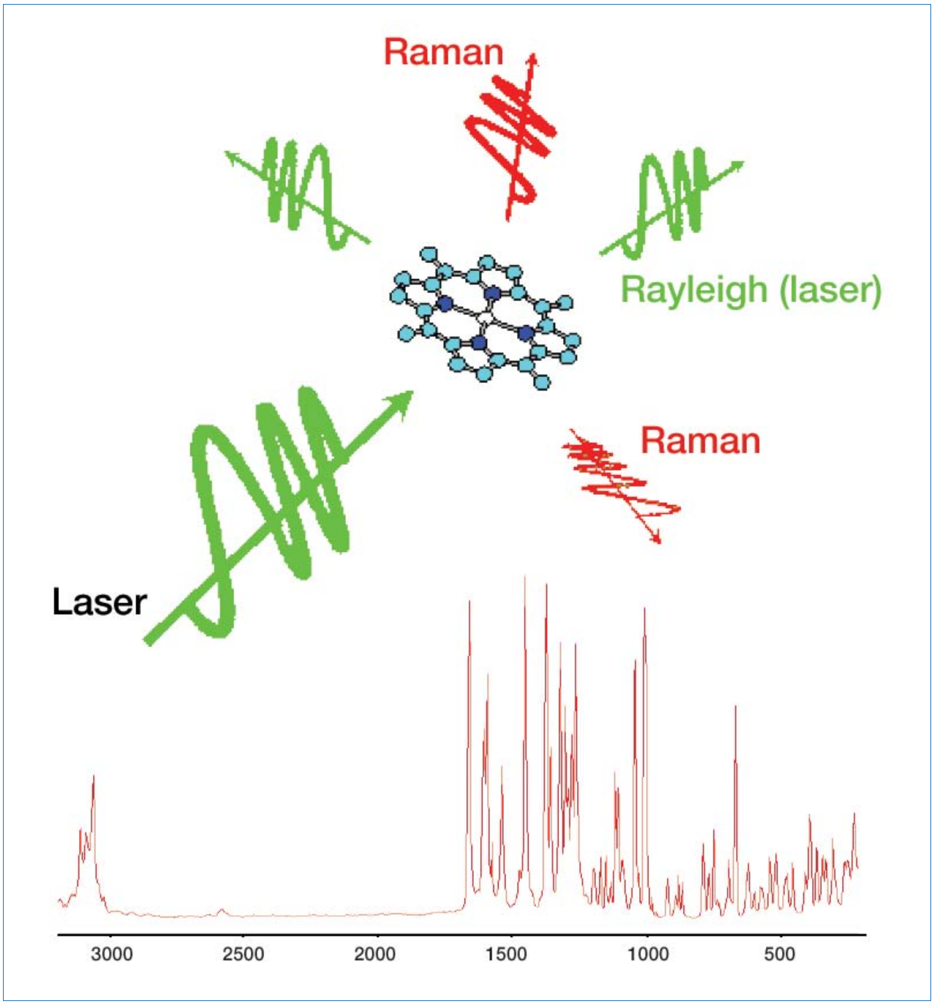
Introduction to Raman Spectroscopy



Top 20 Questions Answered

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PerkinElmer®
precisely.

1. Can Raman spectra be obtained from solids, liquids and gases?

Raman spectra can be obtained from *most* molecular samples, i.e. solids, liquids, gels, slurries, powders, films, etc. Raman spectra can even be obtained from some metals!

It is possible to obtain Raman spectra of gases. However, since the concentration of molecules in gases is generally very low, this typically requires special equipment, such as long pathlength cells. Sometimes an N≡N peak from gaseous nitrogen can be observed.

2. Can I do Raman spectroscopy on mixtures?

Yes, a Raman spectrum is a spectral “fingerprint”. If there are a number of different compounds in a mixture, the resulting Raman spectrum will be a superposition of the spectra of each of the components. The relative intensities of the peaks can be used to give quantitative information on the composition of a mixture of known compounds.

Where the identities of the components are unknown, simple software-based protocols, such as “spectral stripping” and library searching, can be used to identify them. In many cases this is possible even where the initial spectra are too complex to be resolved with the naked eye.

Figure 1 shows an example of a Raman spectrum of a mixture, in this case o-, m- and p-xylene. This spectrum is of 33% p-xylene, 33% o-xylene, 33% m-xylene and has been captured through a glass bottle.

