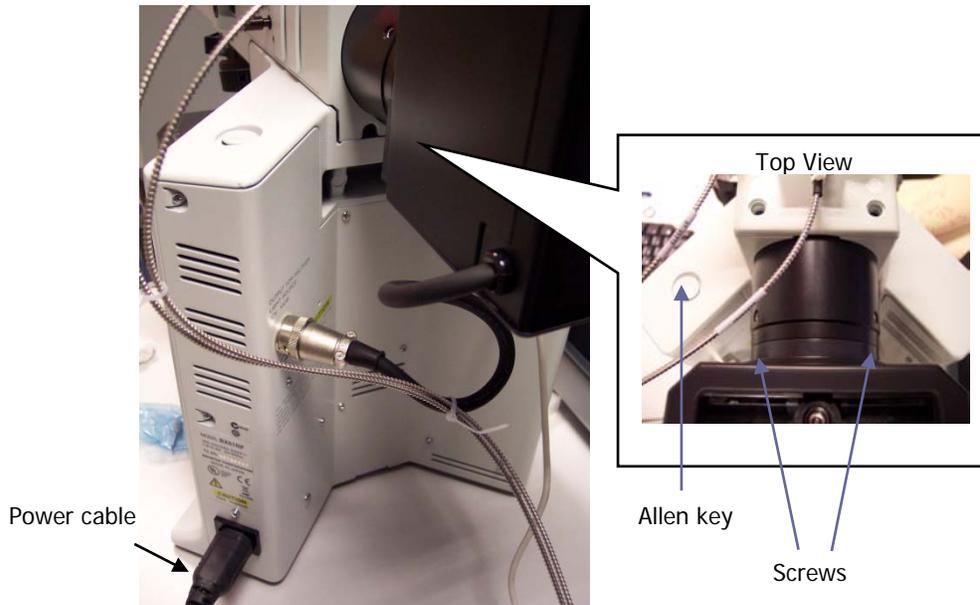


Figure 15 Attaching the illumination source to the microscope

## Connecting the Raman Module to the Microscope

The Raman module is positioned on top of the illuminator module so that the connector bracket is aligned above the mounting hole. Ensure that the left-hand side of the Raman module (as viewed from the front) is aligned with the illuminator module and then tighten the grub screw.

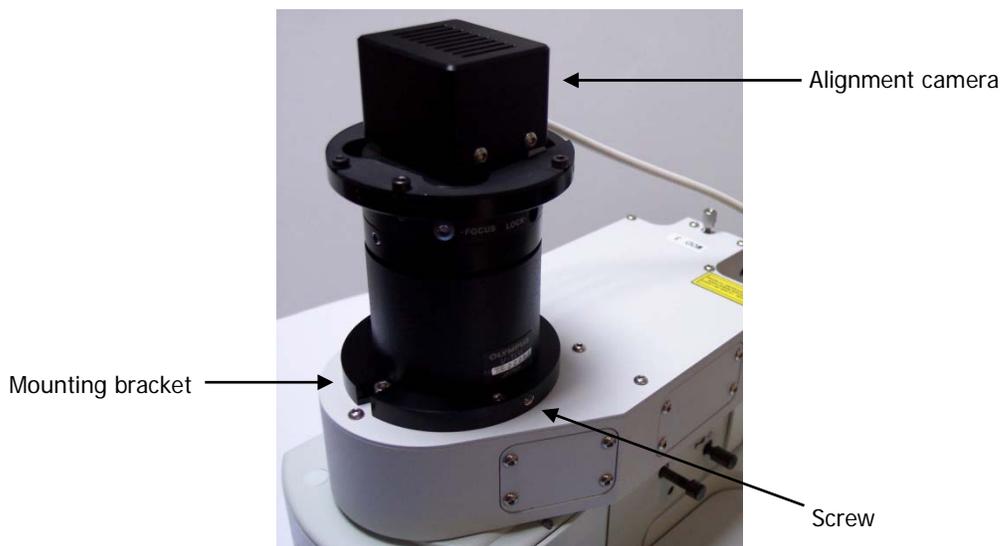


Figure 16 Connecting the Raman module

## Connecting the Alignment Camera to the Raman Module

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

**NOTE:** Do not attach more than two alignment cameras in the Raman system. If you connect the RamanMicro 300 to a RamanStation 400F, this system will have the maximum recommended two alignment cameras.



**Figure 17** Connecting the alignment camera

**NOTE:** Do not connect the USB cable from the alignment camera to the PC until after the software is installed. See *Installing the Instrument in Spectrum Software* on page 43.

## Connecting the RamanMicro 300 to the Spectrometer Module

The microscope is connected to the spectrometer module in the RamanStation 400F or RamanFlex 400 by two fiber optic cables. The excitation fiber transmits the laser energy from the spectrometer to the microscope, and the collection cable transmits the collected Raman data back to the detector.

**NOTE:** A RamanFlex 400 provides two sets of connectors. For a RamanFlex 400F, either set can be used. For a RamanFlex 400, only the left-hand set (below **LED FIBER 1**) should be used to connect a RamanMicro 300.

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

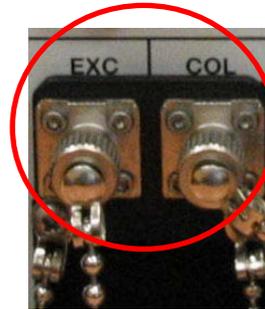
**CAUTION**

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



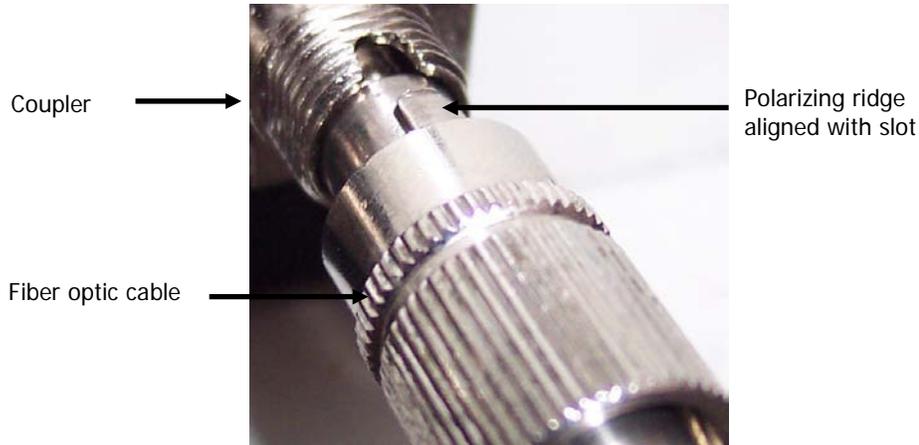
Excitation fiber

Collection fiber



**Figure 18** Fiber optic cables connecting the RamanMicro 300 to a RamanStation 400

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



**Figure 19 Connecting the fiber optic cable**

2. Connect the collection cable (part number L1323339) from the Raman module to the **COL** port on the rear panel of the spectrometer.  
Ensure that the end of the fiber with the white cable marker is attached to the Raman module.  
Make sure that the polarizing ridge on the fiber optic probe cable is aligned with the slot in the coupler casing before carefully pushing the cable into the coupler (Figure 19).

