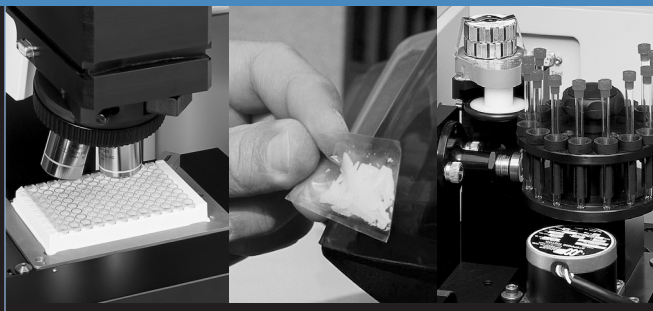
Introduction to Raman Spectroscopy



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Introduction

In 1928, Sir C.V. Raman documented the phenomenon of inelastic light scattering. Radiation, scattered by molecules, contains photons with the same frequency as that of the incident radiation, but may also contain a very small number of photons with a changed or shifted frequency. The spectroscopic process of measuring these shifted photons was later named after Sir Raman, with the shifting of frequency referred to as the Raman effect and frequently shifted light as Raman radiation. By the end of the 1930s, Raman spectroscopy had become the principle method of non-destructive chemical analysis.

Infrared spectroscopy replaced Raman as the preferred method after World War II, when the development of sensitive infrared detectors and advances in electronics made infrared easier to use. Infrared spectroscopic measurements became routine, whereas Raman spectroscopy still required complex instrumentation, skilled operators and darkroom facilities.

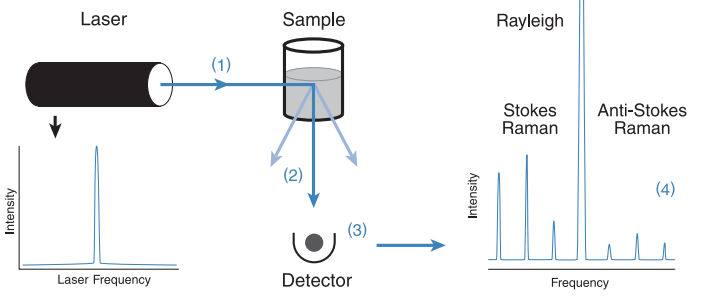
Although the development of lasers in the 1960s spurred renewed interest in the Raman technique, its acceptance was primarily limited to research laboratories. Raman instrumentation still required skilled operators to collect simple spectra, and the process was quite labor intensive.

Later developments, such as the availability of less expensive and more sensitive **Charge Coupled Devices (CCDs)**, the availability of **holographic notch filters** and the advent of **Fourier transform Raman (FT-Raman)**, launched a renaissance of Raman as a routine laboratory technique.

Today, the most advanced modern Raman instruments are completely integrated into a single unit and computer controlled, are interlocked for laser safety, have automated protocols for calibration and offer large spectral libraries. These advances make the collection and utilization of Raman spectra a routine exercise.

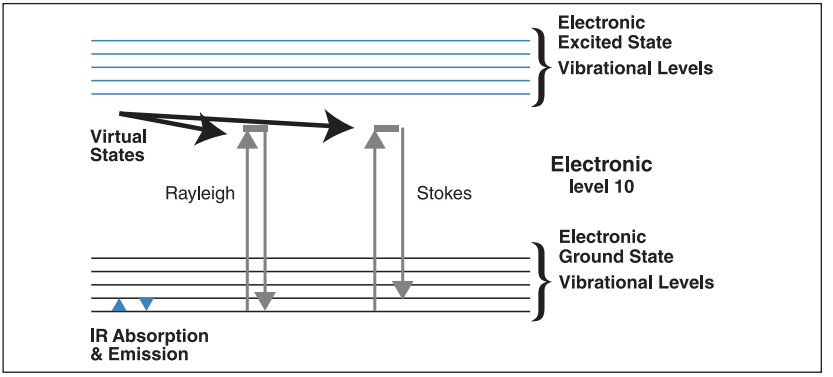
Theory

In Raman spectroscopy, a sample is irradiated with a strong monochromatic light source (usually a laser). Most of the radiation will scatter "off" the sample at the same wavelength as that of the incoming laser radiation, a process known as **Rayleigh** scattering. However, a small amount – approximately one photon out of a million (0.0001%) – will scatter from that sample at a wavelength shifted from the original laser wavelength.