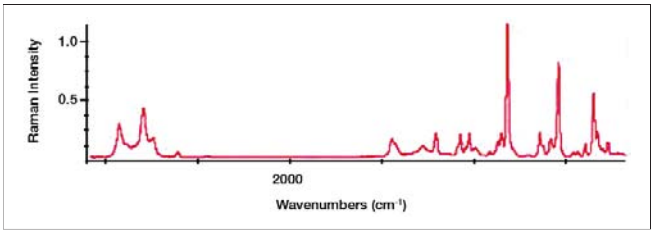

Figure 1. Sample Raman spectrum of a mixture.

3. Is Raman qualitative or quantitative?

Raman is both qualitative and quantitative.

As previously mentioned, Raman spectra are unique molecular “fingerprints”. Spectral library searching can be conducted to allow qualitative analysis.

Quantitative analysis of a mixture is typically performed by measuring the relative intensities of bands that are directly proportional to the relative concentrations of the compounds. Alternatively, chemometric methods can be used. These quantitative analyses can be performed on samples with high concentrations ranging from 90-100% material of interest down to concentration determination at PPB levels.

4. Will a pure chemical overload the detector?

No, Raman detectors, or Charge Coupled Device detectors (CCDs), have a wide dynamic range and users can select the appropriate exposure time for their sample.

5. Does Raman work on aqueous samples?

Yes! Water does give a Raman spectrum, but it is a weak Raman scatter. For example, a 4% alcohol in water solution will yield a Raman spectrum containing signals from both the alcohol and the water. The water spectrum (96%) will be about the same intensity as the alcohol (4%). Trace analysis in water is also possible using Surface Enhanced Raman.

6. How long does a Raman spectrum take to acquire?

This depends on the sample, but all PerkinElmer® Raman instruments can acquire a Raman spectrum of most substances in a matter of seconds. Figure 2 shows a spectrum of a paracetamol tablet taken in just one second.

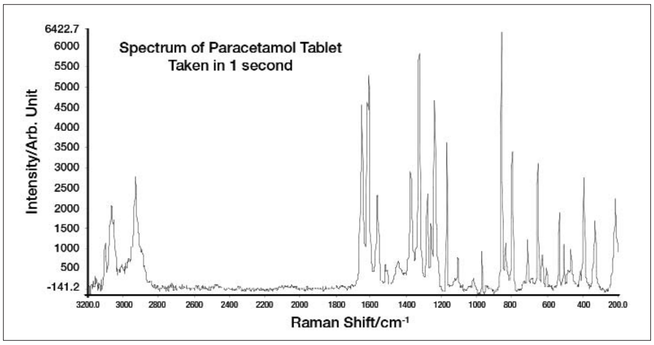

Figure 2. Spectrum of a paracetamol tablet taken in one second.

7. Does Raman require any sample preparation?

Usually, no. Raman spectra can be obtained from bulk solids, liquids, tablets, polymers, paper, etc. with little or no sample preparation. Analysis can also be carried out through many containers such as glass bottles, Pyrex® reaction vessels, plastic containers, blister packs, bags, etc. See Figures 3 and 4.

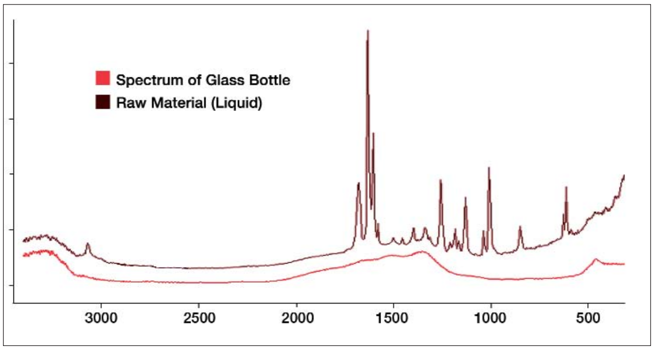

Figure 3. Automatic spectral subtraction from a glass bottle.

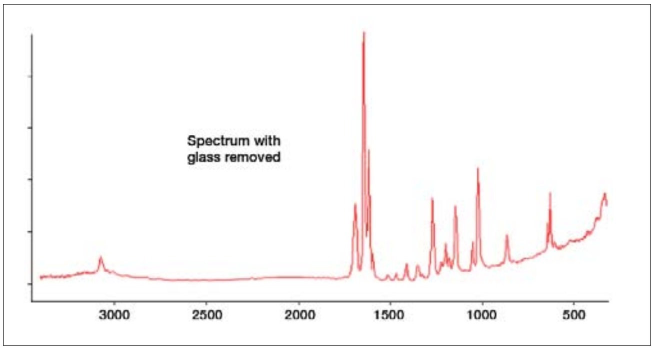

Figure 4. Raman spectrum with glass bottle removed.

