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Application note from the Spectroscopy Products Division

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Combined Raman and FT-IR spectroscopy

Using the Illuminat*IR*™ module for Renishaw's inVia Raman microscopes

The combined inVia Raman and Illuminat*IR*™ FT-IR spectrometer

The Illuminat*IR*™ is a modular FT-IR spectrometer from Sens*IR* Technologies, which may be easily mounted beneath the eyepieces of the research grade, infinity corrected, microscope of Renishaw's inVia instrument (Fig. 1). This enables users to retain full use of the range of optical techniques available with the Leica™ DMLM microscope.

The Illuminat*IR*™ is commonly used with either a diamond contact ATR (attenuated total reflection) or a diffuse reflectance objective. The sample can be viewed, and Raman spectra collected, either through these objectives or through the high quality microscope objectives supplied with the inVia Raman microscope. Simple switching between video view, IR acquisition, and Raman acquisition allows IR and Raman spectra to be acquired sequentially from the same spot on the sample; this switching is fully software controlled on the inVia Reflex instrument.

This document will briefly explain the benefits of combined Raman and FT-IR analysis, and introduce some of the major application areas.

Infrared and Raman spectroscopy

Infrared (IR) absorption and Raman scattering are both commonly used to study and identify substances using the compound's characteristic internal vibrations. Infrared spectroscopy is an absorption process, measuring the fraction of the light absorbed as the wavelength of the light is varied. The incident light is absorbed when the energy of the light closely matches the energy of a vibrational transition in the sample.

A tiny proportion (approximately 1 in 10^9) of the photons incident on a sample interacts with vibrations in the sample and is scattered at higher or lower energy (Raman scattered). Raman spectroscopy involves the measurement of the difference in energy between the incident light and the Raman scattered photons, which corresponds to the energy of the vibrational transitions.

Several 'selection rules' determine whether or not particular vibrations are infrared or Raman active. Providing the energy of the incident light is equal to that of a vibrational transition in the sample, the incident light is then able to excite vibrations in the sample. For a vibrational transition to be IR active, there must be a change in the dipole moment of the molecule during the vibration. If the vibration affects the polarisability of the molecule then the vibrational mode will be Raman active. This theory can be used to predict the number of Raman and IR active vibrations. For instance, theoretical calculations suggest that the complex $K_4Fe(CN)_6$ will have three Raman active vibrations and one IR active vibration. The experimental spectra, shown in Figure 2, show that this is indeed the case.

Figure 1
The IlluminatIR™ module mounted on an inVia Reflex Raman microscope. Inset: Contact ATR objective

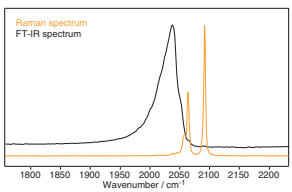

Figure 2
FT-IR and Raman spectra of $K_4Fe(CN)_6$

Vibrations with a large change in dipole moment or polarisability lead to stronger coupling between the photons and the vibrational transitions and hence give stronger IR absorption or Raman scattering, respectively. Therefore, groups such as carbonyls and nitriles give strong IR absorption, while aromatic rings and unsaturation lead to intense Raman bands.

Raman vs. FT-IR

Many of the differences between IR and Raman result from the different wavelengths used. For instance, the size of the illuminated spot directly affects the possible spatial resolution. The introduction of the microscope-based Raman system allowed the laser spot to reach its minimum possible diameter: the diffraction limit. The diffraction limit is proportional both to the wavelength of the light and the efficiency of the optics used. The ability of Raman spectrometers to employ visible laser excitation and high quality microscope objectives results in a diffraction-limited laser spot of < 1 µm. IR systems employ longer wavelengths and hence the theoretical diffraction limit is much larger - around 20 µm. Moreover, IR spectrometers also suffer from less efficient objectives, typically giving an illuminated spot of around 100 µm. Apertures can be used to improve the resolution by spatially filtering the collected light, but with a penalty of lower signal intensity. These differences crucially affect the ability of the instrument to resolve the components of inhomogeneous mixtures.

Fluorescence and phosphorescence are often observed when materials are exposed to visible light. Visible and UV laser excitation sources are of short enough wavelength to induce these emissions, but infrared light is not. Hence FT-IR spectroscopy does not suffer from interference, whereas these emissions can be so strong that the Raman scattered light is completely masked. The introduction of near infrared laser diodes for use in Raman spectroscopy often allows fluorescence problems to be avoided (see Fig. 3). One advantage of the shorter wavelengths commonly used in Raman spectroscopy is that the laser emission may lie close to an electronic transition in the sample. This leads to a resonance enhancement of particular vibrations in the sample, sometimes increasing signal intensity by a factor of 10^6–10^8.

