



Raman Spectroscopy

An Introduction to JASCO Raman Spectrometers

This guide briefly introduces the theory and applications of Raman spectroscopy in materials analysis, using JASCO dispersive and Fourier-transform Raman instrumentation. More detailed theoretical treatments and different instrument configurations, together with advanced Raman techniques are covered in a number of textbooks. The World-Wide Web / Internet is also a recent and growing source for Raman basic background information. Try accessing the following URL's:

http://www.icmm.csic.es/Fagullo/ramw3_e.htm

<http://www-personal.umich.edu:80/~jshaver/virtual/virtual.html>

<http://www.ictp.csic.es/ramanft/erft/home.htm>

Key Points

- **Raman scattering:** light that is weakly, inelastically scattered from any material. A change in the light's energy (wavelength) takes place, that is characteristic of the material's chemical structure. Less than 10^{-6} of any scattered light is inelastically scattered.
- **Rayleigh scattering:** The strong, elastic scattering of light from any material, with zero change in the light wavelength.
- **Stokes scattering:** Raman scattered light that has lost some energy to the scatterer, and so is red-shifted to a longer wavelength. "Normal" Raman spectra contain only Stokes peaks.
- **Anti-Stokes scattering:** Raman scattered light that has gained some energy from the scatterer, and so is blue-shifted to a shorter wavelength. The intensity of anti-Stokes scattering is lower than Stokes, at room temperatures.
- **Micro-Raman:** A high NA microscope objective (100x ~ 150x) and confocal (pinhole) aperture are used to illuminate and collect from a very small sample volume, typically $1\mu\text{m} \times 1\mu\text{m}$ in area and about $\sim 2\mu\text{m}$ depth. Analyzed fluorescing volume is limited.
- **Macro-Raman:** Where a lower NA, longer working distance lens illuminates a larger ($\sim 100\mu\text{m}$ or more) region of sample. Ideally used for homogeneous powders, liquids, gases, etc. Fluorescence can be a limiting factor with visible laser excitation.
- **Dispersive Raman:** The Raman spectrum is analysed by using a diffraction grating or series of gratings, that may be fixed (CCD detector) or scanned (PMT detector).

Background History

Raman spectroscopy is an old analytical technique that is recently undergoing a tremendous revival due to technological advances in lasers, detectors and spectroscopic optical systems. 1930 Nobel Physics Laureate *Sir Chandrasekhara Venkata Raman*, first demonstrated inelastic scattering of light in 1928. When light is scattered by any form of matter, the energies of the majority of the



photons are unchanged by the process, elastic or *Rayleigh* scattering. However, about one in one million photons lose or gain energy that corresponds to the vibrational frequencies of the scattering molecules. This can be observed as additional peaks in the scattered light spectrum. The process is known as *Raman* scattering and the spectral peaks with lower and higher energy than the incident light are known as *Stokes* and *anti-Stokes* peaks respectively. Most routine Raman experiments use the Stokes peaks only, because they are more intense at room temperatures.

Sir C.V. Raman

Very strong monochromatic light sources are needed for Raman spectroscopy (Raman himself used sunlight focussed through a telescope!) but despite the difficulties, by the 1930's the method had gained considerable popularity. Because the Raman scattered light is so weak, early work required slow, photographic collection of the spectra- taking many hours- and with the invention of infrared absorption spectroscopy, the technique lost popularity. The availability of intense laser light sources from the 1970's onwards and increased Raman signal intensities began the method's revival, which was continued by improved detector technology.

From being an expensive, slow and specialist technique only two decades ago, Raman is now an everyday method that is becoming faster, more portable, and less expensive as the necessary lasers, detectors and holographically processed optical components continue to fall in price. Raman spectra can now be acquired in real-time from almost any material both in the laboratory, the factory process line or specialist on-site locations. It is a non-destructive and non-invasive method: samples inside glass bottles or transparent plastic containers can be analysed without breaking the seal or risking contact with toxic samples.