8. Does Raman work through glass or plastic containers?

Yes. Analysis can also be carried out through many other containers such as Pyrex® reaction vessels, plastic containers, blister packs, and many plastic bags.

9. Can I do Raman at high/low temperatures and variable pressures?

Yes, and it is simple! PerkinElmer offers a range of variable temperature cells and fiber optic probes for use at elevated temperatures and pressures. Temperatures up to 500 ˚C and pressures of up to 3000 PSI can be easily accommodated. If needed, temperatures and pressures above this are accessible using customized equipment.

10. Is Raman destructive?

Raman is usually not destructive, but if too much laser power is used or if the power is focused on a small point (in a microscope) it can be destructive, i.e. it can burn your sample. Since FT-Raman systems employ higher power lasers (up to 2 Watts compared to milliwatts), greater care must be taken to ensure the sample does not get damaged. Sample burning is less of a problem with dispersive Raman spectrometers since much lower laser powers are employed. Using a large sample spot or lowering the laser power significantly reduces the risk of sample damage.

11. What type of laser is used in PerkinElmer's Raman instruments?

The PerkinElmer Raman instruments incorporate a line-narrowed, high performance 785 nm stabilized diode laser. A 785 nm laser employed at this wavelength provides excellent sensitivity and minimizes the incidence of sample fluorescence.

Additional laser information:

What is the laser's linewidth?

Linewidth is 0.03 nm.

What is the stimulated emission background (how many dB from the laser center Wavelength)?

See Figure 5 for stimulated emission background examples.

What are the noise level and the long-term instability?

Signal-to-noise ratio > 40 dB, relative intensity noise -100 f < 1 GHz, stability 0.1 nm.

Is it possible to adjust the laser power illuminated at the sample?

Yes, this is software adjustable between 100 mW and 5 mW.

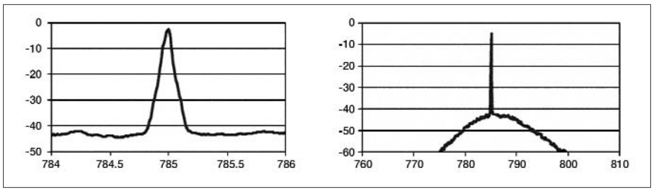

Figure 5. Stimulated emission background examples.

12. What is the line density of your grating?

The PerkinElmer® Raman 400 series instruments incorporate an Echelle spectrograph, which offers full spectral coverage at high resolution, with no moving parts. A technical note explaining how this Echelle spectrograph works is available at *www.perkinelmer.com/raman*.

The Echelle grating line density is not directly comparable with a traditional Raman spectrograph grating line density. The Echelle grating is a very special type of grating at about 60 l/mm. The grating is in the shape of a letterbox and is placed at an extreme angle relative to the incident light. This configuration, in conjunction with a second "cross dispersing" grating, gives a dispersion equivalent to a 1800 l/mm grating in a traditional 1/4 M spectrograph.

The Echelle spectrograph gives complete spectral coverage at high resolution in a single acquisition. To obtain the equivalent range and resolution, a traditional spectrograph equipped with a 1800 l/mm grating would have to acquire five separate spectra and stitch them together into a complete spectrum. The Echelle gratings can do this in a singe acquisition allowing for rapid, high quality, data acquisition.

Our Raman 200 series spectrometers incorporate a traditional (Czerney Turner) spectrograph, common in Raman. These systems utilize a 600 l/mm grating.

13. What type of Rayleigh filtering is used?

Our instruments use ultra steep, high performance edge filters. These offer excellent blocking efficiency of Optical Density (OD) 6 and allow analysis down to 95 cm^{-1}, with no need for user optimization. These edge filters are environmentally stable and have lifetime stability, unlike holographic filters. Overall, we have OD of 12 at the laser wavelength (2 x Rayleigh filters, each with an OD of 6).