(1) Laser light excites the sample.
(2) This light is scattered in all directions.
(3) Some of this scattered light is directed to the detector, which records the Raman spectrum.
(4) This spectrum shows light at the original laser (or Rayleigh) frequency and the Raman spectral features unique to the sample.

As illustrated in the following simplified energy level diagram, a molecule at rest resides in the ground vibrational and electronic states. The electric field of the laser raises the energy of the system for an instant by inducing a polarization in the chemical species. The polarized condition is not a true energy state and is widely referred to as a "virtual state". Relaxation from the virtual state occurs almost instantaneously and predominately returns to the initial ground state. This process results in Rayleigh scatter. Relaxation to the first excited vibrational level results in a Stokes-Raman shift. Stokes-Raman shift scatter is of lower energy (longer wavelength) than that of the laser light. Most systems have at least a small population initially in an excited vibrational state. When the Raman process initiates from the exited vibrational level, relaxation to the ground state is possible, producing scatter of higher energy (shorter wavelength) than that of the laser light. This type of scatter is called anti-Stokes-Raman scatter (not illustrated).



The vibrational states probed by Raman spectroscopy are the same as those involved in infrared spectroscopy. As such, Raman spectroscopy is very similar to the more frequently used Fourier transform infrared (FT-IR) spectroscopic technique. The two **vibrational spectroscopy** techniques are, in fact, complementary. Vibrations that are strong in an infrared spectrum (those involving strong dipole moments) are usually weak in a Raman spectrum. Likewise, non-polar functional group vibrations that give very strong Raman bands usually result in weak infrared signals.

For example, hydroxyl- or amine-stretching vibrations and the vibrations of carbonyl groups are usually very strong in an FT-IR spectrum, and are usually weak in a Raman spectrum. However, the stretching vibrations of carbon double or triple bonds and symmetric vibrations of aromatic groups are very strong in the Raman spectrum. Therefore, Raman spectroscopy is not only used as a stand-alone technique, but is often used in combination with FT-IR for a complete spectroscopic picture of the sample.

Vibrational spectroscopy provides key information on the structure of molecules. For example, the position and intensity of features in the vibrational spectrum can be used to study molecular structure or determine the chemical identity of the sample.

With experience, it is possible to identify the chemical compounds or to study intermolecular interactions by observing the positions and intensity of the Raman bands. However, it is also quite straightforward to identify compounds by spectral library searching. Raman is ideal for library searching because of the extensive spectral information, the unique spectral fingerprint of every compound and the ease with which such analyses can be performed.

Why Raman Spectroscopy?

Raman spectroscopy has major advantages over other analytical techniques. The most important advantages are the ease of sample preparation and the rich information content.

Raman is a light scattering technique, so all that is required for the collection of a spectrum is to place the sample into the excitation beam and collect the scattered light.

There are few concerns with sample thickness (as in transmission analyses) and little contribution from the ambient atmosphere, so there is no need for high-vacuum or desiccated sample holders. Glass, water and plastic packaging each have very weak Raman spectra, making the technique even easier to use. Often, samples can be analyzed directly inside the glass bottle or plastic bag without opening the package and risking contamination. Aqueous samples are readily analyzed without the need to remove water, and because ambient humidity is not a problem, there is no need to purge the instrument.

Furthermore, no two molecules give exactly the same Raman spectrum, and the intensity of the scattered light is related to the amount of material present. This makes it easy to obtain both qualitative and quantitative information about the sample, allowing for spectral interpretation, library searching, data manipulations and the application of quantitative analysis computer methods.

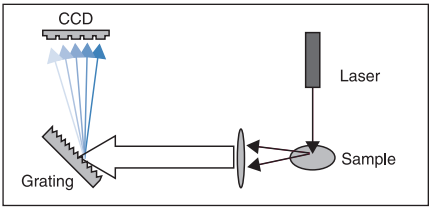
Raman spectroscopy is non-destructive. There is no need to dissolve solids, press pellets, compress the sample against optical elements or otherwise alter the physical or chemical structure of the sample. Thus, Raman has been used extensively for analysis of such physical properties as crystallinity, phase transitions and polymorphs. The lack of sample preparation also minimizes cleanup and the possibility of cross-contamination.