**CAUTION**

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

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

## **Installing the Instrument in Spectrum Software**

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

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

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

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

## Alignment of the Microscope



**WARNING**

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

The RamanMicro 300 system consists of three primary components:

- The microscope
- The Raman module
- The alignment camera module.

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

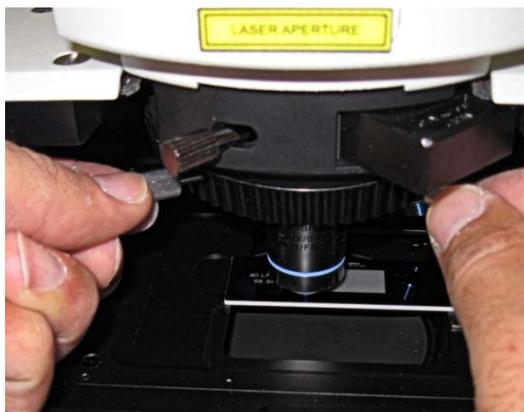
### Aligning the Microscope Objectives

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

The microscope objective nosepiece can fit up to 4 objectives.

1. Select the highest magnification objective lens on the microscope (either the 100x or 50x) by rotating the plastic ring on the objective nosepiece.
2. Select the Bright Field (BF) switch position on the microscope.
3. Place a slide with an easily recognizable feature on it (for example, a graticule slide).
4. In the Spectrum software, select **Raman Instrument** from the Setup menu. The Setup Instrument tabs are displayed.
5. On the Setup Instrument Basic tab, ensure that **Microscope** or **Microscope XYZ Stage**, as applicable, is selected as the Accessory.
6. Select the Setup Microscope or Setup Microscope XYZ Stage tab, as applicable, and then select **Live Video**.
7. If you have a RamanStation 400F, ensure that you are viewing the image for the microscope stage and not the sample compartment.
8. Move the stage until the feature is in focus and in the center of the crosshairs in the Live Video.  
Ensure that the image remains sharp and focused. If not, the tension adjustment on the coarse focus adjuster may not be tight enough.
9. Select the next highest magnification objective.

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



**Figure 20 Microscope objective adjusters**

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



**Figure 21 Feature in center of cross hairs at three objective magnifications**

## ***Aligning the Raman Module***

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

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

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

### ***Aligning the Laser Path***

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

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

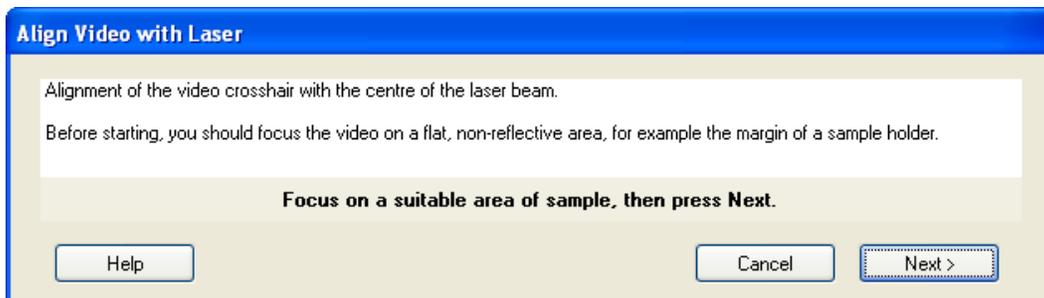
If the system is misaligned, we strongly recommended that you contact a PerkinElmer Service Representative. Changes to the laser path alignment will have a dramatic impact on the performance of your instrument.

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

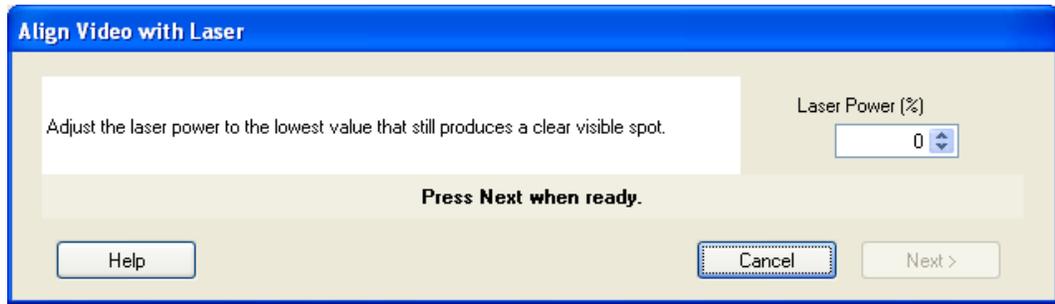
### ***Aligning the Video Camera with the Laser Spot***

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

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



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

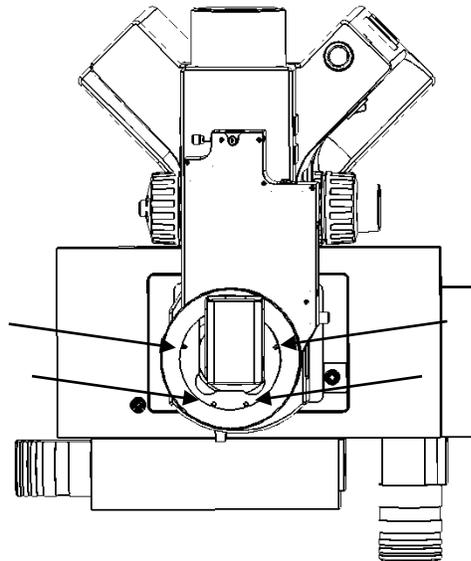


11. Adjust the **Laser Power** until you can see a clear visible spot.
12. If the laser spot is visible and close to the cross hairs, you do not need to physically adjust the camera module. Continue at step 16 to perform a fine adjustment to align the cross hairs with the laser spot.