14. Can you provide detailed specification of the available Charge Coupled Detectors (CCD) for the Raman product line?

PerkinElmer offers a choice of high performance, scientific-grade CCD detectors: Front Illuminated, Open Electrode and Deep Depletion CCDs. A detailed technical note of the various CCDs available can be found at *www.perkinelmer.com/raman*.

15. During analysis of multiple samples, such as in the case of multi-well plates, is the autofocus function available to optimize the focal point on each sample?

Yes, autofocus can be automatically applied at every point. If the system is configured to take a map of each sample in a multi-well plate, autofocus can be applied at the center-point of each map or at every point in each map.

16. Can Raman differentiate between unlike polymorphic forms?

The built-in video camera available on the RamanStation and RamanMicro systems can be used to observe visible morphological changes between different polymorphs. The Raman spectra of polymorphs will be different, but in many cases these variations are small and can occur anywhere in the spectral range. The extended wavenumber range, from 100 to over 3000 cm^{-1}, combined with the high spectral resolution routinely achievable with the RamanStation 400 ensures that any variation in peak position and/or peak intensity is readily measured.

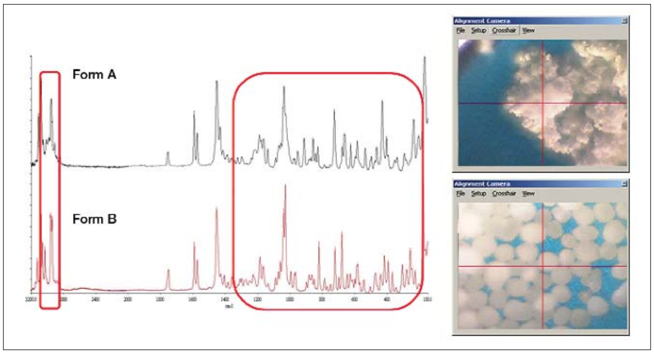

Figure 6. Visible morphological changes between different polymorphs.

17. What type of Raman instruments are available?

There are three types of Raman instruments: benchtop spectrometer, fiber optic spectrometer and Raman microscope. At PerkinElmer, we offer all three types of instruments.

Benchtop: RamanStation™

Fiber Optic: RamanFlex™

Microscope: RamanMicro™

Product brochures and technical specifications for these instruments can be found on our webpage at *www.perkinelmer.com/raman*.

Figure 7. Fiber optic probe.

18. Can the user write their own LabVIEW program to control their system?

Yes, we provide LabVIEW™ drivers that allow full instrument control, including acquisition setup, start acquisition, take background, etc. It is also possible to autofocus and control the motorized XYZ stage using the LabVIEW interface.

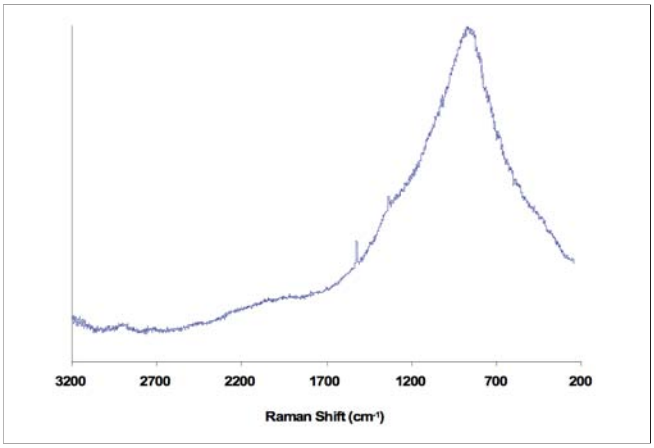

Figure 8. Highly fluorescent Raman spectrum from an ink sample.

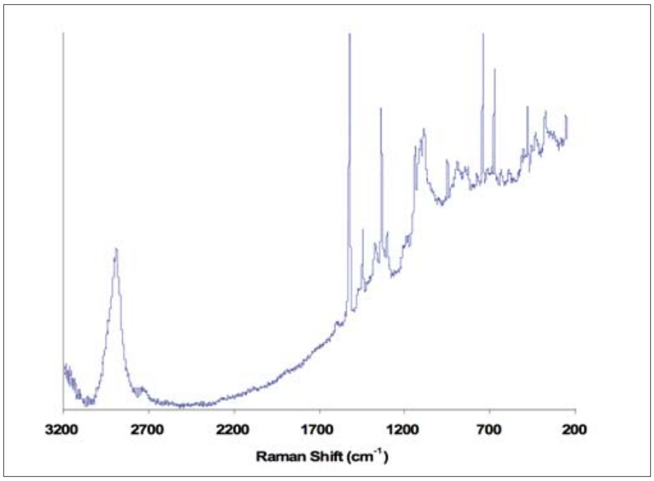

Figure 9. Two minutes of photo-bleaching radically reduces the intensity of the fluorescent background.