Several additional advantages are obtained with Raman spectroscopy over other vibrational techniques due to the fact that its operational wavelength range is usually independent of the vibrational modes being studied. Other vibrational techniques require frequencies that correspond directly to the vibrational modes being studied. Raman is a dream come true for most researchers as it can be performed using any operating range from UV to NIR allowing you to select the most convenient range for your sampling technique in order to yield the best results. Raman allows easy access to vibrational modes associated with frequencies in the far-infrared which can otherwise be very difficult to access. Raman also allows microscopy with spatial resolution as fine as 1 μm and easily executed remote fiber-optics work providing vibrational mode information normally associated with wavelengths ranging from 2 – 100 μm. Achieving results like this using the native frequencies would be a daughnting task, but Raman makes it easy.

Raman spectrometers basically employ one of two technologies for the collection of spectra: **dispersive Raman** and **Fourier transform Raman**. Each technique has unique advantages and each is ideally suited to specific analyses.

Dispersive Raman Spectroscopy

To observe the Raman spectrum, it is necessary to separate the collected Raman scattered light into individual wavelengths. In dispersive Raman instruments, this is accomplished by focusing the Raman signal on a grating, which spatially separates the different wavelengths. This spatially dispersed beam is directed to a **CCD**.



Dispersive Raman usually employs visible laser radiation. Typical laser wavelengths are 780 nm, 633 nm, 532 nm, and 473 nm although others are common. One advantage of using shorter wavelength lasers is the enhancement in the Raman signal that occurs at shorter wavelengths. The efficiency of Raman scatter is proportional to $1/\lambda^4$, so there is a strong enhancement as the excitation laser wavelength becomes shorter.

This would suggest that all Raman should be done with the shortest wavelength lasers available. However, one factor hindering the practice of Raman as a routine tool is the unpredictable **fluorescence** that often occurs. Fluorescence is a very efficient emission several orders of magnitude stronger than the Raman signal, so minor fluorescence can overwhelm the desired Raman measurement.

Fluorescence occurs when the virtual energy level overlaps an upper electronic level, so as the energy of the laser gets higher (shorter wavelength), the likelihood of fluorescence increases. The phenomenon is excitation wavelength dependent, so a sample that fluoresces at one wavelength may not at another. Thus, when selecting an instrument, it is important to look for rapid and effortless exchanges between two difficult excitation lasers.

The **grating** has a strong influence on spectral resolution and instrument throughput. Gratings have many lines or grooves "blazed" into the surface, which disperse the incoming light. The higher the number of grooves on the grating, the wider the dispersion angle of the exiting rays.

It is necessary to have many grooves (for example, 1800 or 2400 lines/mm) for a high-resolution spectrum, in which very closely spaced wavelengths must be distinguished. A smaller number of lines are needed (300 or 600 lines/mm) for a low-resolution spectrum. The higher the dispersion of the exiting rays, the larger the area over which the different wavelengths will lie when they reach the detector surface.

With a fixed detector size, there is a point (resolution) beyond which not all of the Raman wavelengths fall on the detector. In cases of higher dispersion (high resolution), it is necessary to move either the grating or the detector to collect sequential regions of the spectrum.

Grating response is also wavelength dependent, so the dispersion (resolution) across the wavenumber axis is not linear, but instead, the dispersion becomes greater at higher wavenumbers (cm^{-1}). For this reason, spectral resolution must be stated for a specific wavenumber and will vary across the spectrum.

Finally, gratings are blazed for optimum throughput over a relatively narrow wavelength range, so a grating should be selected for the desired resolution and for the correct laser wavelength. Using a single grating for more than one laser wavelength or more than one resolution requires compromises in both instrument throughput and sensitivity. Ideally, gratings should be specifically matched to the laser and experimental conditions of the experiment.

The CCDs commonly used for dispersive Raman are silicon devices with very high sensitivity. The detecting surface of the CCD is a two-dimensional array of light-sensitive elements, called **pixels** (usually each pixel is <30 µm). Each pixel acts as an individual detector, so each dispersed wavelength is detected by a different pixel (or closely spaced group of pixels).

CCD detectors commonly have a large wavelength response region, routinely extending from 400 nm up to approximately 1000 nm. Specialized detectors extend the response up to approximately 1100 nm or down into the UV range. For dispersive Raman with a 780 nm laser, the 3000 cm^{-1} response (corresponding to the C-H stretch region of the spectrum) results in 1018 nm radiation. Many common CCDs have very weak responses for the higher wavenumber response of the NIR laser, and going any higher in laser wavelength rapidly disqualifies the CCD as a viable detector.