Using a Raman probe to examine a bottle's contents

Advantages of Raman Spectroscopy

Although the most widely used structural spectroscopic method is still Fourier-transform infrared (FT-IR), the often problematic sample preparation needed for IR is unnecessary for Raman: most samples are simply placed on the sample stage or holder and measured “*as-is*”. Glass is essentially transparent for the Raman process and materials can be measured inside glass vessels such as bottles, capillary tubes and cells.

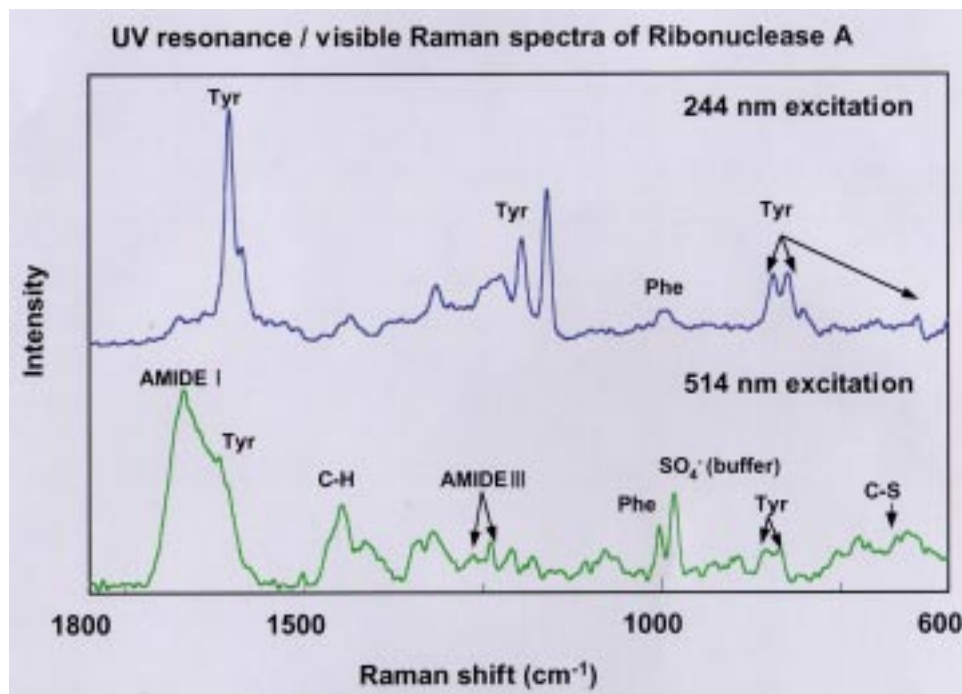
One of the biggest drawbacks with IR spectroscopy is the strong absorption from water, often requiring evacuation of the sample and spectrometer to reduce the background signal levels. Water scatters only weakly in Raman and is essentially transparent in the visible and NIR Raman wavelengths, so aqueous samples are easily measured and no special care is required for background removal. The method is fully non-destructive and the samples can be fully recovered after use.

Various excitation wavelengths are available for dispersive Raman, giving the technique some advantages over FT-Raman that uses NIR lasers. In principle, shorter wavelengths give stronger signals because the intensity of the Raman scattering is proportional to the fourth power of the laser frequency. Changing the excitation from the green argon line (514.5 nm) to blue (488.0 nm) gives a measurable increase in Raman scattering intensity: $(514.5)^4 / (488.0)^4 = 1.24$ but from the near-infrared to deep ultraviolet the difference is even more dramatic: $(785)^4 / (244)^4 = 107.0$ even neglecting any absorption- or resonance-enhancement.

A few years ago all CW (continuous wave) lasers were large, expensive and unwieldy gas-plasma units, but CW lasers can now be made little larger than a matchbox thanks to progress in laser diode technology. Today, the most popular Raman lasers are the compact, air-cooled solid-state units such as the frequency-doubled YAG (1064 nm fundamental) green line at 532 nm, the 785 nm diode laser, and for FT-Raman, the diode-pumped YAG 1064 nm. Only some tens to hundreds of milliwatts of power are needed for most Raman work. This small laser technology is currently advancing very rapidly thanks to the telecommunications industry and spectroscopy applications are benefiting greatly. Other lasers traditionally used are argon ion (Ar^+) gas lasers, giving 514.5 nm, 488 nm and with frequency doubling, 257 nm and 244 nm in the deep UV. For portability and low cost, the first two devices are the best choice.