In contrast to FT-IR spectroscopy, for which aqueous solutions present a major difficulty, water is the ideal solvent for Raman analysis. Whilst water strongly absorbs light at mid-infrared wavelengths, it is a weak Raman scatterer, leading to little or no interference from water in Raman spectra. This also has a major impact on the study of biological samples. Raman is routinely used in the analysis of live microbiological samples, an application for which IR spectroscopy is wholly unsuited.

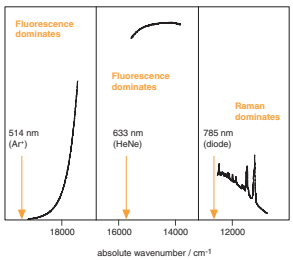

Figure 3
Spectra from a conducting polymer, demonstrating the benefits of near infrared excitation for fluorescent samples.

Sample preparation

The two main techniques employed for FT-IR spectroscopy are transmission and reflectance. Transmission IR requires that a proportion of the incident light can pass through the sample. This is commonly achieved by thinning the sample or using KBr or Nujol® to dilute it. Reflectance methods include contact IR spectroscopy, an extremely powerful technique that uses a diamond placed against the sample to collect the IR spectrum. This allows high quality IR spectra to be acquired from solid samples with little or no sample preparation. However, this technique is not suitable for applications where contact between the collection optic and the sample is undesirable, e.g. for fragile

samples or samples that must be preserved without the possibility of contamination. The diffuse reflectance technique is often used in these instances, however this can lead to misleading artefacts in the acquired spectra. Careful preparation of the sample can eliminate these, but is time consuming and non-trivial.

The nature of the Raman scattering phenomenon eliminates the need for sample preparation. Samples can be analysed on almost any surface, through glass vials and plastic bags, and in aqueous solutions. Raman analysis is therefore a non-contact, non-destructive technique. The majority of samples are simply placed on a microscope slide prior to Raman analysis.

Applications

There is a wide range of current and potential applications of combined FT-IR and Raman spectroscopy. The complementary nature of the techniques, in terms of information content, practicality and sampling, give a huge advantage over each technique applied individually.

Forensics

Raman and FT-IR spectroscopies are extensively used in forensic laboratories around the globe, but combined instruments are a very recent innovation. Both Raman and IR spectroscopy can be used as 'fingerprint' techniques to identify narcotics, cutting agents, narcotic precursors, and explosives. Figure 4 shows Raman and FT-IR spectra of ephedrine (used in the manufacture of methamphetamine) and PETN (a high explosive). The different techniques give similar spectra, with slight differences due to the different selection rules for the absorption and scattering phenomena. Commercial Raman and FT-IR databases enable the user to identify the substance with a high degree of confidence.

The particular strengths of FT-IR analysis for forensic applications include:

- the speed with which high quality, full range, spectra can be collected
- the large number of comprehensive commercial databases available
- the lack of fluorescence from impurities.

Raman, on the other hand:

- allows analysis of particles less than 1 µm in diameter, without sample preparation
- is non-contact, maintaining the integrity of important evidence
- can be used to deconvolve mixed spectra for component analysis, due to high spectral resolution
- can be employed to image the location of illicit substances within a mixture of cutting agents with extremely high spatial resolution.

Pharmaceuticals

Pharmaceutical applications are particularly well-suited to utilise the different information provided by Raman and FT-IR. Most pharmacologically active compounds include both C-O/N bonds and unsaturated carbon-carbon bonds. Many also include regions of aromaticity. Polar bonds such as those of carbonyls, acetyls, amines, and nitriles usually give large dipole moment changes on vibration, and hence result in very strong IR bands. In contrast, the vibrations of multiple C-C bonds and aromatic regions lead to substantial polarisability changes and strong Raman scattering. Therefore, Raman spectroscopy offers the ability to 'fill in the gaps' left by IR spectroscopy, and vice versa.

