LaserFocusWorld®

PennWell®

EDITORIAL DIGEST

Fundamentals of Raman spectroscopy

Raman spectroscopy is a nondestructive and highly versatile technique for analysis of chemicals, both organic and inorganic. It is used in industry, bioscience, medical diagnosis, forensics, and many other areas.

Fundamentals of Raman spectroscopy

by **TRAVIS THOMPSON**

THIS TECHNICAL DIGEST covers the fundamentals of Raman spectroscopy and instrumentation. Topics include the history of Raman spectroscopy, what Raman is and how it works, Raman instrumentation, and common applications.

A brief history

Raman was first discovered by C.V. Raman and K.F. Krishnan in 1928. In 1930, C.V. Raman was awarded the Nobel Prize in physics for this discovery. During the 1930s, Raman was recognized as a principle means of nondestructive chemical analysis. During this time, challenges were also discovered. These included the lack of a good Raman source, lack of a good detector, and interference from fluorescence, which in some cases could overwhelm the Raman signal.

During the 1960s, there was revived interest in Raman due to the advent of lasers, and in 1986, the first Fourier-transform (FT) Raman instrumentation was developed. Fourier-transform Raman provided a way to overcome problems with fluorescence, making the technique much more viable for researchers. In the 1990s, dispersive Raman instrumentation was developed, and included the advancement of compact near-infrared (NIR) lasers, multichannel detectors, and fiber-optic probes. This time also saw the advent of portable integrated dispersive Raman systems. All of this led to Raman becoming a much more viable technique for a variety of different fields and a number of applications.

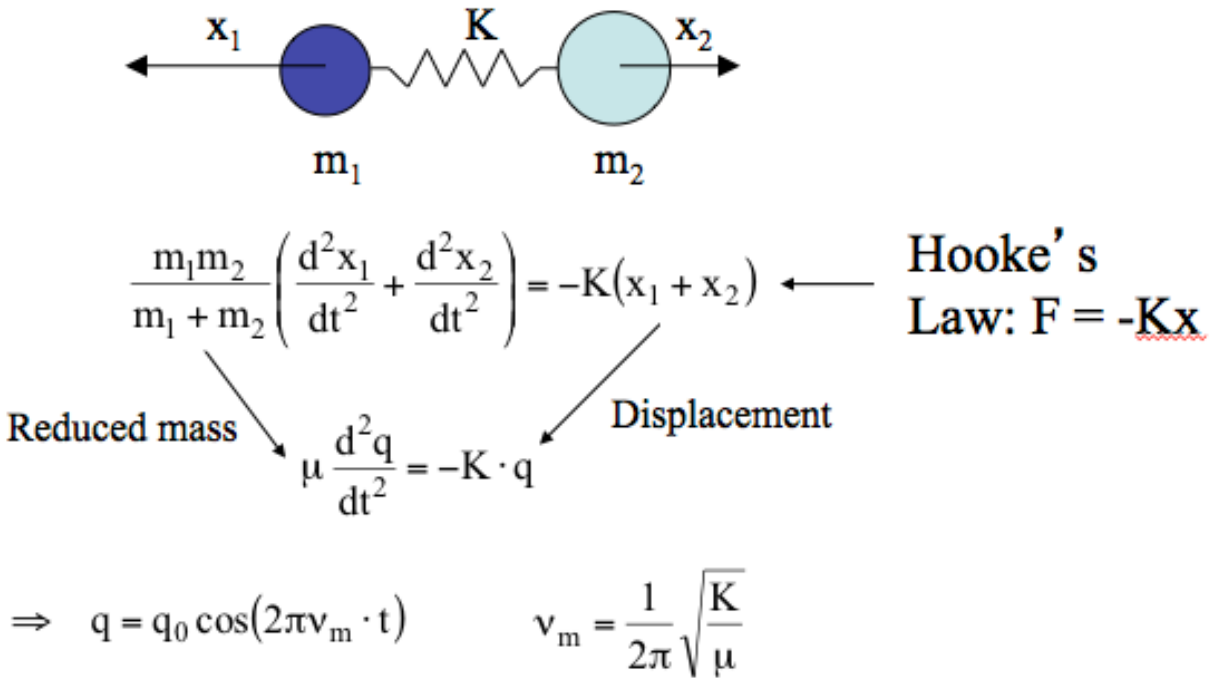
Benefits of Raman

Raman spectroscopy is a form of molecular spectroscopy that involves the scattering of electromagnetic radiation by atoms or molecules. It probes the vibrational, rotational, and other low-frequency modes of molecules; the Raman signal is observed as inelastically scattered light.

There are a number of advantages to Raman spectroscopy. For one, Raman is a nondestructive technique and typically requires little to no sample preparation. The Raman analysis also can be performed directly through transparent containers, including plastic bags, glasses, jars, cuvettes, and so on. Furthermore, Raman can be used for both qualitative and quantitative analysis, and the Raman technique is highly selective, meaning that it is able to differentiate molecules in chemical species that are very similar.

Raman also has the advantage of fast analysis times. A typical analysis can take just a few seconds, and unlike FTIR spectroscopy, Raman is insensitive to aqueous absorption bands.