19. Is fluorescence a problem?

Fluorescence can often contaminate Raman spectra. The use of a Near-IR 785 nm laser radically reduces the possibility of fluorescent contamination, but there can still be some fluorescent problems. It is often the case that photo-bleaching can reduce the fluorescence down to cosmetic levels, and auto-baseline correction can remove the residual baseline. These two facilities are available in the PerkinElmer Spectrum™ software. Figures 8, 9, and 10 illustrate the effects of photo-bleaching and auto-baseline correction. Technical notes explaining photo-bleaching and auto-baseline correction are available at *www.perkinelmer.com/raman*.

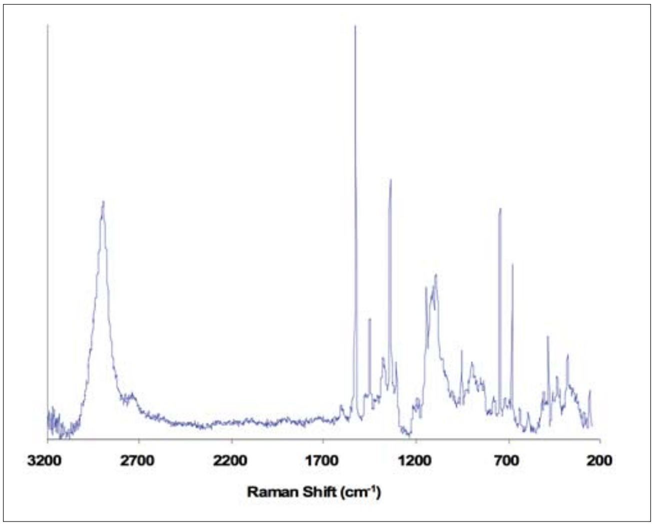

Figure 10. Auto-baseline correction removes residual fluorescent baseline, resulting in fluorescent-free Raman spectrum.

20. What is Raman imaging and what can it be used for?

In Raman imaging, Raman spectra are taken over a predetermined sample surface area in a set sequence and with a defined point interval (spatial resolution).

This set of data is displayed as an image where areas of high Raman intensity are shown in one color (normally red) and areas of lower intensity are shown in a different color (normally blue). This variation in color may simply reflect a difference in concentration of the same material across the sample, or may show variation in chemical composition. Underlying each pixel of the image is the appropriate Raman spectrum associated with that point on the sample.

The Spectrum software allows the user to display these individual spectra and generate chemical distribution images of the components in the sample. A visible image survey of the sample is acquired using the built-in video camera and motorized stage. The area where the Raman image is acquired is comparable with this visible image shown in Figure 11.

This technique will be of interest to any laboratory wanting to better understand the distribution of components within their samples. Obvious examples include distribution of components within pharmaceutical tablets, distribution of minerals over a geological sample and distribution of components within a composite.

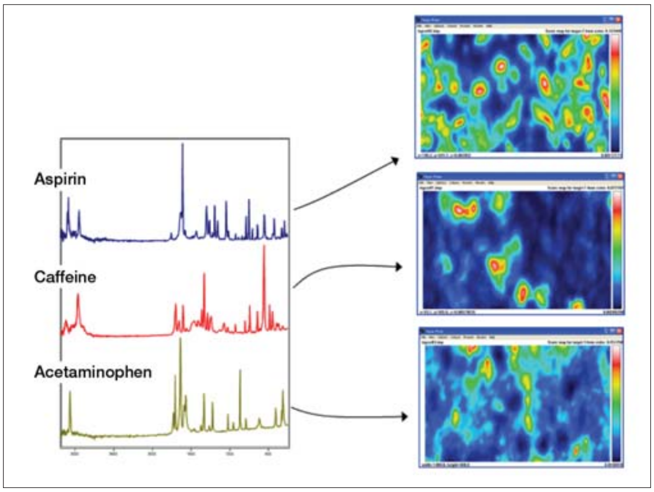

Figure 11. Chemical distribution images of components in a sample.

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