OR

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

13. Loosen the 4 screws on the top plate of the Raman module until the camera is just free to move (Figure 22).

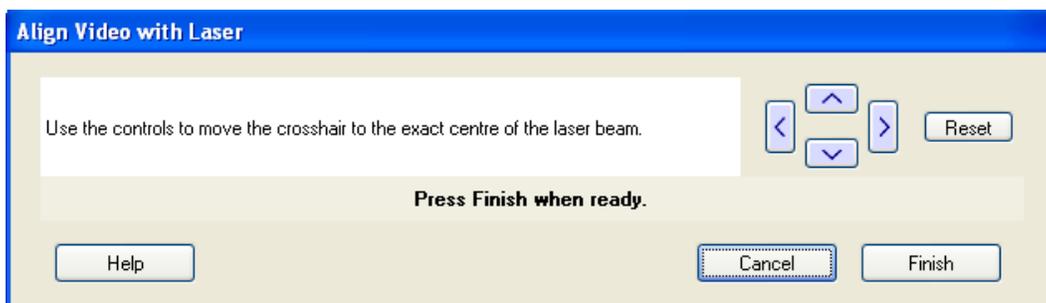


**Figure 22 Top view of the microscope showing the screws used to adjust the position of the camera module**

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

16. Click **Next**.

The following dialog is displayed.



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

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

### ***Aligning the Raman Coupler***

#### **CAUTION**

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

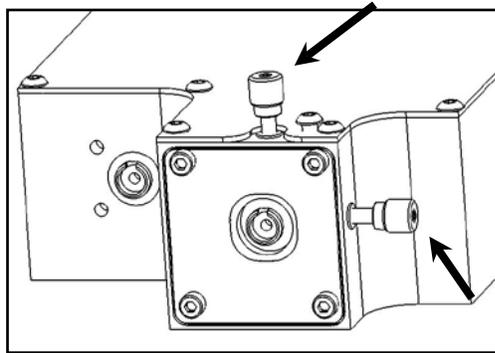
You should only adjust the Raman coupler if the signal throughput has decreased. This may occur if you have moved the unit. The only adjustment to the Raman coupler is the xy-adjuster at the rear of the Raman module (see Figure 23). The procedure for alignment is as follows:

1. Place a flat sample of silicon on the sample stage.  
We recommend that you purchase the sCAL Raman Calibration Standard (part number L1321843) for this alignment procedure.
2. Loosen, but do not remove, the 4 retaining screws at the coupler so that about 0.5 mm of thread is showing.
3. Ensure that the Bright Field (BF) switch position is selected on the microscope, and that the illumination is turned on.
4. In the Spectrum software, select **Raman Instrument** from the Setup menu.  
The Setup Instrument tabs are displayed.
5. On the Setup Instrument Basic tab, ensure that **Microscope** or **Microscope XYZ Stage**, as applicable, is selected as the Accessory.
6. Select the appropriate **Sample Holder** in the drop-down list.
7. Select the lowest magnification objective on the microscope.
8. Ensure that the correct objective it is selected in the Microscope Objective drop-down list on the Setup Instrument Basic tab.

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

14. Click  .  
The RamanMicro 300 will start collecting data in the Live display.

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



**Figure 23 Adjustment of the XY Raman coupler**

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

## Moving the Instrument

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**WARNING**

*The RamanMicro 300 microscope weighs about 20 kg. The spectrometer module used with the RamanMicro 300 microscope accessory weighs about 20 kg. Two people are needed to lift the instrument.*

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

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 64.

**Using the**  
**RamanMicro 300**

## General SOPs

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The RamanMicro 300 accessory when combined with a RamanFlex 400 Series or a RamanStation 400F and Spectrum software is a very flexible system. This chapter demonstrates how to perform a number of tasks. The procedures provided may not be the most straightforward or simple for the task you wish to perform, so treat these Standard Operating Procedures (SOPs) as a starting point and with time you may wish to develop your own SOPs which better suit your specific mode of use. These procedures also act as a “learn by doing” section allowing you to explore the functionality of the software and spectrometer.

The General SOPs demonstrate how to collect data when your samples are on the microscope stage. For step-by-step instructions for calibrating the spectrometer, see *Calibration and Performance Verification SOPs* on page 64.

**NOTE:** The RamanMicro 300 system can be purchased with either a manual or a motorized stage.

### SOP-1: Turning the Raman System On

The RamanMicro 300 must be used in conjunction with the RamanStation 400F or RamanFlex 400 Series instruments.

1. Switch on the PC and monitor, and log in to Windows.
2. Ensure the laser power key on the front panel of the spectrometer is in the ARM position.
3. If applicable, ensure that the laser safety interlock is satisfied.
4. Ensure the instrument power supplies are connected to a mains outlet and that the outlets are switched on.

If your accessory is connected to a RamanFlex 400, there will be one instrument power supply unit (PSU). If your accessory is connected to a RamanStation 400F, there will be one instrument PSU and one PSU for the stage controller box. You also need to ensure that the microscope power cord is connected to a mains outlet. If you have a motorized stage, you will need to switch on the power supply unit.

5. To turn on the instrument, press the PSU power switch ON (Figure 24).

| - ON                      O - OFF



**Figure 24** The PSU power switch

6. Check that the Power LED on the spectrometer 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. Switch on the microscope and the microscope illumination.
8. If you have a motorized microscope stage, switch on the stage controller box.
9. From the Start menu select **All Programs**; the **PerkinElmer Applications** group; the **Spectrum** sub-group and then the **Spectrum** application.  
The Spectrum start-up screen is displayed; followed by a dialog that may require your login details:



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

OR

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

12. Click **OK**.  
Spectrum starts.  
If you selected to connect to the instrument, it will prepare for use by turning on the laser and cooling the detector to its operating temperature (usually  $-50\text{ }^{\circ}\text{C}$ ). Messages will be displayed in the Status bar at the bottom of the workspace, and in the Scan icons on the Measurement bar.

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

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

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

## ***SOP-2: Turning the Raman System 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. If required, switch off the instrument by pressing the power switches on the PSUs to OFF:

| - ON            O - OFF

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

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

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

The instrument will shut down automatically after the appropriate time period. Alternatively, you can shut down the instrument by right clicking on the Raman instrument icon (RamanStation 400F or RamanFlex 400 Series) 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 79 for details of how to shut down the instrument and restart the software.

### ***SOP-3: Setting the Limits of the Motorized Stage***

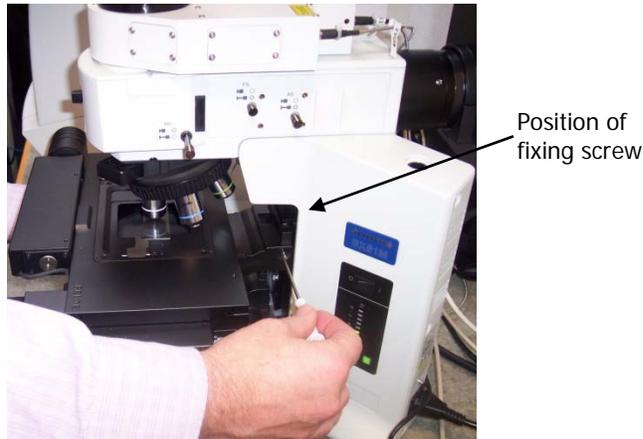
**NOTE:** If you move the stage, you may also have to check the alignment of the microscope objectives. Refer to *Aligning the Microscope Objectives* on page 44.