When considering Raman scattering, we can think about this in one of two ways: The first is the classical-wave interpretation, and the second is the quantum-particle interpretation. Consider the classical-wave interpretation: All electromagnetic radiation has an electric field associated with it. The electric field of the incident light can interact with the molecules in the sample through



$$\frac{m_1 m_2}{m_1 + m_2}\left(\frac{d^2x_1}{dt^2} + \frac{d^2x_2}{dt^2}\right) = -K(x_1 + x_2)$$

$$\mu \frac{d^2q}{dt^2} = -K \cdot q$$

$$\Rightarrow \quad q = q_0 \cos(2\pi\nu_m \cdot t) \qquad \nu_m = \frac{1}{2\pi}\sqrt{\frac{K}{\mu}}$$

FIGURE 1. *In the classical derivation of the Raman effect, the molecular displacement Q is derived as a cosine function.*

the polarizability of those molecules. Polarizability is related to the ability of electronic clouds surrounding the molecule to interact with an electric field. Soft molecules, like those in benzene, tend to be very strong Raman scatterers, while hard molecules like water tend to be very poor Raman scatterers.

We can also think about light as being composed of particles (photons). When photons are directed toward a sample, sometimes they will hit a molecule and bounce off. If that collision is inelastic, and energy is lost by making the molecule vibrate, the photon will have less energy. The number of photons scattered is related to the electronic size of the molecule, so molecules like benzene with large-signal bonds will scatter more than molecules like water, which has small-signal bonds.

Again considering light as a wave, let's explore the derivation of the Raman effect. We can derive Raman scattering by considering the vibrational mode structure of a simple diatomic molecule. Using Hooke's Law in approximation, we can derive the displacement Q as a cosine function (see Fig. 1).

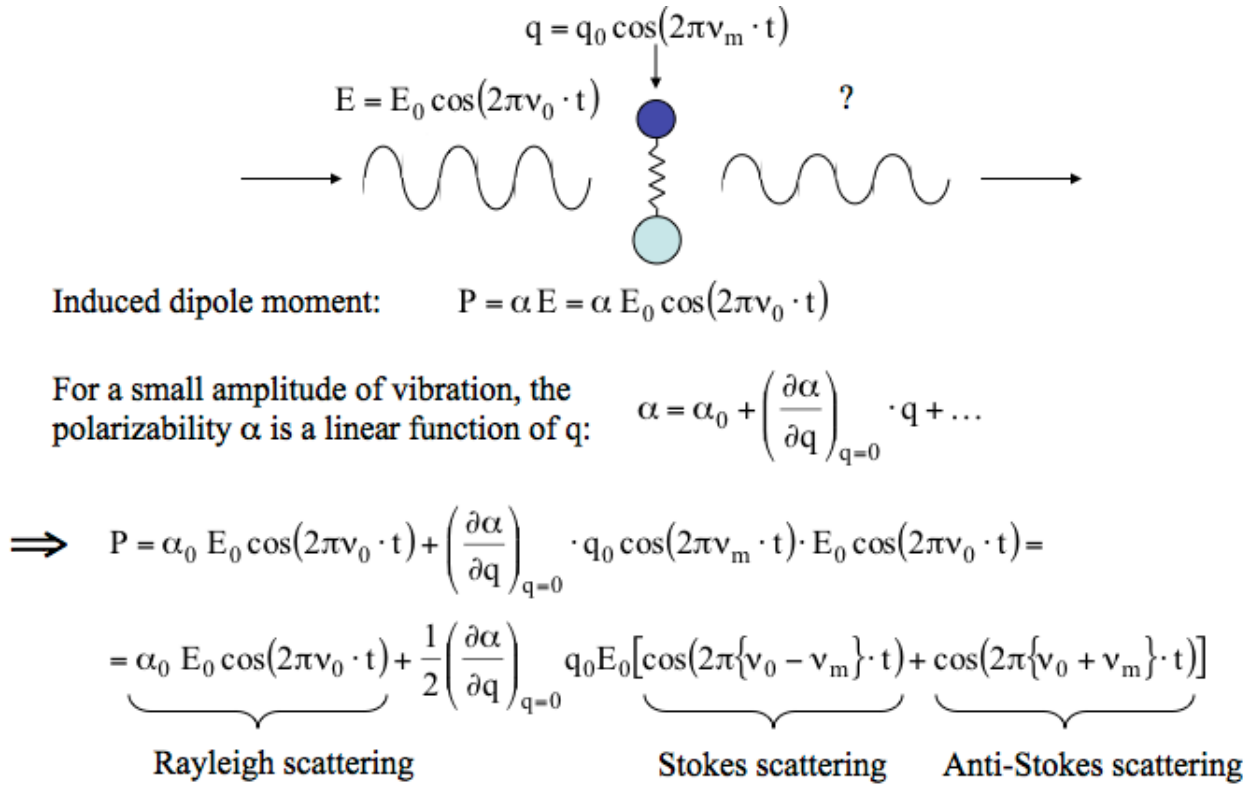


FIGURE 2. *For a small-amplitude vibration, the polarizability is a linear function of Q.*

The size of the molecular vibrations is inversely proportional to mass and directly proportional to the bond strength. Now we can consider the effect of incident light on the displacement of a molecular vibration. When the electric field of the incident light interacts with the molecule, it interferes with the molecule's vibration, inducing a dipole moment. The induced dipole moment is a function of the polarizability of the molecule and the incident electric field.

