Introduction to Raman Spectroscopy

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Introduction

While Raman spectroscopy has long been recognized as a valuable research technique in the years since the phenomenon was first observed by Dr. C. V. Raman in 1928, it is only fairly recently that Raman has emerged as an important analytical tool across a number of industries and applications. No longer designed to appeal only to highly specialized and trained experts, the best of today's Raman instruments are fully integrated and come with built-in system intelligence that frees the user to focus on results and not on having to become an expert in the technology itself. Busy analytical laboratories are now able to adopt Raman spectroscopy without having to devote time to developing the expertise that used to be essential in order to be able to collect high quality data.

Due to its sensitivity, high information content, and non-destructive nature, Raman is now used in many applications across the fields of chemistry, biology, geology, pharmacology, forensics, pharmaceuticals, materials science, and failure analysis. Spectral libraries in excess of 16,000 compounds are now available for direct compound identification.

In many laboratories, infrared and Raman spectroscopy are used as complementary techniques, because each method looks at different aspects of a given sample. While IR is sensitive to functional groups and to highly polar bonds, Raman is more sensitive to backbone structures and symmetric bonds. Using both techniques provides twice the information about the vibrational structure than can be obtained by using either alone.

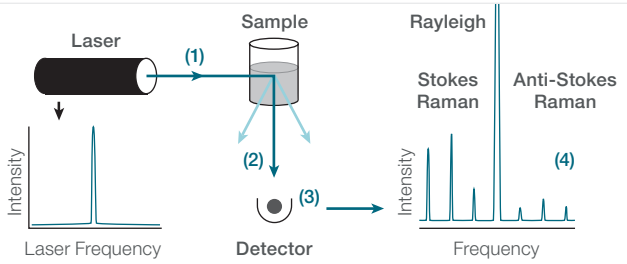
In addition to providing unique information about a sample, Raman offers several additional benefits, including:

- Minimal or no sample preparation
- Sampling directly through glass containers
- Non-destructive analysis, so the same sample can be used in other analyses
- Non-intrusive analysis, permitting study of more labile sample features, such as crystal structure
- Minimal water interference
- No interference from atmospheric CO_2 or H_2O

The objective of this booklet is to provide an introduction to the technique of Raman spectroscopy and its relevance as an analytical tool. The first section contains a simple description of the theory behind the Raman effect, followed by a discussion of the relevance of Raman spectroscopy and its complementarity with other vibrational techniques. This is followed by an overview of the two major Raman instrument designs: dispersive Raman and Fourier transform Raman (FT-Raman). Advantages and key applications are discussed for each of these instruments, followed by a list of desirable features in modern instruments.

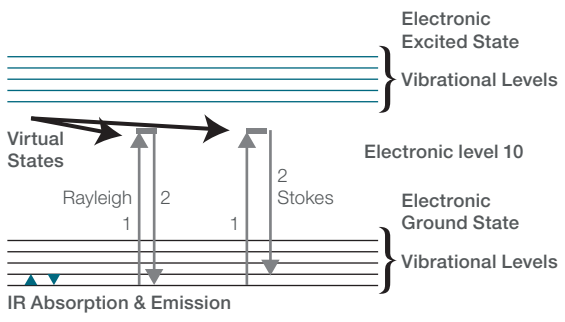
Theory: The Raman Effect

When a sample is irradiated with an intense monochromatic light source (usually a laser), most of the radiation is scattered by the sample at the same wavelength as that of the incoming laser radiation in a process known as **Rayleigh** scattering. However, a small proportion of the incoming light – approximately one photon out of a million – is scattered at a wavelength that is shifted from the original laser wavelength.



(1) Laser light excites the sample.
(2) The 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 (for Rayleigh) frequency and the Raman spectral features unique to the sample.

