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# Powerful Partners Provide Complete Analytical Solutions

**This article examines advances that have been made to combine existing analytical techniques with Raman instrumentation. Some of these methods offer enhanced sample visualization, while others provide complementary vibrational spectroscopic information from a single sample point.**

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**R**aman spectroscopy has matured during the past decade to become a routine analytical technique that can be applied to many application areas. The breakthrough in Raman spectroscopy occurred at the beginning of the 1990s, when new technologies were combined to enable the production of small, highly sensitive, robust, and easy-to-use instruments. The resulting commercial Raman systems typically combine an optical microscope for sampling, a low-power, air-cooled laser for excitation of the Raman spectrum, a notch or edge filter to block the Rayleigh scattered light, and a single spectrograph to disperse the Raman scattered light onto a Peltier-cooled charge coupled device detector (1).

The benefits of using a research-grade microscope, without any loss in performance or flexibility, are extremely important when considering the optical microscopy aspects of the Raman exper-

iment, where good sample visualization often is key to success. Many options exist to enhance the process of finding the point of analysis — for example, viewing in cross-polarized light or using Kohler illumination. Indeed, the sample visualization often is one of the major challenges when performing Raman experiments, particularly when striving to analyze micrometer or submicrometer sample areas with little or no optical contrast against their background.

This article highlights the advances that have been made to combine existing analytical techniques with Raman instrumentation. A variety of hyphenated technologies have been demonstrated over the years including: Raman-high performance liquid chromatography (HPLC), Raman-nuclear magnetic resonance (NMR), Raman-electron spin resonance (ESR), Raman-x-ray fluorescence (XRF), Raman-Fourier-transform infrared (FT-IR),



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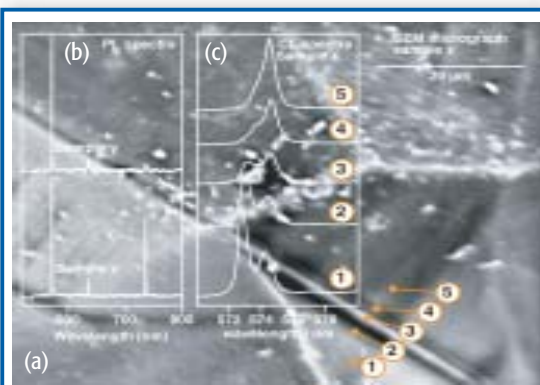
Raman–scanning electron microscopy (SEM), and Raman–atomic force microscopy (AFM). Many of these, despite being demonstrated as proof of principle, have failed to make an effective impact in solving real-world problems. This article will focus on three hyphenated methods that have been shown to have a significant impact for the researcher, namely, Raman with SEM, FT-IR, and AFM and near-field scanning optical microscopy (NSOM). Both the SEM and AFM/NSOM combinations offer the user enhanced sample visualization, while the FT-IR combination

provides complementary vibrational spectroscopic information from a single sample point.

### Raman Spectroscopy Combined with SEM

The combination of Raman and SEM technologies has been attempted by various groups. The benefits of observing the sample using the SEM and providing a single analysis point for both the Raman and energy dispersive x-ray spectroscopy (EDS) are clear for anyone experienced in chemical analysis. The frequency with which samples require both techniques to provide a meaningful and complete analysis is high for many industrial and academic problems.

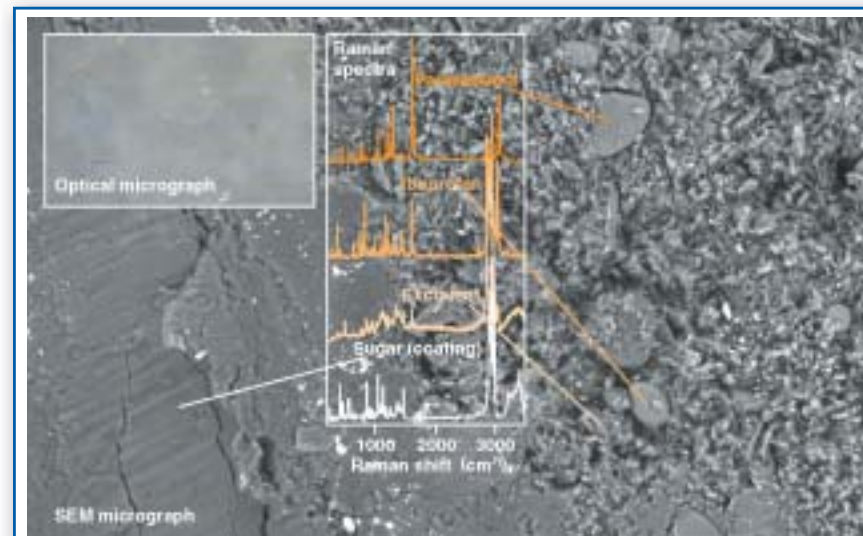
The first real attempt to combine these two methods was made by an academic group of researchers led by Greve at the University of Twente (The Netherlands) (2). This group devised a