For a small-amplitude vibration, the polarizability is a linear function of Q (see Fig. 2). By applying the linear approximation, we see the first term represents Rayleigh scattering; the frequency variable is represented by ν_0, which means there has been no change in the frequency. This is also known as elastic scattering. The second term of linear approximation produces Stokes and anti-Stokes scattering, and these are represented by a change in the frequency, with the frequency variables denoted by negative ν_m and positive ν_m.

Raman from the particle point of view can be visualized by a quantum energy diagram (see Fig. 3). We'll start first with Rayleigh scattering. When incident light hits a molecule, an electron in a ground vibrational state is promoted to a virtual state. It then relaxes and returns to the same vibrational state from which it started. This is denoted by ν_0, which means there has been no change in the frequency; this is elastic scattering.

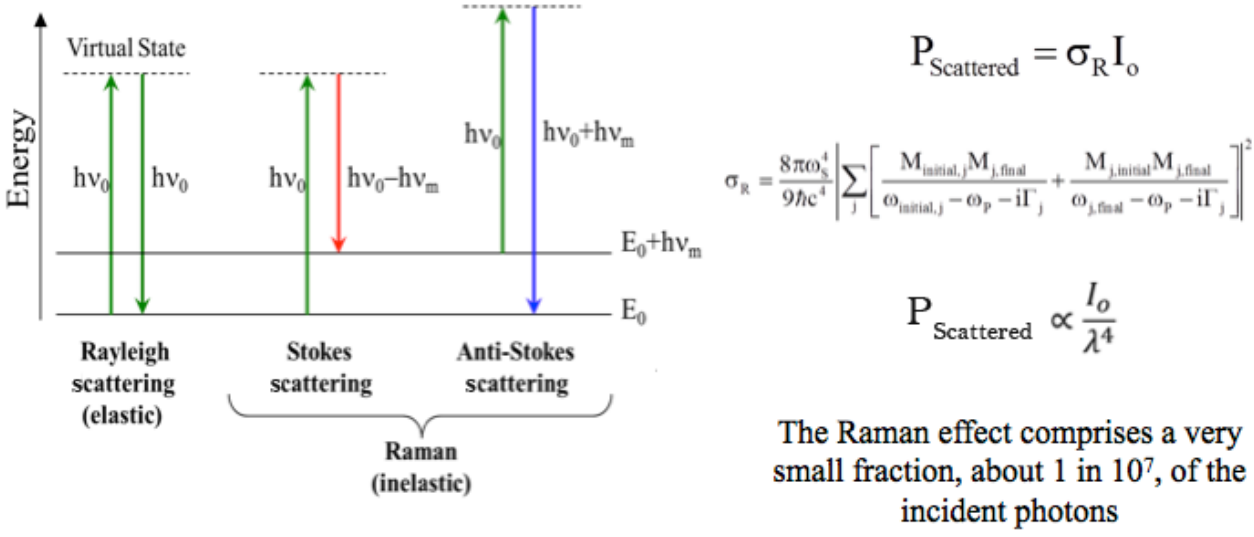


FIGURE 3. *Considering excitation in the form of quanta (photons), Raman scattering can be depicted in a quantum energy diagram.*

Stokes scattering

In Stokes scattering, the electron also begins in the ground vibrational state. It is promoted to a virtual state and then relaxes to an energy state that is higher than the electron's starting state. For anti-Stokes scattering, an electron begins in a vibrational state that is more energetic than the ground state. It is promoted to a virtual state then relaxes back down to the ground vibrational state, which is lower in energy than when it started. Anti-Stokes scattering is denoted by positive ν_m. Of the two, Stokes is more commonly used for most Raman measurements because most electrons are in the ground vibrational state at room temperature.

The power of Raman scatter is directly proportional to the intensity of the incident light and inversely proportional to the expectation wavelength to the fourth power. This is important for a number of reasons. Primarily, Raman itself is a very weak technique: For every 10 million photons that are incident on the sample, only one of those will be Raman-scattered. So it's very important to consider excitation wavelengths when performing Raman measurements.

Because Raman scatter is inversely proportional to the fourth power of the excitation wavelength, the more energetic the excitation wavelength, the more Raman scatter we will be able to observe. For example, if we look at one sample with a 532 nm source and one sample with a 785 nm source, we're going to see more Raman scatter coming back from the system that was analyzed with the 532 nm excitation wavelength.