As illustrated in the simplified energy level diagram, a molecule at rest resides in the ground vibrational and electronic states. The electric field of the laser beam 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 referred to as a "virtual state". Relaxation from the virtual state occurs almost instantaneously and is predominantly to the initial ground state. This process results in Rayleigh scatter, which is scattered light of the same wavelength as the excitation laser. Relaxation to the first excited vibrational level results in a Stokes-Raman shift. Stokes-Raman shift scattered light is of lower energy (longer wavelength) than that of the laser light. In addition, most systems have at least a small population of molecules that are initially in an excited vibrational state. When the Raman process initiates from the excited 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 and is not illustrated.



The vibrational states probed by Raman spectroscopy are similar to those involved in infrared spectroscopy. However, the two **vibrational spectroscopy** techniques are complementary, in that vibrations that are strong in an infrared spectrum (those involving strong dipole moments) are typically weak in a Raman spectrum. Likewise, non-polar functional group vibrations that give very strong Raman bands usually result in weak infrared signals. As an example, hydroxyl- or amine-stretching vibrations and the vibrations of carbonyl groups are usually very strong in an FTIR spectrum and are weak in a Raman spectrum. The stretching vibrations of carbon double and triple bonds and the symmetric vibrations of aromatic groups give a very strong Raman signal.

Raman spectroscopy provides key information about the structure of molecules. The position and intensity of features in the spectrum reflect the molecular structure and can be used to determine the chemical identity of the sample. Spectra may also show subtle changes depending on the crystalline form. With the extensive spectral libraries that are now available, it is very straightforward to identify compounds by spectral library searching.

Why Raman Spectroscopy?

Raman spectroscopy offers some major advantages in comparison with other analytical techniques. 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 and little interference from the ambient atmosphere, so there is no need for high-vacuum or desiccated sample holders. Glass, water and plastic packaging have weak Raman spectra, making the technique even easier to use. Samples usually can be analyzed directly inside a glass bottle or plastic bag without having to open the package and risk contamination. Aqueous samples are readily analyzed without having to remove water, and because there is no interference from ambient humidity, there is no need to purge the instrument.

No two molecules give exactly the same Raman spectrum, and the intensity of the scattered light is proportional to the amount of material present. Thus Raman provides both qualitative and quantitative information about the sample, allowing for spectral interpretation, library searching, data manipulation and the application of chemometric methods.

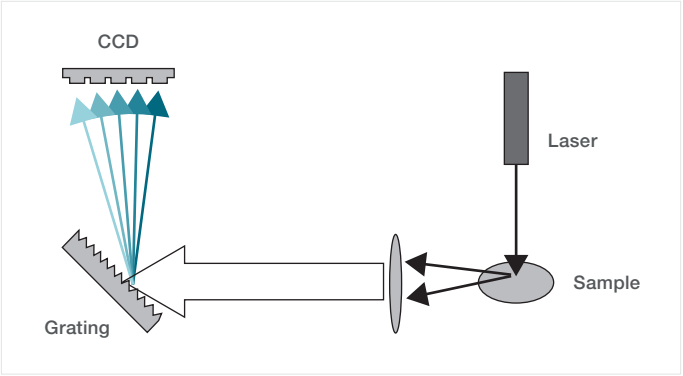
Raman spectroscopy is non-destructive. There is no need to dissolve solids, press pellets, compress the sample or otherwise alter its physical or chemical structure. This makes Raman spectroscopy ideal for investigating physical properties such as crystallinity, phase transitions and polymorphs. The lack of sample preparation also minimizes cleanup and the possibility of cross-contamination.

Raman spectroscopy also has several additional advantages. Unlike other vibrational techniques, its operational wavelength range is usually independent of the vibrational modes being studied. Since Raman spectroscopy measures the shift in frequency from that of the excitation laser, it can be performed using any operating range from UV to NIR. It thus permits access to vibrational mode information normally associated with wavelengths ranging from 2 – 200 µm. This makes Raman ideal for the study of inorganic materials that have vibrational frequencies in the far-infrared that are otherwise difficult to reach. Dispersive Raman microscopy using visible excitation wavelengths delivers 1 µm spatial resolution, and is widely used in the analysis of micron-level sample contaminants.

Raman spectrometers are based on one of two technologies: **dispersive Raman** and **Fourier transform Raman**. Each technique has its unique advantages and each is ideally suited to specific types of analysis.