**Figure 1.** Analysis of a diamond film prepared by chemical vapor deposition (CVD): (a) SEM micrograph showing linear feature; (b) photoluminescence (PL) spectra collected from two bulk samples (x and y) at  $-172\text{ }^{\circ}\text{C}$ ; and (c) cathodoluminescence (CL) spectra collected at  $-172\text{ }^{\circ}\text{C}$  across the linear feature in sample x. (Sample provided by J. Butler, Chemistry Division, Naval Research Laboratory, Washington DC.)

method of transferring the sample from the electron beam to a Raman analysis point within the SEM chamber. This method, while effective, suffers from the inability to view the secondary electron image from the sample and record Raman data simultaneously. Precise sample positioning under the two analysis points also can present problems. However, application data focusing on biological applications have been published, demonstrating the potential of the combined analysis approach.

More recently, a truly combined Raman SEM system has become available commercially. This system enables the user to view the secondary electron image, with its enhanced depth of field, contrast and resolution performance compared to optical microscopy, while simultaneously recording the Raman spectrum from a  $1\text{ }\mu\text{m}$  to  $2\text{ }\mu\text{m}$  analysis area (similar to EDS). The



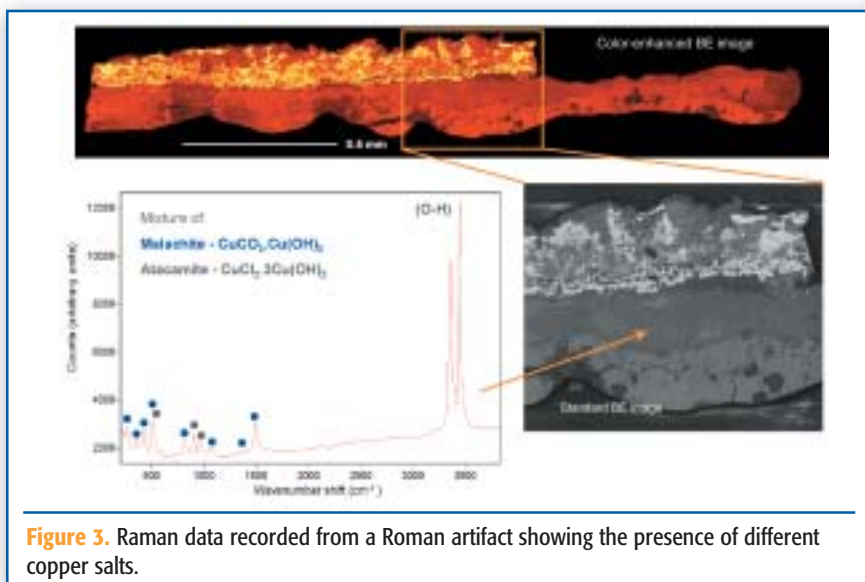
**Figure 2.** Raman spectra obtained from a pharmaceutical formulation showing the presence of the different constituents together with the secondary electron image of the sample.

advantage of imaging the sample area under investigation and recording the chemically specific Raman data together with the elementally specific EDS information without repositioning the sample is clear. The additional chemical and structural information, which is readily accessible from organic material by Raman spectroscopy, overcomes the major limitation of EDS detectors. Inorganic materials benefit from Raman spectroscopy also, where information such as the phase and composition of metal oxides offers significant advantages over EDS.

During the short time this combined methodology has been available, many application areas have been identified as significant beneficiaries of the technology. These areas have gained not only from the combination of Raman spectroscopy with SEM, but also the ability to measure both photolumines-

cence and cathodoluminescence spectra from samples contained within the SEM chamber.