There is a tradeoff here. In one example, a fluorescence region spans from about 275 to 975 nm (see Fig. 4). So if we provide excitation in that range, it is likely that we could promote fluorescence. In some cases, the fluorescent signal could be strong enough to completely overwhelm the Raman signal. Now suppose that a sample is analyzed using three different wavelengths: 532, 633, and 785 nm. For the 532 nm spectrum (green), we see a very large fluorescence background and we're able to see some of the Raman, but we're seeing this just as small blips on the fluorescence spectrum. When we move to 633 nm excitation (red), we see less fluorescence background, and we start to see more of those Raman bands. Finally, when we move to 785 nm (blue), we have essentially gotten rid of the fluorescence background, and now we are just seeing the Raman signal.

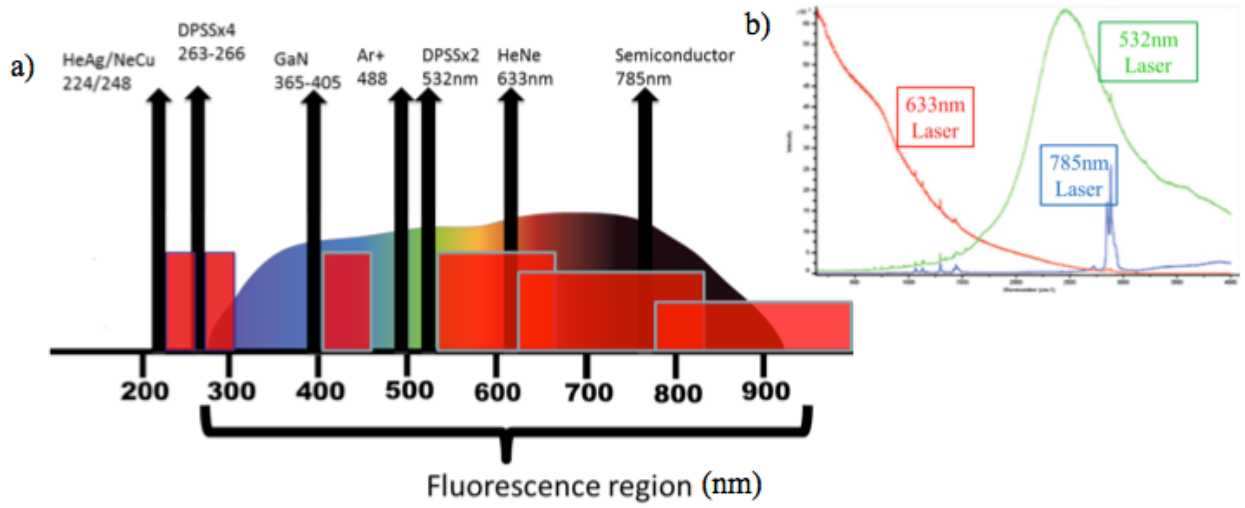


FIGURE 4. *Excitation and fluorescence wavelengths are shown in a region spanning 275 to 975 nm (a). A sample analyzed at three different wavelengths—532 (green), 633 (red), and 785 nm (blue)—shows differing spectra for each wavelength (b). The lack of fluorescence for 785 nm reveals the Raman signal.*

So there is always a tradeoff between excitation wavelength, the power of Raman scattering, and fluorescence, and this will vary depending on the type of sample that is being analyzed. A good general rule of thumb is that 532 nm excitation is well suited to inorganic chemicals and 785 nm excitation to organic chemicals.

There are a few things that can be done to circumvent fluorescence completely. One of those is to use a deep-ultraviolet (DUV) excitation wavelength. Because fluorescence doesn't appear until roughly 275 nm by exciting at wavelengths below that, the Raman signal will be spatially offset from the fluorescence. The problem with this is that DUV lasers are very energetic, so there is the likelihood that the sample could be damaged or burned.

If we look at the other end of the spectrum, we could use an excitation wavelength such as 980 or 1064 nm. What this will do is again get us outside of that fluorescence region, but the downside here is that the power of Raman scatter is going to go way down. So in some cases, we may have to use more power on the sample in order to see enough Raman signal coming back.

Inside a Raman spectrometer

There are three main components in a Raman spectrometer unit: the laser, the spectrometer itself, and the sampling interface. A good Raman laser will have a number of different characteristics, including narrow linewidth, small form factor, low power consumption, and an extremely stable power output. This last one is key, and it is important for a Raman laser to have not only a stable power output but also a stable wavelength output. If we're conducting Raman measurements using a 785 nm source, we want to ensure that the source is only emitting 785 nm.

The next component is the spectrometer: Key performance factors here are high resolution, low noise, small form factor, and low power consumption. It is critical that the appropriate detector be used, depending on which excitation laser is being employed. For UV excitation, typically a photomultiplier tube (PMT) or CCD is chosen. For visible excitation, a standard CCD is typical, and for NIR excitation, an indium gallium arsenide (InGaAs) array is typical.

The third component is the sampling interface. One interface used in many Raman spectrometers is the fiber-optic probe, which provides a very flexible sampling interface. In addition, fiber-optic probes can be easily adapted to a variety of sampling chambers, such as liquid flow cells, gas flow cells, and optical microscopes. A very important feature of a fiber-optic probe is a high-optical-density Raman cutoff. When we're looking at the Raman spectrum, we want to ensure that we're blocking out as much of the laser wavelength as possible so that we can observe the Raman shift. It is very important that we can look at the Raman shift very close to the laser line because many materials have very important spectral features very close to the line.