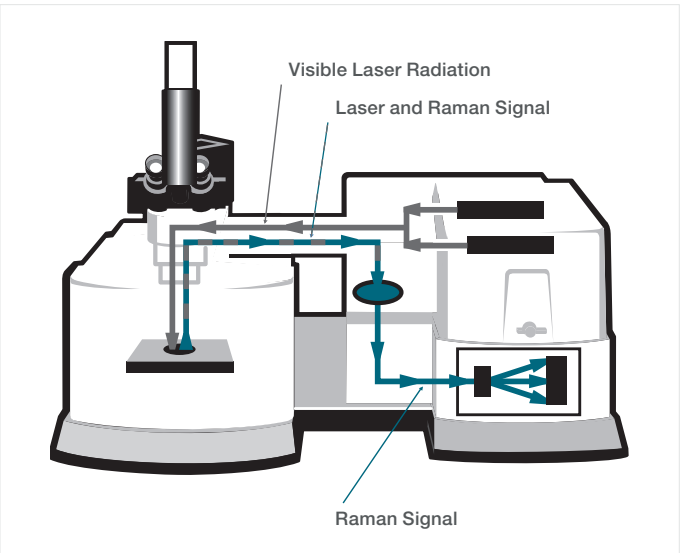
Dispersive Raman Spectroscopy

To observe the Raman spectrum, it is necessary to separate the collected Raman scattered light into its composite wavelengths. In dispersive Raman instruments, this is accomplished by focusing the Raman scattered light onto a diffraction grating, which splits the beam into its constituent wavelengths. These are directed onto a silicon charge-coupled device or **CCD** detector.



Dispersive Raman usually employs visible laser radiation. Typical laser wavelengths are 780 nm, 633 nm, 532 nm, and 473 nm; others are also used. The intensity of the Raman scatter is proportional to 1/λ4, so short excitation laser wavelengths deliver a much stronger Raman signal. Although this would suggest that all Raman should be collected using the shortest wavelength lasers, **fluorescence** is also much more likely to occur under these conditions. It is possible to use software and other strategies to correct for low-level fluorescence interference and still be able to obtain usable Raman spectra. However, strong fluorescence saturates the CCD and makes Raman measurements impossible. Fluorescence is excitation wavelength-dependent, so a sample that fluoresces at one wavelength is less likely to do so at another. When selecting a Raman spectrometer, it is important to look for one that integrates multiple laser sources and makes it easy to exchange lasers rapidly and effortlessly.

Spectral resolution determines the amount of detail that can be seen in the spectrum. If the resolution is too low, it may be impossible to distinguish between closely related compounds. When the resolution is unnecessarily high, the data become noisy without providing any increase in useful information. Spectral resolution is determined by the diffraction grating dispersion and by the optical design of the spectrograph. Gratings have many lines or grooves blazed into the surface, which disperse the incoming light. The higher the number of grating lines per unit length, the broader the dispersion angle and the higher the spectral resolution obtained. With a fixed detector size, there is a resolution beyond which not all of the Raman wavelengths fall on the detector in one exposure. To achieve higher resolution, it is necessary to move either the grating or the detector to collect sequential segments of the spectrum. Spectral resolution can also be increased by extending the optical path length of the spectrograph or by improving the spectrograph design.



Gratings are designed for optimum throughput over a relatively narrow wavelength range, so gratings should be selected for the desired resolution and for the correct laser wavelength. Using a single grating for more than one laser wavelength requires a compromise in instrument throughput and sensitivity. Ideally, gratings should be matched specifically to each laser.

The CCDs commonly used for dispersive Raman are highly sensitive silicon devices. The detecting surface of the CCD is a two-dimensional array of light-sensitive elements, called **pixels** (typically 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 and down into the UV range. This means that the longest excitation wavelength that can be used with a silicon CCD detector, without loss of the higher shifted wavenumber portions of the Raman spectrum, is about 780 to 785 nm. (A 3300 cm^{-1} Stokes shift from 780 nm corresponds to 1050 nm.)