In general, absorption at the excitation wavelength is not required for Raman scattering to occur and a variety of different laser wavelengths, from blue through green, red and the near-IR, can all give similar results from many samples. Raman scattering produces spectral emission at a constant wavenumber *shift* from the exciting light, so a green laser of 532 nm should give a qualitatively identical spectrum to that obtained from 785nm light. Recently, there is increasing interest in enhanced Raman scattering, either by using a special sample substrate (*surface enhanced Raman scattering, SERS*) or by exciting with *e.g.* ultraviolet light that is absorbed by particular structural features at frequencies close to a Raman active mode, resulting in intensified Raman emissions.

This latter method is of interest in the biological sciences for *e.g.* protein structural studies, in conjunction with other techniques like NMR, FTIR and CD. Although these ultra-short wavelength sources are still relatively expensive (they can cost more than the base Raman spectrometer), their increasing use in the semiconductor industries will probably ensure the prices continue to fall.



Raman spectra from ribonuclease A obtained by UV (244nm) and visible (532nm) laser excitation. Exciting different vibrational modes allows detailed probing of the biopolymer structure.

Fluorescence

Intense irradiation with visible light can cause unwanted fluorescence that is stronger than the Raman signal. This problem is particularly noticeable in natural products and organic materials, as well as polymers. One way to avoid or minimise it is by using the FT-Raman method and longer (less energetic) excitation wavelengths. The 1064nm laser practically avoids exciting fluorescence at the expense of lower Raman efficiency but the FT-IR spectrometer and detector are reasonably efficient at these wavelengths. FT-Raman spectrometers are usually attachments or modifications of FT-IR instruments, with the laser source, sample chamber (it can be micro- or macro) and a NIR optimised detector. JASCO's RFT-600 FT-Raman attachment interfaces with the FTIR-620 main unit to provide a powerful Raman and FT-IR dual-purpose system.

Although Raman scattering is a very weak process, the combination of an intense light source and sensitive detector overcome most of the problems. Particularly, micro-Raman, where a high NA objective lens focusses on a small region of sample, the amount of sample actually required for a spectrum is extremely small- only a few cubic microns in volume. Very small impurity particles can be detected even when embedded in a different (transparent) matrix because the different Raman signals are resolved by the spectrometer.

Sensitivity

The volume sensitivity of Raman is therefore very high and with the advantage of total sample recovery and usually no sample damage it is a good method where the sample amount is limited. Care needs to be taken with labile, strongly-absorbing samples, in which case the laser power must be attenuated to a suitable level and perhaps longer acquisition times used.

Sample holders are available to cover most eventualities. Thermally labile samples such as proteins can be cooled with TE (Peltier) stages and cryogenic (liquid N₂ or He) samples stages can be fitted. At room temperature, powders, liquids, all solids and gases can be measured with either microscopic or macroscopic (~mm) resolution. For studies of ceramics and inorganic materials, high-temperature microscope stages accommodate temperatures up to ~1500° C. Usually these exceptional conditions can be accommodated with alternate objective lenses on the microscope turret.

For situations where the sample cannot be taken into the instrument, the excitation laser and scattered Raman signal can be fiber optic coupled to the spectrometer. Probes such as the JASCO RMP-100 allow powerful in-situ Raman measurements at a range of wavelengths with semi-micro capability using a long working distance lens and built-in colour CCD-TV camera for sample viewing. Other probes allow for immersion measurements in various liquid samples.