Raman spectral information

A Raman spectrum can provide multiple types of information. First of all, every molecule or chemical species has its own unique Raman spectrum; this can be thought of as a molecular fingerprint. This allows us to develop databases of known standards that can later be used for the identification, or verification, of unknowns.

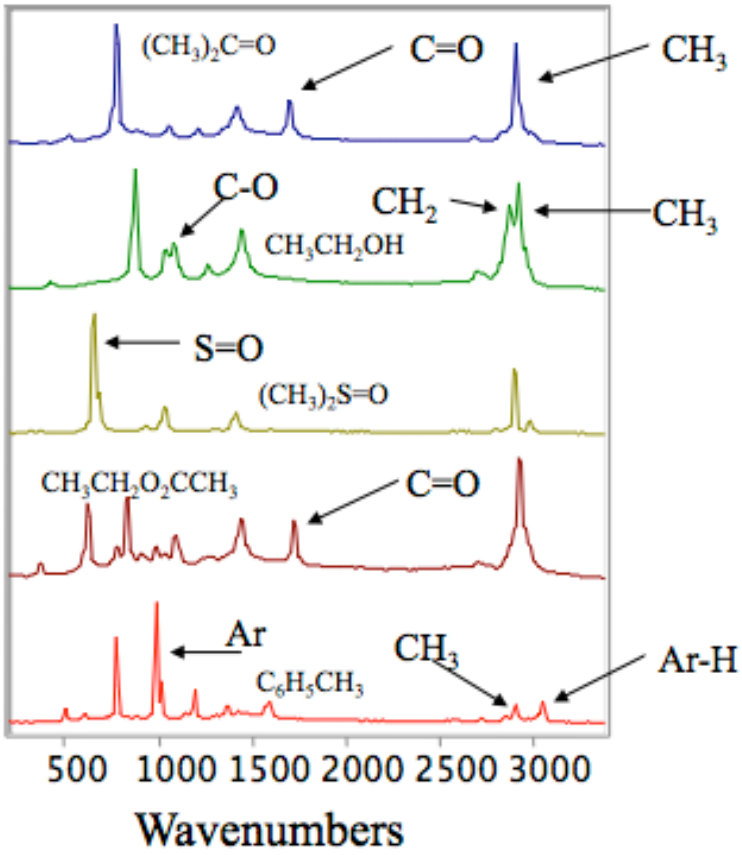


FIGURE 5. *Raman spectra are shown (from top) for acetone, ethanol, dimethyl sulfoxide (DMSO), ethyl acetate, and toluene.*

Raman also provides structural information (see Fig. 5). A number of different chemicals are shown here with their Raman spectrum; the first is acetone. Looking at the acetone spectrum, we see different bands. Each one of these bands is related to a specific vibrational, rotational, or other low-frequency mode, and each one will always be in the same position regardless of whether we're using 532, 785, or 1064 nm excitation light.

So for acetone, we will always see the carbon-oxygen double bond at roughly 1550 wavenumbers no matter what laser line is used. If we look a little bit higher in the spectrum, we see a band that is attributable to a CH_3 stretch. Again, it doesn't matter which excitation wavelength is being used; this particular CH_3 band for acetone will always be observed in this location.

Below acetone in the figure is ethanol. One of the bands shown is the carbon-oxygen single bond, while higher up in the spectrum we're seeing bonds that are CH stretches. Below ethanol is dimethyl sulfoxide (DMSO), showing the sulfur-oxygen double bond. We can use the same approach to the two spectra below that, ethyl acetate and toluene. The importance here is that each one of these bands in a Raman spectrum is due to a specific mode of that chemical. This information can be a little overwhelming, especially with spectra that have ten or more Raman bands. However, band-assignment tables are readily available, which can help give an idea of exactly what each band means.

Raman can also be used to track changes in frequency shift as well as to look at differences in peak bandwidth. One good example is looking at a crystalline silicon sample. The crystalline silicon band—the main, most intense band—is located at roughly 520 wavenumbers. If we stress or strain the silicon, we're going to see that band shift, depending on how much stress or strain we've applied.

Chemometrics analysis

Once the data are collected, how do we make good use of them? The way to do this is by using chemometrics analysis. Chemometrics is the use of mathematical and statistical methods for the analysis of chemical or spectral data. There are really two types of key information here: qualitative and quantitative. Qualitative is correlation analysis for material identification and/or verification. As previously mentioned, we can build databases of known standards then use those databases for the identification of unknowns, and we can also obtain quantitative information. So this is multivariate analysis of complex systems with large data sets.

This allows us to uncover hidden trends or outliers of those classifications; we can also develop models to predict response in unknown sets—for example, constructing calibration curves for the identification of unknown concentrations. All of this information allows us to better optimize experimental design.