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

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

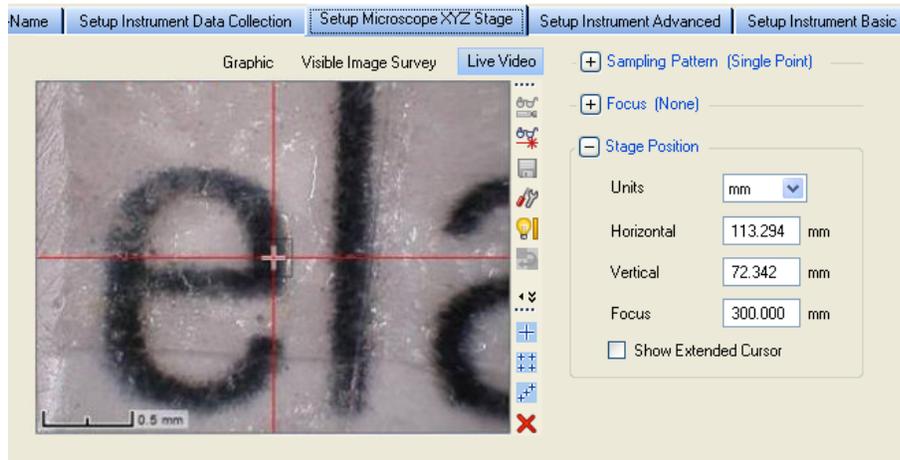
1. Ensure the instrument is switched on by following SOP-1.  
If the stage controller box was switched off, you will be required to reset the stage before using the motorized stage.
2. Select **Raman Instrument** from the Setup menu.  
The Setup Instrument tabs are displayed.
3. Select the Setup Instrument Basic tab.

4. Ensure that **Microscope XYZ Stage** is selected in the Accessory drop-down list. The options available will depend upon your instrument configuration.
5. Ensure that the correct **Microscope Objective** is selected in the software.
6. Ensure that the pre-focusing lever on the coarse adjustment control is on the off position.  
The pre-focusing lever restricts the travel of the coarse adjustment control, and is located on the left-hand side of the microscope. The lever is in the off position when it is rotated counter-clockwise away from you when facing the microscope. Refer to the Olympus manual provided with your microscope for further information.
7. Loosen the fixing screw on the side of the microscope (Figure 25).



**Figure 25 Adjusting the height of the motorized stage**

8. Manually move the stage down until the stage is at its lowest position.
9. Re-secure the fixing screw to fix the height of the stage.
10. Select the Setup Microscope XYZ tab.



**Figure 26 Setup Microscope XYZ Stage tab showing the Live Video view**

11. Select  **Toolbox** and then select **Reset Stage** from the sub-menu.

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

**CAUTION**

*Be very careful when moving the stage not to touch the objectives.*

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

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

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

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

**CAUTION**

*Once you have reset the stage you should use the joystick ONLY to raise or lower the stage. DO NOT try to adjust the stage manually, or you will have to reset the stage.*

*When using the joystick, take care not to continue using the motors when the stage has reached the lower or upper limits of travel. To see if you have moved outside the limits, check the z-axis focus dials on the right-hand side of the microscope. If the two wheels counter-rotate, you will need to reset the stage. If you continue to move the stage, the cable can become trapped and the motor could be damaged.*

## SOP-4: Sample Positioning using a Manual Stage

1. Lower the microscope sample stage to allow sufficient clearance for your sample and the longest objective.
2. Place the sample on a microscope slide and place it on the stage.

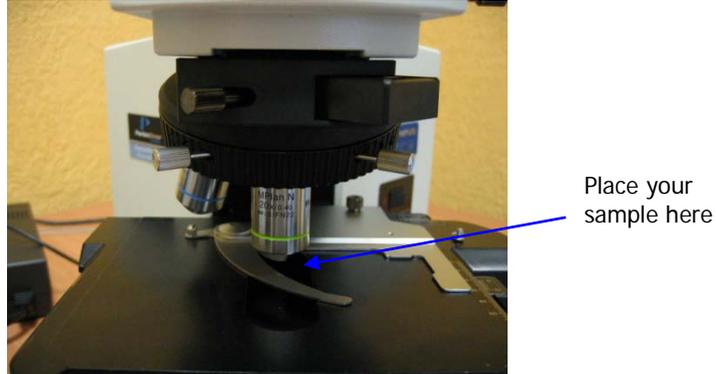


Figure 27 Positioning the sample under the objective lens

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

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

4. Move the stage so that the sample is approximately lined up below the objective lens.
5. Select the Bright Field (BF) switch position on the microscope.
6. Switch on the illumination source.  
The switch is located on the right side of the microscope frame.
7. Set the required light intensity using the adjustment knob on the right side of the microscope frame base.
8. Select **Raman Instrument** from the Setup menu.  
The Setup Instrument tabs are displayed.
9. Select the Setup Instrument Basic tab.

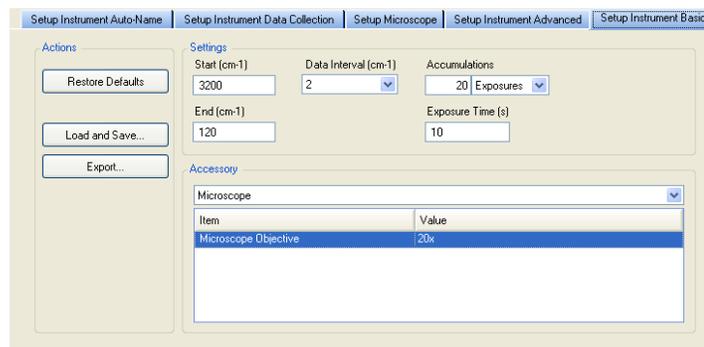


Figure 28 Setup Instrument Basic tab with the Microscope accessory selected

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



**Figure 29 Setup Microscope tab**

14. Focus on the sample using the microscope z-axis control knobs.  
Take care not to crush the sample or dirty the objectives. The working distances of the objective lenses supplied as standard are 20 mm (5x), 1.3 mm (20x) and 0.38 mm (50x).
15. Accurately position the sample in the x and y directions using the stage control knobs.
16. If higher magnification is required, select the next most powerful objective lens on the microscope.
17. Select the appropriate objective lens from the **Microscope Objective** drop-down list.
18. Focus on the sample and again move the sample in x and y directions, if necessary.  
The crosshairs indicate the spot where analysis takes place.
19. When the sample is correctly positioned, switch off the illumination source.
20. Select the Dark Field (DF) switch position on the microscope.
21. If the microscope light-proof enclosure is fitted, close the doors.
22. Click  .  
The RamanMicro 300 will start collecting data in the Live display.  
You can use Monitor to help you optimize the Exposure time and Laser power for your sample, and adjust the focus of your sample to maximize the Raman signal. The data displayed on screen when monitoring cannot be saved. To save data you must collect spectra.
23. Click  to exit the Live display.