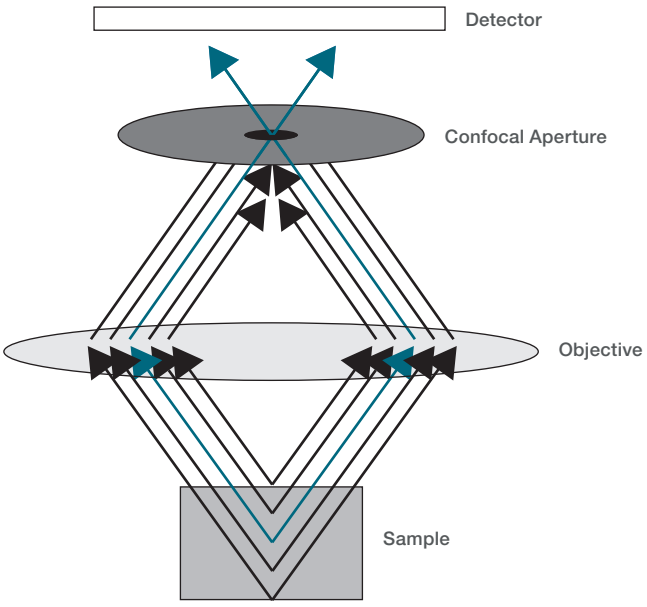
The low end cutoff of the Raman spectrum is determined by the ability of the Rayleigh filters to exclude Rayleigh scattering (light at the excitation laser wavelength) from the spectrograph. The performance of a Raman spectrometer at the low shifted wavenumber end of the spectrum also depends on the adequacy of the laser line filters. Good modern instruments should be able to attain at least a 100 cm^{-1} cutoff. Since inorganic compounds display important Raman bands below 100 cm^{-1}, an instrument that can deliver a 50 cm^{-1} low end cutoff is ideal.

Raman Microscopy

Dispersive Raman microscopy is ideal for the analysis of very small samples. Since spatial resolution is diffraction-limited, the short excitation laser wavelengths used for dispersive Raman are optimal for analyzing small sample features. With 532 nm excitation, a modern Raman microscope can achieve sub-micron spatial resolution. The analysis of small defects in the polymer films used in liquid crystal displays is a typical application.

In order to achieve micron-level spatial resolution, it is critical that a Raman microscope is optimally aligned. In order to target and analyze a micron-sized particle, the visual light path, the excitation laser beam path and the Raman scatter beam path from the sample to the spectrograph detector must all be targeted precisely on the same spot. Since all instruments will become misaligned at the micron level as a result of exposure to thermal shifts and other environmental changes, the instrument design must make it easy for the user to perform precision alignment on a routine basis in order to ensure optimal performance.

Placing a sufficiently small aperture in the focal plane of the microscope makes it possible to perform confocal microscopy. Light rays from surrounding regions of the sample are blocked by the aperture and only rays from the optical focal point pass through the aperture to the detector. This is a powerful technique for nondestructively probing the depths of transparent samples without having to cross-section them. It is widely used to investigate inclusions and for analyzing polymer laminates.