A BWID qualitative software package is used for the development of spectral libraries or databases and is also used for later identification of unknowns. Using BWID, we can preprocess some of the data using things such as baseline corrections, sample smoothing, derivatives, and others. We can then develop methods or models using partial-least-squares regression or other algorithms and use these models or methods to predict unknowns.

Industries using Raman spectroscopy

In the pharmaceutical industry, one very big application for Raman is the identification and verification of incoming raw materials. Again, we can build databases then use those databases for the identification of unknowns. In addition, Raman can be used for the analysis of tablets, liquids, and gel caps. Because Raman is a nondestructive technique and requires little to no sample preparation, we can perform the analysis directly on those samples, even through the gel caps.

Raman is also used for process analytical technology (PAT), which is pharmaceutical manufacturing line monitoring and control—for example, real-time monitoring of drying, coating, and blending. Another application is the identification and analysis of active pharmaceutical ingredients, additives, and excipients, as well as drug-identification control with regard to purity and quality.

Raman is an important tool in counterfeit-drug analysis. Figure 6 shows spectra of Tegretol and the generic form of the drug, carbamazepine. This analysis was conducted in the field; we did not even have to open up the blister packs (plastic packaging) when we were looking at these chemicals. Visually, you don't see a whole lot of differences between the two spectra (shown in red and black); however, if we use a package such as BWID, we can differentiate these two very quickly and easily, referring to a number of different spectral features.

In the food and agriculture industries, Raman can be used for measuring chain length and extent of saturation of fatty acids in edible oils, as well as in meat-product quality analysis. One important use for Raman is in the analysis of food contaminants; this is actually done using a technique called surface-enhanced Raman scattering (SERS). As mentioned before, Raman is very selective but not very sensitive. There are ways to overcome this weak sensitivity; one of them is SERS. With this technique, nanoparticles—typically of gold or silver—are positioned close to the area of interest. Essentially the nanoparticles enhance the return Raman signal, and that enhancement can be very large, ranging anywhere from 10^4 to 10^{16}. Using SERS for the analysis of food contaminants allows us to characterize bacteria, antibiotics, toxins, and other contaminants.

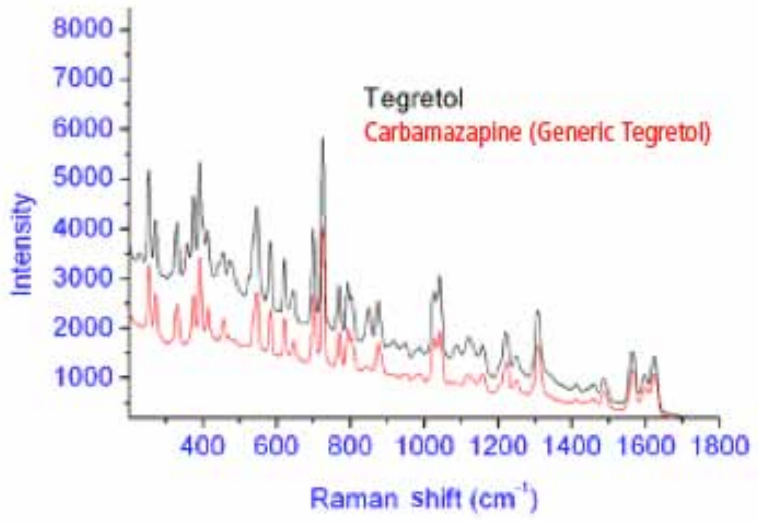


FIGURE 6. *The Raman spectrum for a pharmaceutical, Tegretol, is shown along with the spectrum for the generic equivalent (carbamazepine). While the differences in the spectra are not visually obvious, a software package (BWID) easily distinguishes the two.*

Raman can also aid in the analysis of components in grain kernels. And as in the pharmaceutical industry, it is routinely used for the identification and verification of incoming raw materials.

In forensic analysis, Raman is employed, for example, in the nondestructive characterization of narcotic drugs. Raman has several advantages when it comes to the identification of narcotics. It is nondestructive, so we're not destroying the sample in the process of analyzing it. Second, Raman is highly selective, meaning that there is a lower likelihood for false positives, or false negatives. Third, Raman analysis is very rapid, so we can analyze materials in just a few seconds.

Explosives, including PETN, RDX, TNT, and others, can be identified via Raman as well as the binding agents within explosive materials. It is also useful for the identification and analysis of toxic solvents in biowarfare agents—so for hazmat teams and first responders, it is a very good tool for the analysis of unknown, potentially harmful agents. Raman instrumentation is portable and very easy to use, and as it can be used with databases, we don't have to concern ourselves too much with spectral interpretation. Analyses can be performed by a nontechnical user; the information can be collected quickly, allowing first responders to take appropriate precautions depending on the types of materials they're finding on the scene.