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

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

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

## SOP-5: Sample Positioning using a Motorized Stage



### WARNING

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

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

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

2. Select the Bright Field (BF) switch position on the Olympus illuminator module.
3. Switch on the illumination source.
4. Select **Raman Instrument** from the Setup menu.  
The Setup Instrument tabs are displayed.
5. Select the Setup Instrument Basic tab.

The screenshot shows the 'Setup Instrument Basic' tab with the following settings:

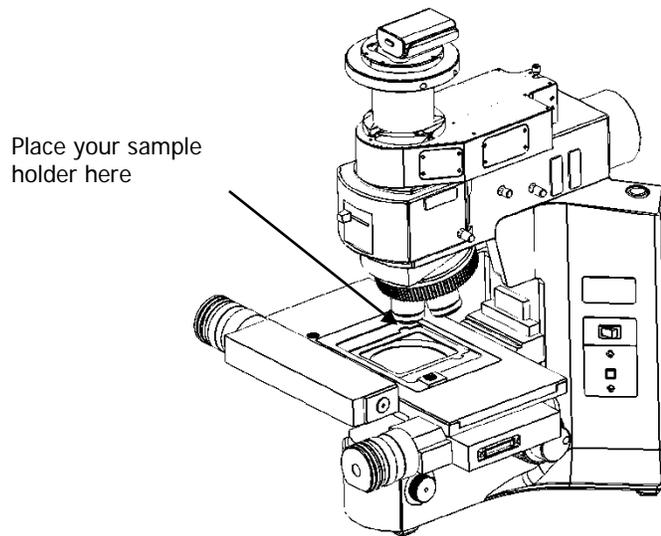
- Actions:** Restore Defaults, Load and Save..., Export...
- Settings:**
  - Start (cm<sup>-1</sup>): 3620
  - Data Interval (cm<sup>-1</sup>): 2
  - Accumulations: 10 Exposures
  - End (cm<sup>-1</sup>): 250
  - Exposure Time (s): 10
- Accessory:** Microscope XYZ Stage
 

Item	Value
Microscope Objective	5x
Sample Holder	Microscope Slide

**Figure 30 Setup Instrument Basic tab with the Microscope XYZ Stage accessory selected**

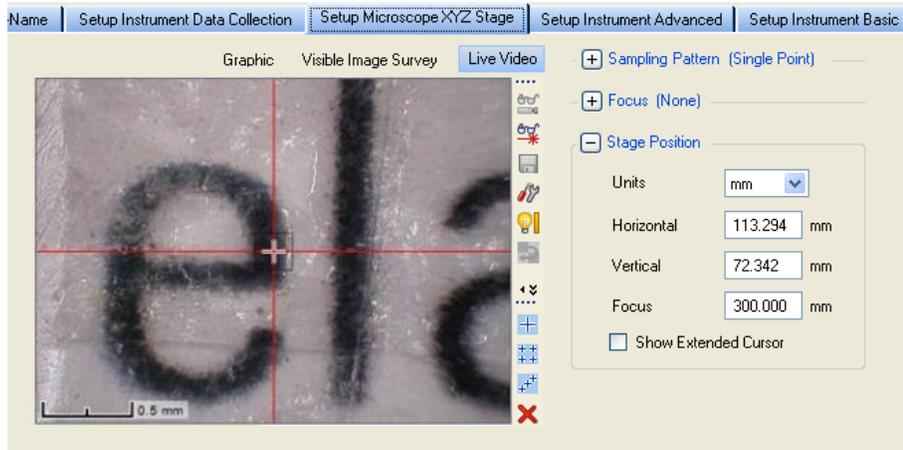
6. Ensure that **Microscope XYZ Stage** is selected in the Accessory drop-down list.  
The options available will depend upon your instrument configuration.
7. Select **5x** from the **Microscope Objective** drop-down list.

8. Load your sample into a suitable sample holder.
9. Select the appropriate Sample Holder in the drop-down list, and follow the instructions on screen to load the sample holder onto the sample stage.



**Figure 31 Sample position on motorized stage**

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



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

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

The crosshairs indicate the spot where analysis takes place.

The joystick operates in the following manner:

- Left / Right joystick movement gives x movement of the stage.
- Top / Bottom joystick movement gives y movement of the stage.
- Twisting the joystick gives z movement of the stage:  
Left twist (counter clockwise) gives upward stage movement;  
Right twist (clockwise) gives downward stage movement.

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

12. If higher magnification is required, select the next most powerful objective lens on the microscope.  
The microscope is supplied with 5x, 20x and 50x objective lenses.
13. Select the appropriate objective lens from the **Microscope Objective** drop-down list.
14. Focus on the sample and, if necessary, move the stage in *x* and *y* directions to accurately position the sample.
15. When the sample is correctly positioned, switch off the illumination source.
16. Select the Dark Field (DF) switch position on the Olympus illuminator module.
17. If the microscope light-proof enclosure is fitted, close the doors.

18. Click .

The RamanMicro 300 will start collecting data in the Live display.

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

19. Adjust the **Laser power (%)** in the Live display to an appropriate value for your sample.
20. Enter an **Exposure time (s)**.  
Changing the exposure time will automatically acquire another background.

21. Click  to exit the Live display.

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

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

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

## SOP-6: 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.

1. Ensure that the instrument is switched on by following SOP-1.  
If the stage controller box was switched off, you will be required to reset the stage before using the motorized stage.
2. Start the Spectrum software and then select **Raman Instrument** from the Setup menu. The Setup Instrument tabs are displayed in the Dialog pane. They enable you to choose the accessory, and setup and save the experimental parameters.
3. Select the Setup Instrument Basic tab.