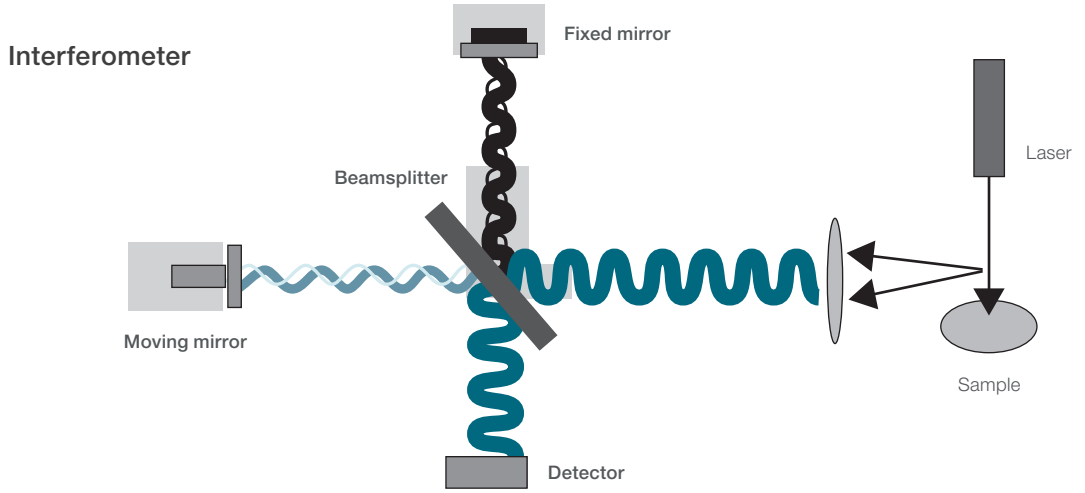


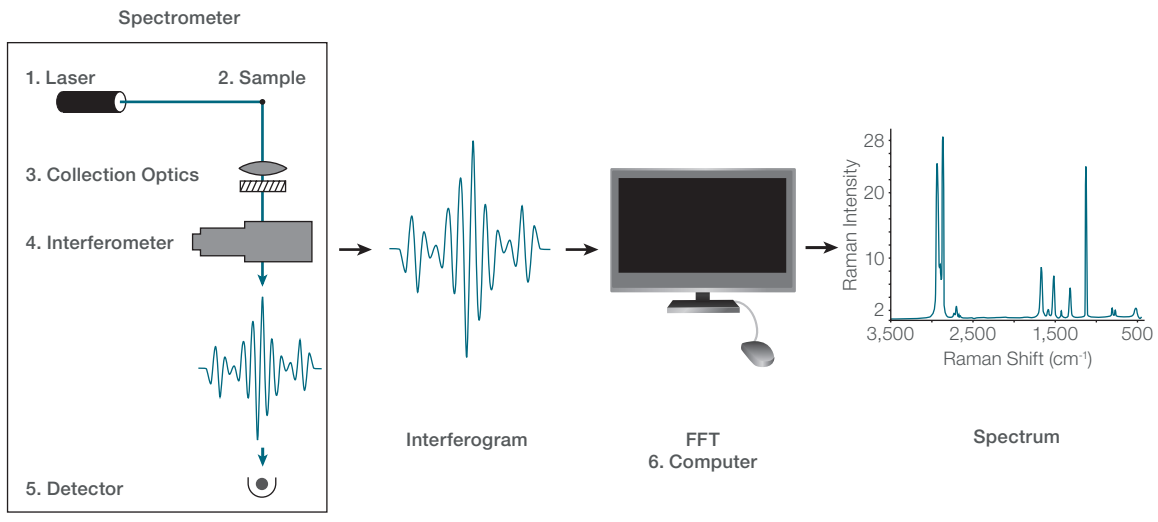
FT-Raman Spectroscopy

In place of visible excitation lasers, an FT-Raman spectrometer uses a laser in the near infrared – usually at 1064 nm. At this wavelength fluorescence is almost completely absent, however because of the $1/\lambda^4$ relationship between Raman scattering intensity and wavelength, the Raman signal is weak. In addition, silicon CCD detectors cannot be used in this region of the spectrum. FT-Raman uses sensitive, single-element, near-infrared detectors such as indium gallium arsenide **(InGaAs)** or liquid nitrogen-cooled germanium **(Ge)** detectors. An **interferometer** converts the Raman signal into an interferogram, permitting the detector to collect the entire Raman spectrum simultaneously. Since at low signal levels the spectral noise is predominantly detector dark noise and is independent of the intensity of the Raman signal, delivering the entire spectrum at once onto the detector greatly improves the signalto- noise ratio. Application of the Fourier transform algorithm to the **interferogram** converts the results into a conventional Raman spectrum.

In addition to freedom from fluorescence interference, another advantage of FT-Raman spectroscopy is its exceptionally good x-axis (shifted wavenumber) accuracy as a result of the internal interferometer calibration supplied by the built-in helium-neon laser. Both of these attributes make FT-Raman the ideal technique for collecting spectra for reference libraries.