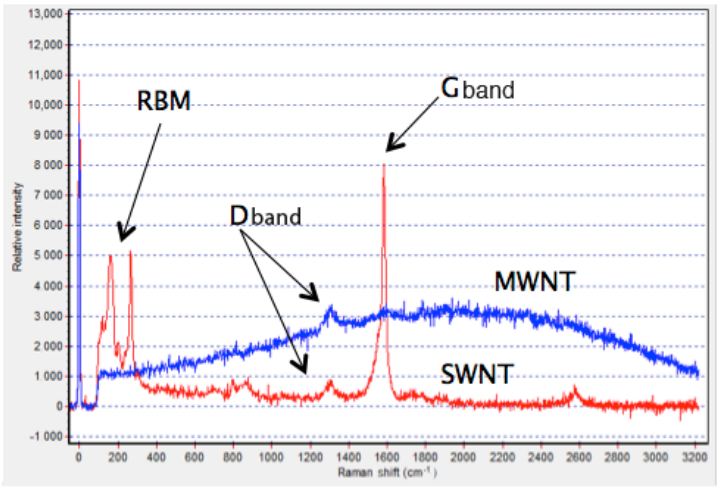
Raman also has a place in the analysis of trace forensic evidence such as fibers, fabrics, pigments, inks, and so on. One instrument that is often utilized for this kind of analysis is the Voyage Raman microscope. When we're looking at trace evidence and dealing with very small samples, a microscope allows us to gain more information visually; we can then use Raman to characterize the samples.

Carbon analysis

Raman can also be employed for the analysis of carbon. It is used for analyzing diamond, graphite, and carbon nanotubes (CNTs). In fact, it is a very good tool for analyzing CNTs as it provides a wealth of information (see Fig. 7). The graph on the right of the figure shows spectra of a single-walled nanotube (red) and a multiwalled nanotube (blue). At the low end of the spectrum, the first bands we see are attributable to so-called radial breathing modes (RBMs), which are vibrational modes. These bands are important because they provide information

on the diameter of the carbon nanotubes.

Higher in the spectrum, we see D and G bands. The first one, the D band, is indicative of an amorphous character, or disorder, in the sample. The G band, often called the graphite band, is indicative of ordered, or crystalline, character. If we look at the single-walled nanotube spectrum, we see an intense G band and a weak D band, which is typical as single-walled nanotubes tend to be very ordered—very crystalline in structure. If we look at the multiwalled nanotube spectrum in blue, we do not see the G band, and we only see the D band. Again, this is quite typical: With multiwalled nanotubes, we tend to see a more amorphous character as compared to single-walled nanotubes.



- RBM – Radial breathing modes
- D band – disorder band, amorphous character
- G band – Tangential mode, crystalline character

FIGURE 7. *Raman is well suited for analysis of various forms of carbon. It can distinguish single-walled from multiwalled carbon nanotubes and determine their diameters.*

As with CNTs, we can obtain information as to the physical properties of a graphene, but we can also use Raman to determine the thickness of the graphene layer.

In gemology and mineralogy, Raman is used as a noninvasive identification tool for gemstones. All molecules and chemical species have their own unique Raman fingerprints, and gemstones are no different. We can construct databases of gemstones and then use those databases later on for either unknown identification or known verification. Raman can also help identify gemstone polymorphs (a polymorph is essentially two different molecules with the same chemical makeup but different structures). In addition, gemstone origin can be determined through Raman-microscopy analysis of inclusions.

Anticounterfeiting is another gemology and mineralogy application. A common

example would be distinguishing diamond from zircon. For diamond, only one band is visible, which is located at 1328 wavenumbers. Zircon, however, has a number of different bands; even visually it is easy to distinguish zircon from diamond. For less obvious cases, the GemRam portable Raman spectrometer is useful, as it includes a database of roughly 400 different gemstones.

The advantages of Raman as a nondestructive test method requiring little to no sample preparation serve art and archeological analysis very well. Raman is routinely used for the analysis of pigments, ceramics, surface coatings of statues, and other artifacts. In one example, two spectra of pigments were collected using the innoRam (see Fig. 8), a portable Raman spectrometer that includes a very sensitive CCD detector. With this instrument, we have the ability to lower the laser power to very low levels to ensure that we're not burning or damaging the samples in any way. Obviously, as we lower the power we're going to see less Raman scattering coming back, so the sensitive CCD helps to collect more of that low scatter. Excitation at 532 nm is typically best for inorganic substances such as these pigments; the 532 nm laser is a bit more energetic than other longer-wavelength lasers, so we must be mindful of the power level to ensure we will not damage the sample.

Bioscience and medical diagnosis

In the biomedical area, Raman is used to detect subtle changes within biomolecules such as those occurring in drug interactions, tissue healing, cosmetics, and disease diagnosis. It is also used for intracellular SERS localization and interaction, immunoassays using SERS and Raman readers, the identification of drug binding to cells, drug DNA and cellular interaction analysis, the investigation of micro-organisms in single cells, yeast-cell classification, the detection of single bacteria, and oxygenation measurements of blood and tissue.