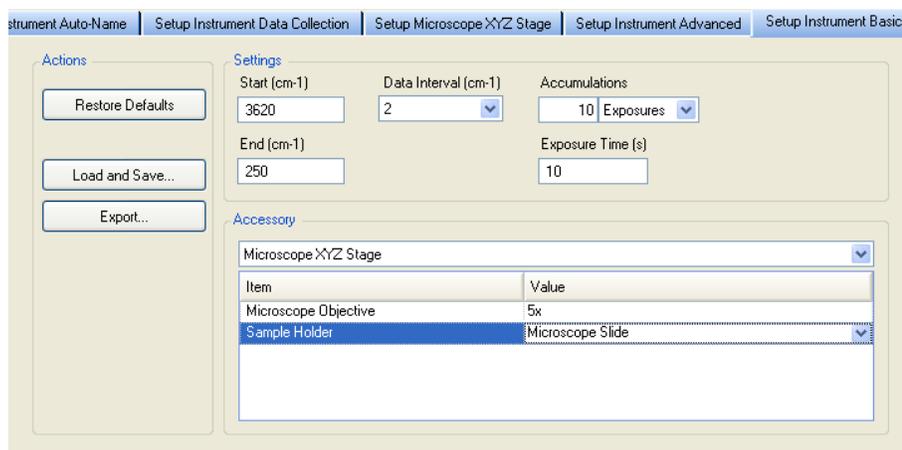


Figure 33 Setup Instrument Basic tab

4. Select **Microscope** or **Microscope XYZ Stage** as appropriate from the **Accessory** drop-down list.  
Depending on the Accessory you have selected, further options may become available.
5. Ensure that the appropriate **Microscope Objective** and **Sample Holder** are selected.
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  $\text{cm}^{-1}$  to 100  $\text{cm}^{-1}$ ), 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. This can be done 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 ( $\text{cm}^{-1}$ ).  
The default data interval is 2  $\text{cm}^{-1}$ . 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.  
To display the Setup Instrument tabs, select **Raman Instrument** from the Setup menu. The tabs are displayed in the dialog pane.
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 motorized stage, select the Setup Microscope XYZ Stage tab, and then select the **Sampling Pattern**.  
Select the sampling pattern type from **Single Point**, **Super Macro Point**, **Custom Grid** and **Custom Line**. Any spectra collected using SuperMacro, Custom Grid or Custom Line sampling patterns are coadded to produce one spectrum.  
For more information, see the Spectrum on-screen Help.
14. If you have a motorized stage, select any **Focus** options.  
You can select to perform a **Raman AutoFocus** or **Video AutoFocus** before each sample measurement.
15. When you have finished defining your experimental parameters, position and optimize conditions for your sample as described in SOP-4 and SOP-5.
16. Click  .

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

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

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

## **Calibration and Performance Verification SOPs**

Several different automated calibration tests are included in the software:

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

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

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

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

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

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

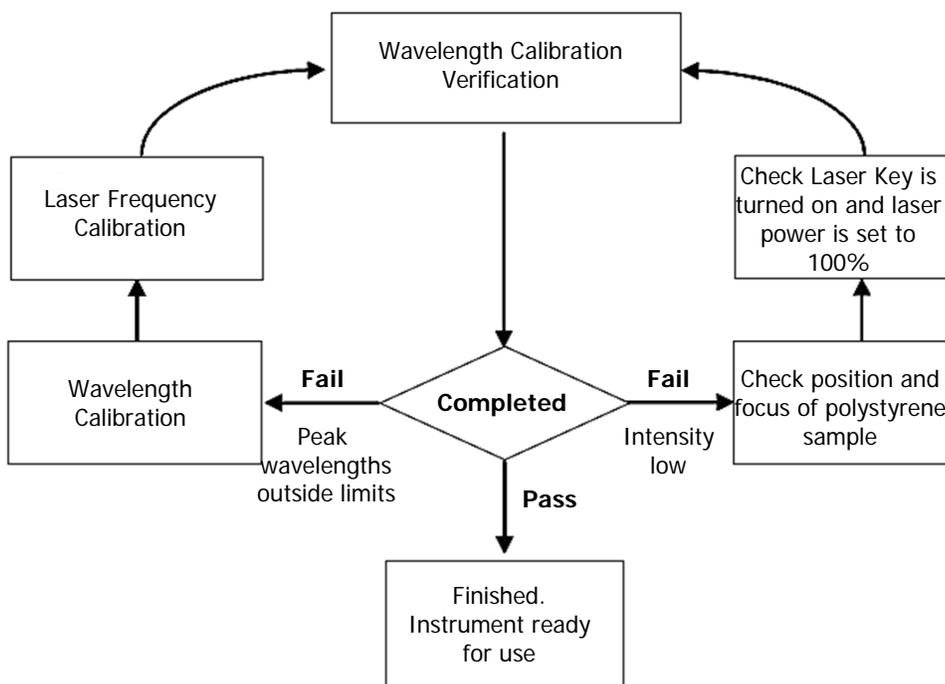
### ***SOP-7: Calibration/Performance Verification***

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  $2\text{ cm}^{-1}$  or smaller.

On completion of the wavelength calibration verification, the results of the verification procedure is displayed. A spectrum detailing the outcome of the verification (or calibration) 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, the laser key is not switched on, or the laser power is too low. Then repeat the wavelength calibration verification to ensure that instrument has been brought back within specification. If the instrument fails the wavelength calibration verification again, please contact PerkinElmer.

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

### ***SOP-8: Wavelength Calibration Verification***

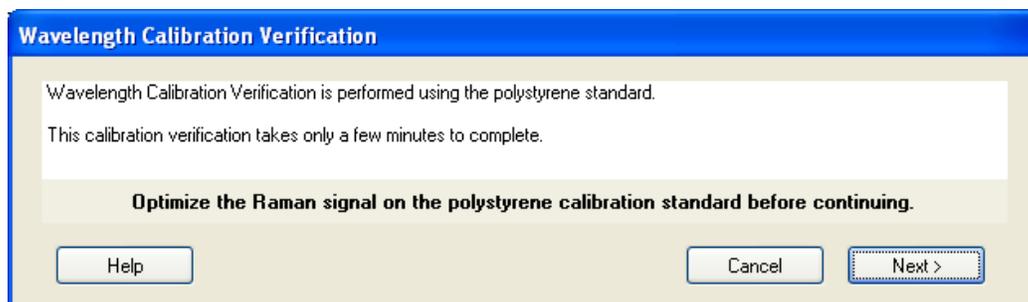
To perform the Wavelength Calibration Verification with the Microscope 50x objective selected:

1. Ensure that the instrument is switched on.  
If not, follow SOP-1.
2. Select the 50x objective lens on the microscope.
3. Place a polystyrene sample (L1181228) in a suitable sample holder.
4. Select **Raman Instrument** from the Setup menu.  
The Setup Instrument tabs are displayed.

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

13. Select .

The Wavelength Calibration Verification dialog is displayed.



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

The instrument is now ready for use.

In the unlikely event that the wavelength calibration verification should fail, follow Figure 34. If the wavelength calibration verification fails for the RamanMicro 300 Accessory, this does not necessarily mean that the performance of any other accessories attached to your instrument is also affected.