Figure 1 illustrates the capability of the Raman–SEM combination to provide a range of spectroscopic data from a diamond film prepared by chemical vapor deposition. The figure depicts the SEM micrograph of the sample, which under white-light illumination of an optical microscope shows no distinguishing features. A linear feature on the film is well resolved in the secondary electron image. Photoluminescence spectra taken from two bulk samples x and y measured at  $-172\text{ }^{\circ}\text{C}$  show distinct differences in linewidth (broader from sample y, indicating a less perfect crystal structure than for sample x). The silicon vacancy peak seen at  $740\text{ nm}$  is considerably weaker in sample y, indicating a lower silicon take-up during the growth process. The cathodoluminescence



**Figure 3.** Raman data recorded from a Roman artifact showing the presence of different copper salts.

spectra shown in Figure 1c represent data taken from different points across the linear feature seen in sample x. These differences are attributed to different amounts of strain across the boundary feature.

Within the pharmaceutical industry Raman spectroscopy has become a new and emerging method for investigating problems such as formulation inhomogeneities, polymorphism, and reaction screening.

Figure 2 depicts data recorded from a pharmaceutical product containing both paracetamol and ibuprofen. As with the previous example, no contrast is seen when viewing the sample under the conventional optical microscope. The enhanced depth of field provided by the secondary electron image gives sufficient contrast to identify and perform a Raman analysis on discrete areas on the sample. The data shown provide excellent spectral discrimination among the components present

within the formulation. The additional possibility of performing such analysis with low-vacuum and environmental SEMs offers a very significant potential for obtaining spectroscopic and image information from dynamic systems.

Figure 3 represents data acquired from a metallic oxide film which has spalled from a Roman statue of the god Mars. This example demonstrates the power of Raman spectroscopy over EDS in providing detailed compositional information from inorganic oxides and salts, rather than simple elemental data. From the Raman spectra it is relatively easy to identify particles of both malachite and atacamite within the sample.

Other application areas for which Raman–SEM has shown early potential benefits include polymer science, material science, semiconductor technology, contamination analysis, nanotechnology, forensic science, corrosion science, and life science. Unfortunately it is

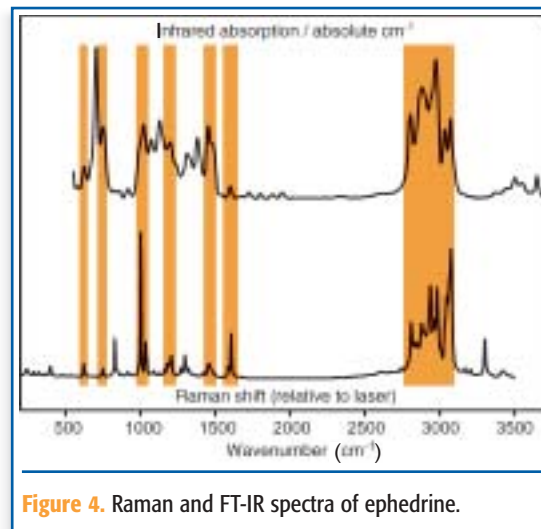
beyond the scope of this article to illustrate each of these applications.

### Raman Microscopy Combined with FT-IR

There is a wide range of current and potential applications of combined FT-IR and Raman spectroscopy. The complementarity of the techniques, in terms of information content, practicality and sampling, gives a huge advantage over each technique applied individually. The addition of an FT-IR (for example, the IlluminatIR from SensIR Technologies Inc., Danbury, CT) is for many Raman microscope vendors a simple add-on that can be installed on-site as an upgrade to an existing Raman system.

The Raman–FT-IR combination offers users confocal Raman microscopy and infrared microscopy in a single instrument. Eliminating the need to have two separate instruments saves users space, money, and time by allowing both techniques to be performed on one system.

The FT-IR commonly is used with either a diamond-contact attenuated total reflection (ATR) or a diffuse reflectance objective with an effective analysis area of  $\sim 20 \mu\text{m}$ . The sample can be viewed, and Raman spectra collected, either through these objectives or through the high quality microscope objectives usually supplied with the Raman microscope. Simple switching between video view, IR acquisition, and Raman acquisition allows IR and Raman spectra to be acquired sequen-



**Figure 4.** Raman and FT-IR spectra of ephedrine.

tially from the same spot on the sample; this switching can be fully software controlled in some instruments.