RMP-100 Raman Micro-Probe allows high-resolution Raman spectra to be obtained at locations remote from the spectrometer

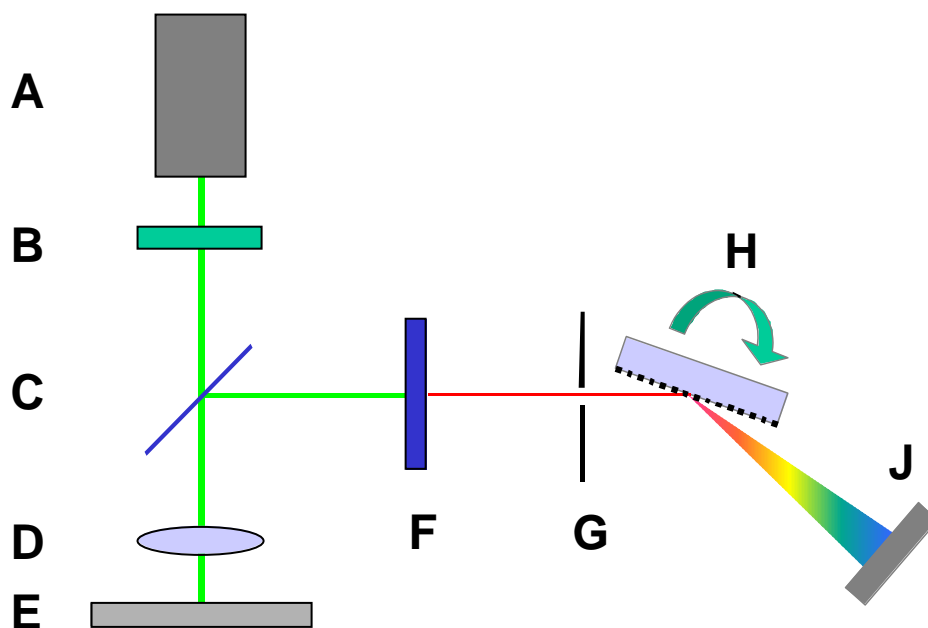
Probe can be hand-held or attached to either a small x-y-z stand or a camera tripod for ease of use. Fiber optics connect the probe to the laser and spectrometer.



Basics of Raman spectrometer instrumentation

The key components of the instrument are:

1. Laser light source (A, B)
2. Sample placed in the laser light path (E)
3. Optics to focus and collect the scattered light (C, D)
4. Spectral filter to remove the laser line and Rayleigh scattering (F)
5. Spectrometer(polychromator) (H)
6. CCD detector (J)



Basic components of a dispersive laser Raman spectrometer

Light from the laser (A) is filtered to remove serious wavelengths (such as from the gas plasma or pump light diodes) by either a system of prisms or an interference band-pass filter (B). The strictly monochromatic light is focussed onto the sample using a microscope (D), or else passed directly through it, depending on the geometry (micro, macro, back-scattering, *etc.*). Various kinds of beam-splitter (C) can be used to direct the Raman scattered signal emerging from the sample / lens into the spectrometer system. The strong Rayleigh scattered light within $10\sim 100\text{ cm}^{-1}$ of the laser line, is removed before the spectrometer entry by either a holographic notch filter, a long-pass edge filter or a separate zero-dispersion monochromator system. The weak Raman scattering is introduced to the dispersion grating optics (H) via a variable width slit (G) that adjusts the spectral resolution. An electro-mechanical drive system under PC control positions the grating at the correct angle to illuminate the CCD detector (J) with the required portion of the spectrum.