One very important use for Raman is in molecular-level detection of cervical, lung, breast, and other cancers. For example, Raman is currently being tested for the identification of breast cancer in lymph nodes via an assessment in the operating room. Currently, visible breast cancer is first removed from the patient and samples of lymph nodes are then collected for postoperative analysis. If cancer is detected in this postoperative testing, the patient must then undergo additional surgical procedures to remove the nodes in the affected area. Initial

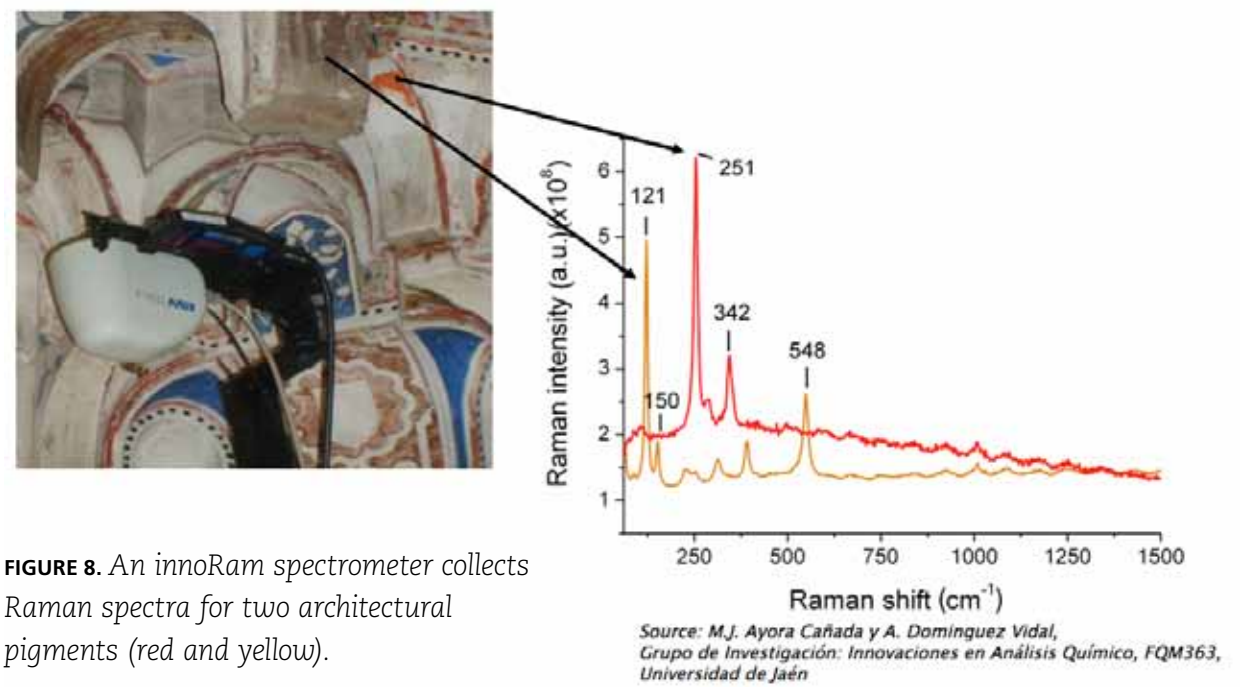


FIGURE 8. *An innoRam spectrometer collects Raman spectra for two architectural pigments (red and yellow).*

testing has shown that Raman can be used to detect differences in tissue composition. This would allow the surgeon to immediately remove all affected lymph nodes during the first surgical procedure. Obviously, the benefit here is that the patient does not have to undergo a number of different surgical procedures to ensure all of the cancer has been removed.

Conclusion

Having considered the advantages of Raman spectroscopy—such as its nondestructive testing capabilities, selectivity, ability to be utilized with little sample preparation, and its flexibility for both qualitative and quantitative analysis—it becomes easier to understand how the method can be so widely applied to various fields across research, medical science, and industry. Given that many of applications covered in this digest were developed fairly recently, it is safe to say that other applications of Raman spectroscopy are waiting in the wings.

Travis Thompson is a Raman spectroscopy product specialist at B&W Tek, Newark, DE; e-mail: TravisT@BWTek.com.

Company Description:

B&W Tek is an advanced instrumentation company producing optical spectroscopy and laser instrumentation, as well as laboratory, portable and handheld Raman systems. We provide spectroscopy and laser solutions for the pharmaceutical, biomedical, physical, chemical, LED lighting and research communities. Originally established as a producer of green lasers in 1997, we've grown into an industry-leading, total solutions provider; coupling our core technologies with custom design and manufacturing capabilities.

Since the company's establishment, we've emphasized strong vertical integration for better efficiency and faster growth. These values allow us to provide you with higher quality products that still fit into your budget. B&W Tek uses core components that are designed and manufactured in-house to create total solutions for a wide range of applications.

In addition to designing, manufacturing and assembling all of our own products, we also have the knowledge and expertise needed to guarantee that our products will fit the demands of your application. We feel that providing instrumentation is just part of our commitment towards providing your solution. Our research and development team consists of over 30 engineers in varying disciplines, each with an advanced degree in their field, and we're eager to share our information and experience with you.

www.bwtek.com

LINKS:

- Handheld, Portable, & Laboratory Raman Spectrometers
- Miniature TE Cooled Spectrometers for Raman & Fluorescence
- Fiber Coupled InGaAs NIR Spectrometers
- Miniature Spectral Irradiance Meters
- Spectrometer & Raman Technical Information