To get the most from your Raman spectrum, it is important to get the full 100 – 3100 cm^{-1} spectrum at all laser wavelengths.

Raman Microscopy

It is advantageous to couple the strength and flexibility of Raman spectroscopy with a microscope that allows analysis of very small samples. The goal of microscopy is to analyze the smallest samples possible and to distinguish the substance of interest from its surroundings. This is known as spatial resolution, and in microscopy, the highest spatial resolution is attained using small pinholes or "apertures" somewhere in the microscope.

To reach higher resolution, it is necessary to use smaller apertures. As light passes through these smaller apertures, diffraction becomes the limiting factor. Therefore, the limit to spatial resolution is diffraction, which is wavelength dependent according to the following equation:

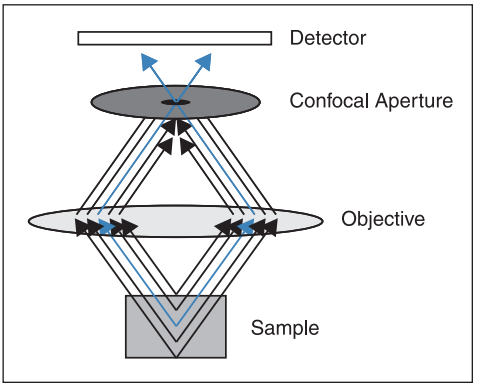
$$D = \frac{1.22\ \lambda}{n.a.}$$

where n.a. is the numerical aperture of the collection optics.

Shorter excitation wavelengths provide the highest spatial resolution, so dispersive Raman microscopy offers excellent spatial resolution (<1 µm).

In addition, by placing a sufficiently small aperture in the focal plane of the microscope, it is possible to perform confocal microscopy, in which light rays from surrounding regions of the sample are blocked by the aperture and only rays from the optical focal point pass to the detector. This is a useful technique for non-destructively probing the depths of the sample without cross-sectioning.

Confocal microscopy is done best by dispersive Raman microscopy with short wavelengths because diffraction at longer wavelengths limits how small the confocal aperture can be, thus limiting the z-axis resolution. The technique is quite useful for analyzing polymer laminates, stacked structures and inclusions, as long as fluorescence is not an issue.



FT-Raman Spectroscopy

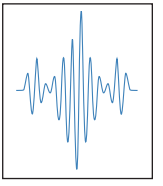
FT-Raman was developed to overcome some of the problems occasionally encountered in dispersive Raman spectroscopy. One important advantage of FT-Raman lies in the near lack of sample fluorescence.

An FT-Raman instrument typically employs a 1 µm excitation laser, an **interferometer** and a high-sensitivity near-infrared detector. By using the longer wavelength excitation laser, there is less energy supplied, so the virtual state is lower and less likely to overlap an upper electronic state. This greatly reduces fluorescence interferences.

Indium gallium arsenide **(InGaAs)** or liquid nitrogen-cooled germanium **(Ge)** detectors are typically used for FT-Raman spectroscopy. These detectors are very sensitive, but are still less sensitive for near-infrared radiation than the silicon CCD is for visible radiation. The advantages of the Fourier transform technique are necessary to provide the sensitivity to extract functional spectral information from this lower intensity signal.

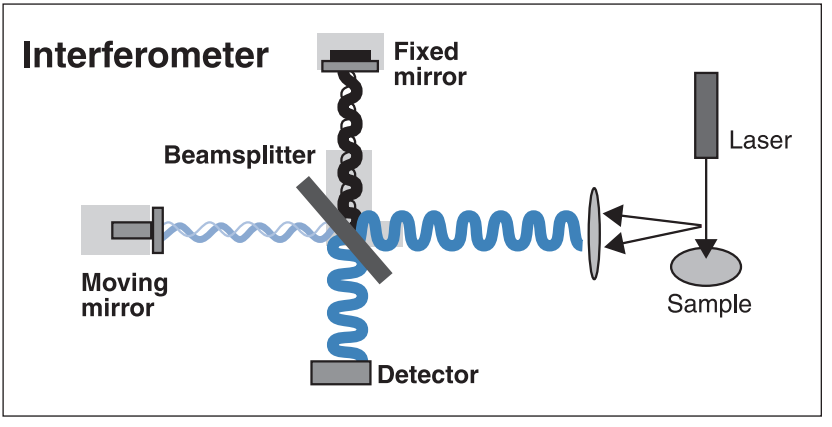
By virtue of the Fourier transform technique, FT-Raman offers:

- High resolution with minimal throughput loss
- Measurement of all wavelengths at once
- Increased signal-to-noise by signal averaging
- Superior wavelength accuracy due to the internal calibration inherent to an interferometer

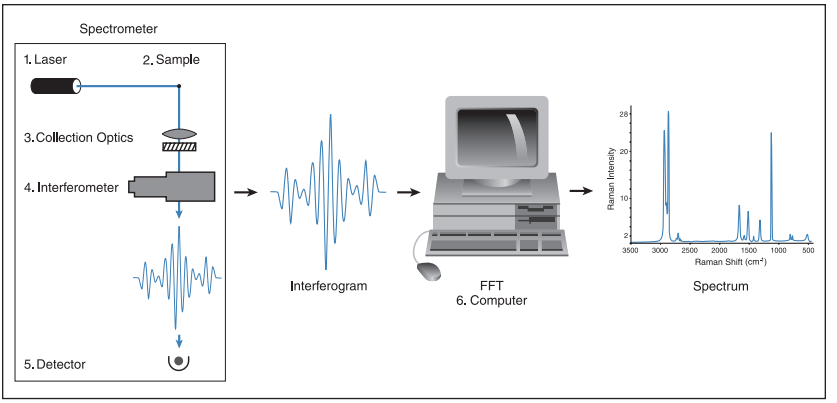


FT-Raman spectroscopy uses an interferometer to produce an **interferogram**, which "encodes" the unique frequencies of the Raman scattering into a single signal. The signal is measured very quickly (usually in one second), making signal averaging fast and accurate.

The interferometer employs a beamsplitter optimized for near-infrared radiation, which divides the incoming Raman scatter into two optical beams, one transmitted and one reflected. The reflected beam travels to and reflects off a flat mirror that is fixed in place. The transmitted beam travels to and reflects off a flat mirror attached to a mechanism that allows the mirror to move a short distance (typically a few millimeters) away from the beamsplitter.



The two beams recombine at the beamsplitter where, because they traveled different distances to and from the mirrors, they constructively and destructively interfere with each other. The moving mirror has a constant frequency and fixed motion, so this interference is modulated. The resulting interferogram has the unique property that every data point (a function of the moving mirror position) has information about every frequency of the Raman scatter collected from the sample.



Vibrational spectra are typically presented as frequency spectra (a plot of intensity at each individual frequency) because the measured interferogram signal is not readily interpreted. The individual frequencies are decoded using the well-known mathematical technique called Fourier transformation. The computer performs this transformation, and the desired spectral information is presented.

Dispersive Raman or FT-Raman?

The answer, in fact, could easily be both. The resulting spectral information is practically the same for both dispersive Raman and FT-Raman, and both offer all the advantages of Raman. However, one technique is recommended over the other for some situations. As a general rule, dispersive Raman offers more when it comes to mircoscopic applications due to its higher sensitivity. FT-Raman also offers more for bulk material analyzes because of their lack of fluorescence, wavelength precision, and the cost effectiveness of the technique. Here are some additional guidelines to help you select between dispersive or FT-Raman.

Dispersive Raman Key Applications

Dispersive Raman spectroscopy has been applied to many types of samples. Shorter laser wavelengths and more sensitive CCDs make the technique ideal for **minor component analysis**, offering low detection limits for such applications as impurity analysis in solutions, polymers or environmental sampling.

Major applications for visible Raman are in the **semiconductor and microelectronics** industries where silicon and various coatings are routinely analyzed. Raman analysis of processed silicon devices yields information concerning silicon stress, shear and other properties important to product quality. And with ever decreasing device size, the spatial resolution of dispersive Raman is very important. FT-Raman is not applicable here because silicon oxide (and many metal oxides) exhibits fluorescence upon near-infrared excitation. Most dispersive Raman analyses on silicon should be done with a 633 nm or shorter wavelength laser, to move sufficiently far from the near-infrared laser-induced fluorescence.