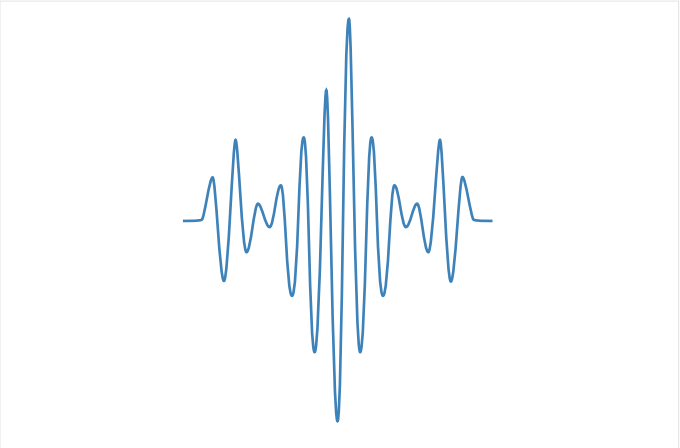
FT-Raman spectroscopy is particularly well-suited for bulk sample analysis and can be configured to accept samples in most common formats, including vials, cuvettes, tubes, plastic bags, bottles, powders, films and solids. The Thermo Scientific iS50 FT-Raman Module features a full-size sample compartment, integrated video camera and 1064nm excitation power of over 1W. With a minimum laser spot size of less than 60 µm, the MicroStage in this module provides microscope capabilities and sufficient spatial discrimination to be able to analyze heterogeneous samples. In addition, multiple samples in well plates or arrays can be set up to be measured automatically.





Dispersive Raman or FT-Raman?

The answer easily could be both. The resulting spectral information is essentially the same for both techniques. As a general rule, the major advantage of FT-Raman is its absence of fluorescence interference. In addition, if the user already possesses an FTIR spectrometer, it is frequently convenient and economical to add an FT-Raman spectrometer to the unit. Since dispersive Raman operates at visible wavelengths, the Raman signal is much stronger, the technique is more sensitive and it delivers higher spatial resolution. This makes dispersive Raman the best choice for small particles and **minor component analysis.**



Typical FT-Raman Applications

FT-Raman is the best choice in situations where samples fluoresce or are likely to contain minor impurities that may fluoresce. For this reason, FT-Raman is widely used in forensic analysis. The ability to sample through containers and evidence bags maintains sample integrity by avoiding the need to break the container seal. It has been used to analyze illicit drug substances, clandestine lab samples, explosives and fibers. Street drugs and clandestine lab samples frequently are contaminated with materials that fluoresce with visible laser excitation. These samples are analyzed successfully using FT-Raman.

In the pharmaceutical industry FT-Raman is used for identification of unknowns, incoming raw material characterization, final product quality monitoring and quantitative analysis. FT-Raman and dispersive Raman spectroscopy have both been used for polymorph characterization and the analysis of surface and bulk structure in combinatorial chemistry. FT-Raman is often used in preference to dispersive Raman because pharmaceutical compounds tend to fluoresce strongly at visible wavelengths.

Pure polymers do not typically fluoresce, but the minor additives, anti-slip agents and plasticizers often do when excited with visible lasers. FT-Raman is thus frequently the method of choice for these samples.

Typical Dispersive Raman Applications

Dispersive Raman spectroscopy has been applied to many types of samples. Micron-level spatial resolution combined with the ability to do confocal analysis and with the higher sensitivity obtained at visible laser wavelengths, makes this technique ideal for minor component analysis, defect analysis, sample inclusions and failure analysis applications. Dispersive Raman is also widely used within the pharmaceutical and life sciences. Increased emphasis on single crystal studies is best met with dispersive Raman. Dispersive Raman spectroscopy is an excellent technique for comprehensive polymorph screening during drug development.

Inorganic analysis and sample identification in **geology** and **gemology** more commonly use dispersive Raman, because this method avoids the metal oxide fluorescence that may occur with FT-Raman spectroscopy. Confocal analysis is an excellent way to probe inclusions in gems and semi-precious stones. This information can sometimes be used to identify the point of origin of these samples. 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.