## SOP-9: Wavelength Calibration

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

1. Ensure that the instrument is switched on.  
If not, follow SOP-1.
2. Attach the external neon calibration accessory (L1320220) to the rear panel of the spectrometer module.  
Push the large connector into the socket labeled **CALIBRATION** (RamanFlex 400 Series) or **CAL ACCY** (RamanStation 400F) and carefully attach the small fiber optic cable to the **COL** coupler below the **LED FIBER 1** connector port (Figure 35).



**Figure 35 Attaching the calibration accessory**

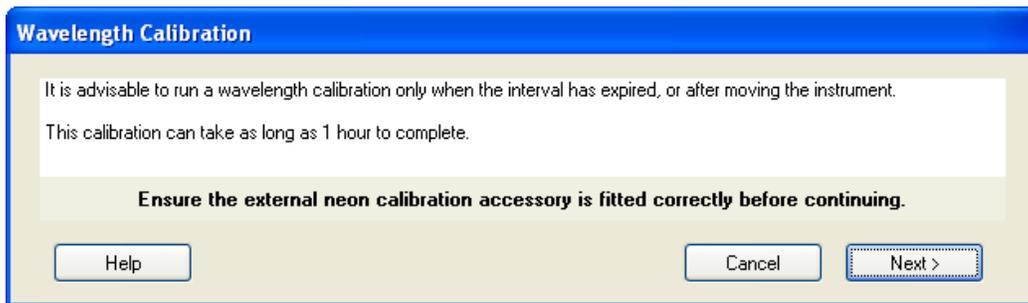
**NOTE:** As wavelength calibration is not required on a regular basis, the external neon calibration accessory is not included in the basic RamanMicro 300 package. The external neon calibration accessory is included in the RamanMicro Calibration Kit (L1320266), which can be purchased separately.

3. Rotate the objective nosepiece until mid-way between two objectives.
4. Cover the microscope with a dark cloth or, if fitted, close the light-proof enclosure doors and ensure that caps are placed on any external fiber optic probes or accessories, to minimize stray light from interfering with the calibration. If you have a RamanStation 400F, ensure that the sample compartment is also closed.
5. Select the Dark Field (DF) switch position on the Olympus illuminator module and switch off the illumination.
6. Select **Raman Instrument** from the Setup menu.  
The Setup Instrument tabs are displayed.
7. Select the Setup Instrument Advanced tab and click **Toolbox**.  
The Adjustments Toolbox dialog is displayed.



8. Select .

The Wavelength Calibration dialog is displayed.



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

The instrument is now wavelength calibrated.

## ***SOP-10: Laser Frequency Calibration***

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

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

1. Ensure that the instrument is switched on.  
If not, follow SOP-1.
2. Select the 50x objective lens on the microscope.
3. Place a polystyrene sample (L1181228) in a suitable sample holder.
4. Select **Raman Instrument** from the Setup menu.  
The Setup Instrument tabs are displayed.
5. On the Setup Instrument Basic tab select **Microscope** or **Microscope XYZ Stage** as appropriate from the **Accessory** drop-down list
6. Select **Microscope 50x** from the Objective drop-down list.
7. Select the appropriate **Sample Holder** in the drop-down list and load the sample holder onto the sample stage, following the instructions on-screen if you have a motorized stage.

8. Select the Dark Field (DF) switch position on the microscope and switch off the illumination.
9. Cover the microscope with a dark cloth or, if fitted, close the light-proof enclosure doors and ensure that caps are placed on any external fiber optic probes or accessories, to minimize stray light from interfering with the calibration.
10. Select the Setup Instrument Advanced tab, and ensure that the **Laser Power** is set to 100%.
11. Focus on the sample of polystyrene.

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

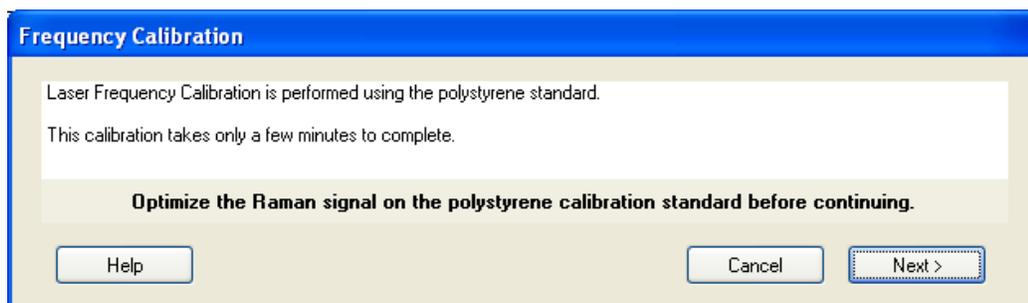
If you do not have a motorized stage, you may find it useful to Monitor your sample to view the Raman signal while manually adjusting the focus controls.

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

The Adjustments Toolbox dialog is displayed.

13. Select .

The Frequency Calibration dialog is displayed.



14. Ensure that you have followed the instructions on the dialog, and then click **Next**.  
The instrument will now perform the laser frequency calibration routine. This will take a few minutes. When the calibration is complete, the result is displayed.

The instrument is now laser frequency calibrated.

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

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

## SOP-11: Intensity Calibration

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

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

The NIST intensity correction is valid in the recommended operating temperature range 20 °C – 25 °C. If you move the instrument to an environment outside this temperature range, that is 15 °C – 20 °C or 25 °C – 35 °C, we strongly recommend that you perform a NIST intensity correction at this new temperature. Contact your PerkinElmer Raman Business Unit.

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

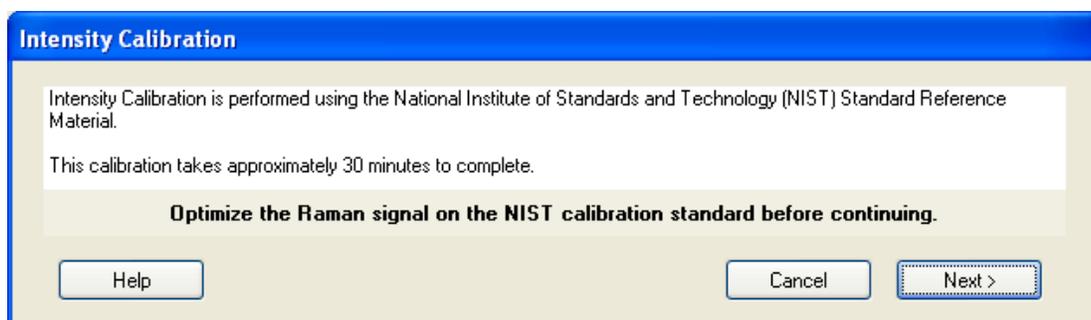
**NOTE:** As intensity calibration is not required on a regular basis, NIST Standards are not included in the basic RamanMicro 300 package. Standard Reference Materials can be purchased from PerkinElmer (L1321831). Your instrument will be intensity calibrated prior to shipping and during installation

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

1. Ensure that the instrument is switched on.  
If not, follow SOP-1.
2. Select the 50x objective lens on the microscope.
3. Place the NIST standard in a suitable sample holder.
4. Select **Raman Instrument** from the Setup menu.  
The Setup Instrument tabs are displayed.
5. Select **Raman Instrument** from the Setup menu.  
The Setup Instrument tabs are displayed.
6. On the Setup Instrument Basic tab select **Microscope** or **Microscope XYZ Stage** as appropriate from the **Accessory** drop-down list
7. Select **Microscope 50x** from the Objective drop-down list.
8. Select the appropriate **Sample Holder** in the drop-down list and load the sample holder onto the sample stage, following the instructions on-screen if you have a motorized stage.
9. Select the Dark Field (DF) switch position on the microscope and switch off the illumination.