Dispersive Raman is becoming more and more popular within the pharmaceutical and life sciences arenas as well. The increased emphasis on single crystal studies within pharmaceuticals and the value of mapping biological tissues with high spatial resolution has created a need that dispersive Raman is best suited to address. There is a possibility of fluorescence with these materials so a long-wavelength laser such as the 780 nm is recommended. A longer wavelength 830 nm laser can be used to avoid fluorescence from strongly fluorescing biological materials, but detector limitations with this laser result in significant sacrifices in spectral range.

Raman spectroscopy offers the ability to measure vibrational spectra of **aqueous samples**. Though FT-Raman has been used to analyze aqueous samples, water has strong interactions in the near-infrared. Therefore, laser radiation and Raman scatter are both susceptible to absorbance by water. Dispersive Raman, with visible laser excitation, is often more sensitive for aqueous samples because water absorbance of the radiation is not present.

Dispersive Raman is often very powerful for analyzing very **dark samples**, such as carbon black loaded or highly colored samples. Many other techniques suffer from total absorbance or sample heating, which is often not present when using the dispersive Raman technique, owing to lower laser powers that can be used.

Inorganics analysis and identification in areas such as **geology** and **gemology** are more commonly done using dispersive Raman because it is often free of the metal oxide fluorescence background that may be seen in FT-Raman. The confocal approach has also been used to probe inclusions in gems and stones by focusing on the region within the body of the material.

Polymer laminates, layered paint samples and other samples in which depth or cross-sectional information is desired are prime candidates for confocal analysis with dispersive Raman microscopy. As long as there is no fluorescence, the highest axial resolution and sensitivity can be obtained at 532 nm. But longer wavelengths can also be used with success.

FT-Raman Key Applications

FT-Raman is the best choice in situations where samples fluoresce or are likely to contain minor impurities that may fluoresce. Significant success has been realized in the **pharmaceutical industry** for unknown identification, incoming raw material characterization, final product quality and quantitative analyses using FT-Raman. There have also been many successes investigating **polymorphs** and analyzing surface and bulk structure in **combinatorial chemistry**. FT-Raman has been successful over dispersive Raman because these pharmaceutical compounds often fluoresce, even at 785 nm excitation, but give nice spectra with 1 µm excitation.

Along the same lines, FT-Raman has experienced great success in **forensic** analyses through sample containers or evidence bags, negating the need to break the container seal. It has been used to analyze illicit drug substances, clandestine lab samples, explosives and fibers. In particular, street drugs and clandestine lab samples often fluoresce with visible laser excitation but can be analyzed by FT-Raman.

Pure **polymers** do not typically fluoresce, but the minor additives, anti-slip agents and plasticizers often do when excited with visible lasers. FT-Raman can typically be used for most of these polymer samples.

FT-Raman has gained acceptance in chemical analysis in such industries as **pulp and paper**, **textiles** and **petrochemicals**. It has been very useful for identification of unknowns because of the availability of Raman libraries. The FT-Raman technique is inherently internally calibrated, so wavenumber accuracy is exceptional. For quantitative and qualitative (library searching) analyses, it is necessary to have good wavenumber accuracy.

FT-Raman methods for routine analysis, whether **raw materials identification** or **product assay**, are easily developed and automated. Due to the comprehensive control software, the instruments are very user friendly and the protocols are intuitive, minimizing the need for trained spectroscopists to collect and interpret Raman data.

Conclusion

Raman spectroscopy is a powerful analytical technique offering many advantages. Overall, Raman offers ease of use because little sample preparation is required, and the spectral information can be used for identification or quantification.

Both dispersive Raman and FT-Raman have advantages that are unique for certain types of samples. Of course, in many industries, such as pharmaceuticals, polymers, petrochemicals and forensics, dispersive Raman and FT-Raman can be applied. Likewise, in the absence of backgrounds, either Raman technique can be used for geological samples, dark samples or aqueous solutions.

In all applications, it is extremely important to test the authentic samples to which the technique will be applied. Often, simulations or synthetic "standards" will not exhibit the interferences or backgrounds that the real samples do, and an evaluation using these "standards" may provide incorrect results.

Only an instrument manufacturer offering both techniques can provide the most accurate and efficient evaluation for your application. In this way, the final choice between dispersive Raman and FT-Raman is based on the best answer for your analysis, and you are not forced into the limited product offering of a supplier who can only provide half the power of Raman spectroscopy.

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