Selecting an Instrument

Since most modern Raman instruments are purchased to be used as analytical tools, instrument design should focus on making Raman easy to use without compromising performance. A well-designed instrument lets its user focus on results and not on having to become a Raman expert. The following list includes some of the things to look for:

Easy to Use

- Features such as autoexposure, smart backgrounds and autofocus rely on the instrument and software intelligence to optimize data collection parameters. This is analogous to the way that the expertise required to achieve professional-level photography has been embedded into digital cameras.
- Components such as lasers, gratings, Rayleigh filters and sampling accessories should be easy to exchange, either through automation and a mouse-click, or by employing SmartLock technology to ensure reproducible results. Smart components are readily recognized by the system software, their identities recorded for full traceability and the user is alerted if components are incompatible with each other.

Laser Safe

- Instruments where the user cannot be exposed to a direct laser beam are classified as Class I laser safe and can be used in open laboratories. Class IIIb laser-safe instruments have to be used in restricted environments in order to prevent inadvertent exposure to the laser beam and are therefore much less convenient to use.

High Performance

- High spatial resolution: one of the key reasons for purchasing a dispersive Raman microscope is to take advantage of its micron-level spatial resolution. In order to achieve this performance consistently, the instrument design must make it easy to maintain optimal alignment. The Thermo Scientific DXR3 Raman line of microscopes and spectrometers employ a rapid, patented, software-driven alignment method that makes it easy to incorporate instrument alignment into routine laboratory maintenance procedures.
- Full spectral range: in order to take full advantage of the power and information content of Raman spectroscopy, spectrometers should cover the full spectral range; from 50 cm^{-1} at the low end to 3500 cm^{-1} at the high end of the spectrum.

Reproducibility: In the Past Raman had a Reputation for Poor Reproducibility, However this can be Addressed by Careful Design

- The excitation laser power can vary considerably from one laser to the next and from one instrument to the next. Incorporation of a laser power regulator that permits the user to specify and regulate the laser power at the sample eliminates this cause for non-reproducible Raman scattering intensity.
- Automatic intensity correction compensates for the wavelength dependence of the silicon CCD detector response and results in reproducible peak intensities whatever the excitation wavelength.
- X-axis non-reproducibility is the result of inadequate spectrograph and laser calibration. Automated, software-driven multipoint laser and spectrograph calibrations are essential for reliable instrument-to-instrument comparisons. This is particularly important for successful sample identification using library searching. Periodic automatic x-axis calibration can be employed to ensure that the instrument corrects for any such variation. Thermo Scientific DXR3 Raman microscopes and spectrometers have this feature built-in.
- Heterogeneous samples, such as tablets or powders will give non-reproducible results if the excitation laser spot size is smaller than the heterogeneous components. Sampling devices for these applications should be designed to raster over the surface of the sample so that the excitation beam and the Raman scatter are collected over a representative portion of the sample surface. The Thermo Scientific DXR3 SmartRaman Spectrometer employs VDPS (Variable Dynamic Point Sampling) technology to automate this.

Conclusion

Raman spectroscopy is a technique which has evolved from what used to be regarded as an interesting research phenomenon into a valuable analytical tool. As a complementary technique to FTIR, Raman offers benefits that include minimal sample preparation, the ability to sample through containers, non-destructive analysis, easy analysis of inorganics, and minimal interference from water. Modern FT-Raman and dispersive Raman spectrometers are easy to use, freeing analytical chemists to focus on results and not on having to become an expert in the technique. Improvements in instrument design, particularly in instrument calibration and reproducibility, now make it easy to use Raman spectroscopy to identify unknown samples using spectral library searching. Raman is also used quantitatively, in QC environments, in forensics, in the analysis of cultural artifacts, gems and gemology, materials research, pharmaceuticals and an ever-expanding range of applications and industries.

Learn more about Thermo Scientific DXR3 Raman microscopes and spectrometers at **thermofisher.com/RamanDXR3**