10. Cover the microscope with a dark cloth or, if fitted, close the light-proof enclosure doors and ensure that caps are placed on any external fiber optic probes or accessories, to minimize stray light from interfering with the calibration. If you have a RamanStation 400F, ensure that the sample compartment is also closed.
11. Select the Setup Instrument Advanced tab, and ensure that the **Laser Power** is set to 100%.
12. Focus on the appropriate NIST standard with the dull surface facing upwards.  
If you have a motorized stage you can use the joystick or Stage Position controls on the SetupMicroscope XYZ Stage tab. Alternatively, click  **Raman AutoFocus** to focus using the strongest Raman signal. The Focus Limits should be set to **Full Range**.  
If you do not have a motorized stage, you may find it useful to Monitor your sample to view the Raman signal while manually adjusting the focus.
13. When the polystyrene sample is optimally focused for Raman signal, select the Setup Instrument Advanced tab and click **Toolbox**.  
The Adjustments Toolbox dialog is displayed.

14. Select .  
The Intensity Calibration dialog is displayed.



15. Ensure that you have followed the instructions on the dialog, and then click **Next**.  
The instrument will now perform the intensity calibration routine. This will take some time. At the end of the calibration, the result will be displayed.  
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 (ARM).
16. Repeat steps 2–15 for each objective lens.
17. Remove the intensity correction standard and store safely.

## Summary

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

## ***Additional Parts and Upgrades***

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### ***Microscope Objective Lenses***

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

### ***Sampling Accessories***

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

**SERS Consumables**

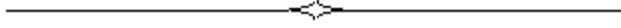
<b>Part Number</b>	<b>Description</b>
L1329300	SERS gold colloid – 50 nm particles (20 ml)
L1329307	SERS gold colloid – 100 nm particles (20 ml)
L1329308	SERS gold colloid – 200 nm particles (20 ml)
L1329301	Aggregating agent – MgSO <sub>4</sub> 0.16 M in milli-Q water (20 ml)
L1329303	Standard crystal 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**

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

**Microscope Light-Proof Enclosure**

<b>Part Number</b>	<b>Description</b>
L1320161	RamanMicro 300 enclosure kit



# **Maintenance and** **Troubleshooting**

## Maintenance

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

### ***Cleaning the Spectrometer and Power Supply Units***



**WARNING**

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

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

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



**WARNING**

*Do not directly clean electrical contacts.*



**WARNING**

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



**WARNING**

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

## ***Maintenance and Service of the Microscope***

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

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

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

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

## **Replacing the Stage Controller Fuse**

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



### ***Electrical hazard***

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

*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

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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

<b>Problem:</b>	<b>During the installation procedure the USB devices are not recognized.</b>
<b>Description</b>	This may mean that there is not enough power on the USB sockets on your PC.
<b>Solution</b>	Ensure that your PC has USB 2.0 sockets. If you are not sure, use a USB 2.0 power hub.
<b>Problem:</b>	<b>You are prompted for the CCD drivers.</b>
<b>Description</b>	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.
<b>Solution</b>	Refer to the <i>Spectrum Administrator's Guide</i> (L1050095) for details of how to reinstall the drivers.
<b>Problem:</b>	<b>Cannot collect data because an interlock is breached.</b>
<b>Description</b>	This means that, for some reason, the interlock system is detecting an open circuit.
<b>Solution 1</b>	Ensure all interlocks, including any remotely interlocked doors, are closed properly.
<b>Solution 2</b>	Ensure your external interlock is wired up correctly and that any interlocked room entrances or sampling enclosures are closed.

### Software

<b>Problem</b>	<b>Spectrum software stops responding.</b>
<b>Description</b>	When you are using the Software, for some reason it stops working, that is, mouse clicks have no effect.
<b>Solution</b>	Hold down the CTRL+ALT+DELETE keyboard buttons to display the Windows Security dialog. Click <b>Task Manager</b> and then click the <b>Processes</b> tab. In the list under <b>Image Name</b> look for <b>IRWinLab.exe</b> (to find this you may have scroll through the list using the scroll bar on the right side) click on this, then click <b>End Process</b> . Then select <b>Raman.exe</b> and then click <b>End Process</b> . If a Task Manager Warning dialog is displayed click <b>OK</b> . Re-start the software. If you are unsure how to do this please refer to SOP-1. <b>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.</b>

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**Problem:**      **The motorized stage does not move to the expected position on a sample holder when requested.**

**Solution 1**      Go to the Setup Instrument Basic tab and ensure that the selected sample holder is correct. If it is not, then select the appropriate sample holder. Check the Graphic view on the Setup Microscope XYZ Stage tab – it may be that you were directing the stage to a prohibited region of the sample holder (hashed area on the Graphic view).

**Solution 2**      Go to the Setup Instrument Basic tab and ensure that the selected microscope objective is correct. If the wrong microscope objective is selected then the wrong scale will be displayed on the Setup Microscope XYZ Stage tab

**Solution 3**      If the Accessory options are correct, it may be that the stage needs to be reset. On the Setup Microscope XYZ Stage tab, click  **Toolbox** and then select **Reset Stage**. The motorized accessory will now reset itself.

**Solution 4**      If the above solutions do not solve your problem, you should contact your PerkinElmer Service Representative.

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

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

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

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**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

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## ***Decontamination***

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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:

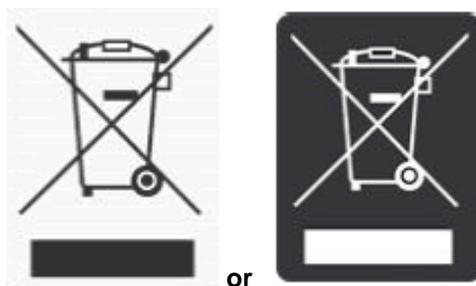
<http://las.perkinelmer.com/OneSource/decontamination.htm>

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

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

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

## **WEEE Instructions for PerkinElmer Products**



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

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

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

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

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

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