Raman and FT-IR spectroscopies are used extensively in forensic laboratories, but the combined instruments are a relatively 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). The different techniques give similar spectra, with slight differences because of 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 avail-

able; and the lack of fluorescence from impurities, which is a common problem in Raman spectroscopy.

Raman spectroscopy, on the other hand, enables the analysis of particles <1  $\mu\text{m}$  in diameter, without sample preparation. It is a non-contact technique, maintaining the integrity of important evidence, and its high spectral resolution enables the deconvolution of mixed spectra for component analysis. Its high spatial resolution can be employed to image the location of illicit substances within a mixture of cutting agents.

Pharmaceutical applications, as stated previously, are particularly well-suited to utilize the different information provided by Raman and FT-IR. Most pharmacologically active compounds include both C–O/N bonds and unsaturated C–C 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 polarizability changes and strong Raman scattering. Therefore, Raman spectroscopy offers the ability to fill in the gaps left by IR spectroscopy, and vice versa.

Figure 5 shows the Raman and IR spectra of a common pharmaceutical product, nedocromil sodium. Various differences between the Raman and IR spectra are evident. This type of

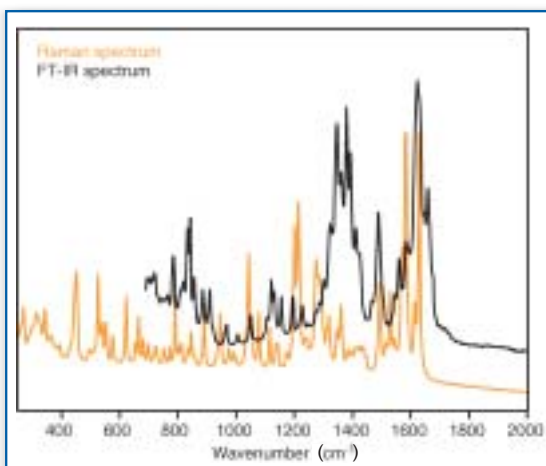


Figure 5. Raman and FT-IR spectra of nedocromil sodium.

compound typically contains 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 crystallinity of the sample, and can be used to investigate polymorphism, hydration state, 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 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 sharper natural linewidths of Raman bands is also important for these applications. For instance,  $\text{CO}_3^{2-}$  ions give very strong, broad IR absorption, which can mask much of the spectrum. However, carbonate ions give intense, sharp, Raman bands, allowing observation of the rest of the spectrum.

There is a wide selection of application areas for combined Raman and FT-IR spectroscopy, including art restoration and archaeology, catalysts, and polymers. As before, it is beyond the scope of this article to illustrate all of these in any depth.

### Raman Microscopy Combined with AFM/NSOM

The combination of near-field scanning optical microscope with a Raman microscope system first was reported as early in 1995 when Batchelder and co-workers (3) designed and integrated a system in their laboratories. Since then other research groups have worked to combine the individual components, while some instrument companies have worked together to offer a total solution under common software operation.

The reasons for combining either AFM or NSOM with a Raman micro-

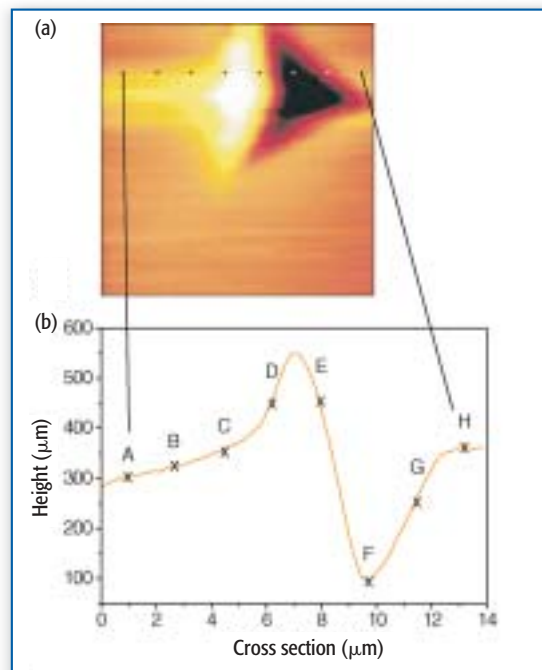


Figure 6. AFM height map (a) and cross section (b) across a nanoindentation in silicon.