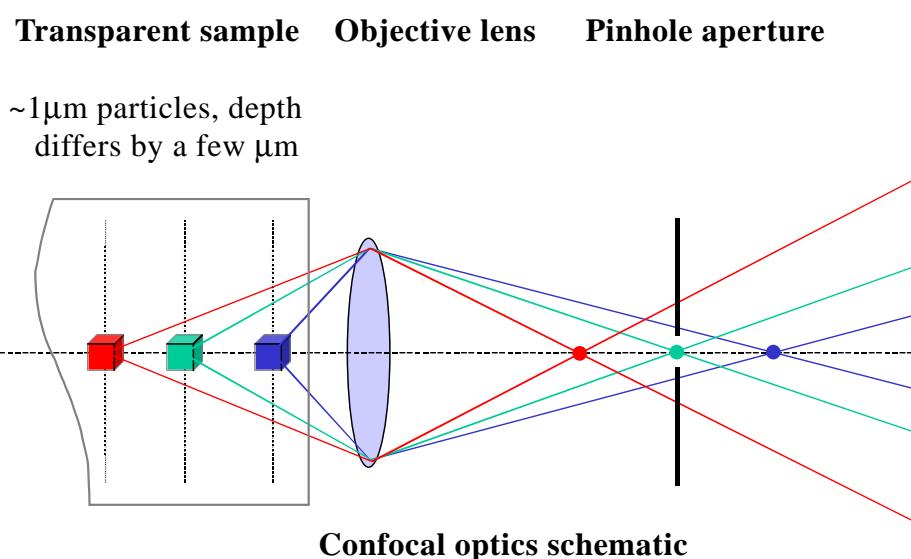
Sampling configurations

Both microscopic and macroscopic sampling is available. The microscopic Raman method, using visible light, can examine regions as small as $\sim 2 \mu\text{m}^3$ by the “confocal” optical technique (explained below). Finally, automated sample stages and computer control allow spectral “mapping” of samples at micron spatial resolution, to produce two-dimensional Raman images of materials.

Micro-Raman has several perhaps not obvious additional benefits compared to macro. Because the high NA (>0.95) objective lens used focusses the excitation laser at a large angle and also collects scattered light from a very small effective volume, only a small amount of the fluorescence excited by visible laser light is collected. This, combined by the higher Raman efficiency of visible wavelength excitation and CCD detectors can avoid much of the signal loss due to fluorescence without resorting to NIR excitation and the FT-Raman geometry.

Confocal optics

The use of a confocal “pinhole” aperture in the back focal plane of the objective lens, prevents “out of focus” light from entering the spectrometer. This spatially filters the Raman signal, allowing the signal from a specific depth in the sample to be isolated. A transparent, Raman scattering sample that contains a buried impurity can be “depth profiled” by changing the objective lens focal position and collecting spectra at each position, in steps of a micron or more. The spectra can be processed to show a depth profiled spectral “map” or “section” through the material, showing the location of the buried impurity. Also, a sample contained within a scattering material, for example a plastic vial, can be analysed by focussing the laser spot inside the container, the confocal aperture eliminates the vial’s background spectrum.



The Objective lens illuminates the sample and collects Raman scattering along the same axis. Light from the “green” inclusion particle in the transparent sample is correctly focussed and the Raman signal passes through the pinhole aperture. Scattering from the red and blue particles is rejected by the aperture and is not analysed.

Optimising the Raman spectrometer system

The spectrometer grating and detector form the heart of the system and are usually carefully matched to the laser wavelength to optimise both spectral resolution and “coverage” of the Raman spectrum by the CCD. The grating will only disperse light over a certain and limited wavenumber range, depending on the incidence angle. For example, there is no point in only using half the 1100 pixels of a CCD with narrowly scattered light, nor of using a grating that has a useful dispersion much greater than the width of the detector.

Depending on the angle of the grating (H), a certain portion of the Raman spectrum will be dispersed onto the detector (J). This segment of the spectrum is acquired more or less instantaneously, depending on the signal intensity and the detector’s performance. For example, a grating that allows about 700 cm^{-1} of spectrum, at wavelengths near to the exciting light, illuminates the 1100 pixel detector. Each pixel “sees” about 0.7 wavenumbers of signal. In order to record more spectrum, we either move the detector and record another segment, or must use grating with fewer lines. In the latter case, the spectral resolution (pixels / wavenumber) is impaired. A typical acquisition comprises several such segments integrated by the software into one seamless spectrum.

Because it is convenient to be able to use both NIR 785 nm and green 532 nm excitation, some systems are fitted with dual gratings (for example, the JASCO NRS-1000) in order to “optimise” the coverage and resolution with one detector. However, it is impractical attempting to optimise systems for very different excitation such as deep UV to NIR, as other factors such as lens and beam-splitter materials problems arise. CCD detectors will operate, with varying efficiency, from deep UV to NIR by careful selection of the window and chip type. In practise, changing a visible Raman system to UV operation typically involves either changing several major optical components or else accepting compromises and practically, separate systems are a more realistic solution. One answer to this is provided by JASCO’s dual-purpose NRS-100 VUV system, combining two source / sample optical paths with a single, dual-grating polychromator and CCD system.



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