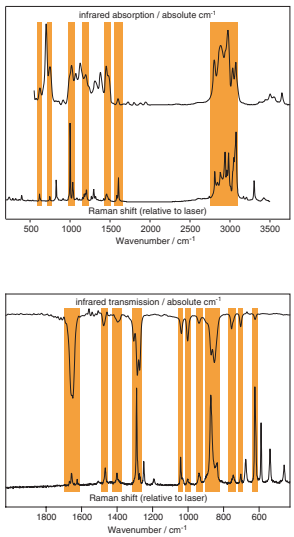

Figure 4
Raman and FT-IR spectra of ephedrine (top) and PETN (bottom)

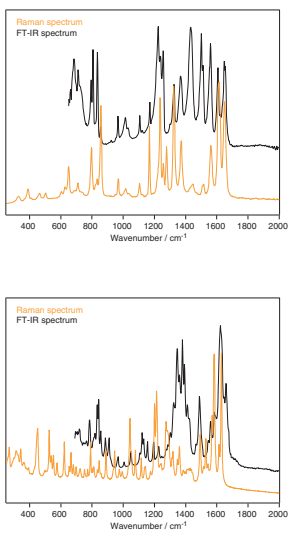

Figure 5
Raman and FT-IR spectra of paracetamol (top) and sodium nedocromil (bottom)

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Figure 5 shows the Raman and IR spectra of two common pharmaceutical products, paracetamol and nedocromil sodium. Various differences between the Raman and IR spectra of each compound are evident . These compounds contain benzene, pyran and quinoline rings, as well as amide, ketone, hydroxyl, carboxyl, and alkyl substituents. The spectral differences demonstrate the varied absorption and scattering properties of these moieties.

These vibrational spectroscopy techniques can be used not only to identify pharmaceutical compounds, but also to probe their physical state. Both Raman and IR spectra contain information on the crystalinity of the sample, and can be used to investigate polymorphism, hydration state (Fig. 6), and crystal symmetry. The ability of FT-IR to acquire full range spectra in very short integration times is extremely useful, as is the sensitivity of the technique to the local environment of the molecule. The higher spectral resolution of Raman spectroscopy, permits the subtle band splitting to be observed as the crystal symmetry changes.

Both techniques can be used to image the location of the various components in solid dosage forms, and the speed of IR can be augmented with the spatial resolution of Raman according to particular analysis needs. The high spectral resolution of Raman is also important for these applications. For instance, CO_3^{2-} ions give very strong, broad IR absorption which can mask much of the spectrum. However, carbonate ions give intense, sharp, Raman bands, allowing the rest of the spectrum to be observed.

Other applications

Below are a selection of the many and varied application areas for combined Raman and FT-IR spectroscopy.

- Art restoration and archaeology:

  Raman and IR spectra of a white paint flake are shown in Figure 7. The IR spectrum is dominated by bands related to the organic fillers and lacquers. The Raman allows easy identification of the inorganic pigments used.

- Catalysts:

  Figure 8 shows the vibrational spectra of a common square planar palladium complex. Both Raman and FT-IR can be used to investigate the metal oxidation state, the ligand structure, the geometry of the complex, and the intermediate compounds formed during catalysis.

- Polymers:

  The Raman and FT-IR spectra of polyethylene terephthalate (PET) are shown in Figure 9. The 1613 cm^{-1} Raman band is related to a vibration with strong aromatic character, and hence is absent from the IR spectrum. Vibrational spectroscopy can be interpreted to reveal physical properties of polymers. Raman and IR spectroscopy can be used to investigate density, layer thickness, crystallinity, surface interactions and solvent effects.

Summary

The IlluminatIR™/inVia combination offers users confocal Raman microscopy and infrared microscopy in a single instrument. The IlluminatIR™ module can be fitted to Renishaw's Raman microscopes at the time of purchase, or retrofitted in the field. Eliminating the need to have two separate instruments saves users both space and money, and saves them time by allowing both techniques to be performed on just one instrument.

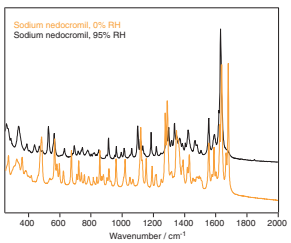

Figure 6
Raman spectra of sodium nedocromil at different relative humidity

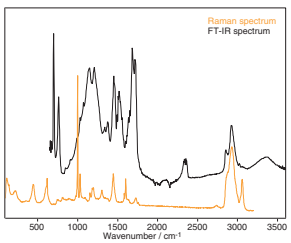

Figure 7
Raman and IR spectra of a white paint flake

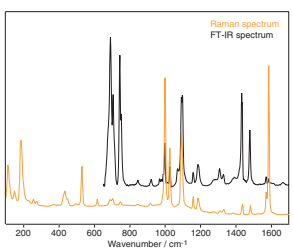

Figure 8
Raman and IR spectra of a square planar palladium complex

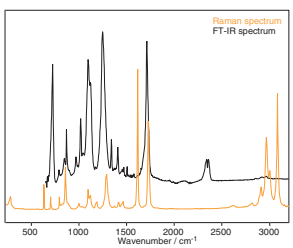

Figure 9
Raman and IR spectra of polyethylene terephthalate (PET)