Experiments performed in collaboration with Professor Y. Gogotsi (Department of Materials Engineering, Drexel University), and R. Noons, R. Forrest, S. Ward and R. Devonshire (Department of Chemistry, University of Sheffield).

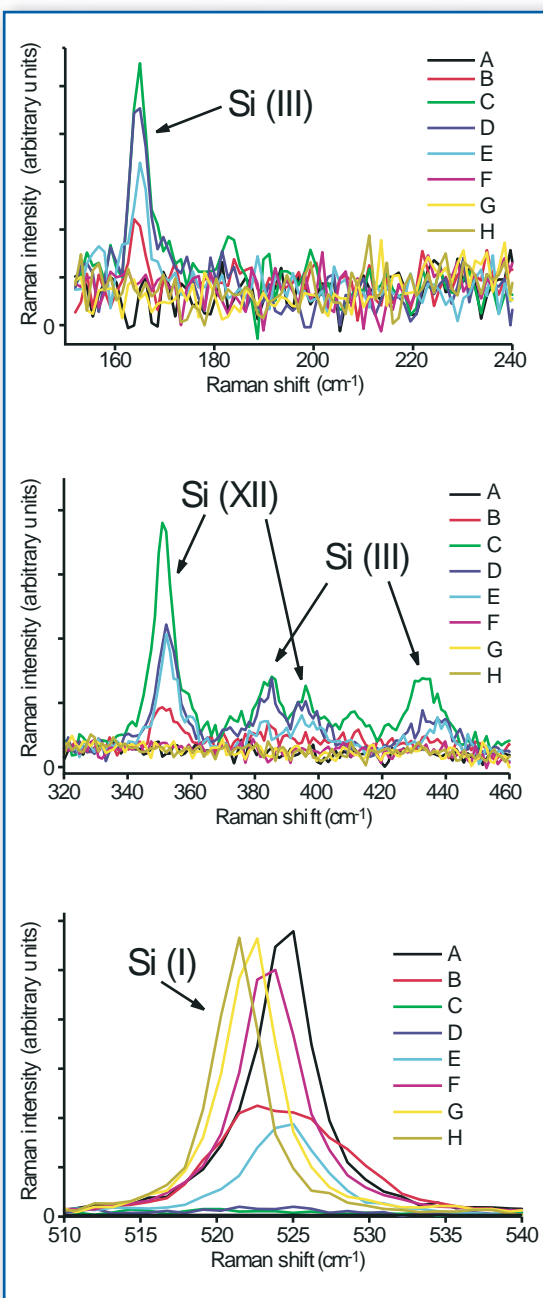
scope are subtly different. The AFM method provides the user with a different visualization technique and can be used to identify the location of sample features not readily discernible using conventional optical microscopy. The NSOM method provides a way to break through the diffraction-limit barrier encountered when focusing lasers with normal refractive optics. Typically a Raman microscopy attachment will provide  $\sim 1 \mu\text{m}$  laser spot size at the sample, whereas the drawn tapered fibers usually used in NSOM reduce the spot size

to ~ 100 nm. The main difficulty with this approach is the very low number of photons that are available for Raman analysis.

The benefit of combining the worlds of AFM and Raman spectroscopy is illustrated in Figures 6 and 7. A 14  $\mu\text{m}$  x 14  $\mu\text{m}$  AFM image of a nanoindentation in silicon is shown in Figure 6, along with a cross section through the indentation. The lettered points on the AFM cross section represent the points at which Raman spectra were collected.

The AFM image shows that the indentation has caused plastic deformation of the silicon, but does not give any indication whether the deformed regions have undergone any phase changes. However, the Raman spectra (Figure 7) clearly show the presence of different phases of silicon, along with shifting and broadening of the main silicon (I) 520- $\text{cm}^{-1}$  Raman band caused by residual stresses (4).

Polymer science is another field where the combination of scanning probe and far-field Raman offers great promise. For example, NSOM measurements with linearly polarized light can reveal micro domains and thus the degree of crosslinking in polyethylene



**Figure 7.** Far-field Raman spectra taken from the eight positions indicated in Figure 6.

and polybutadiene films. Mechanical properties also can be measured. The AFM is able to monitor the elasticity of the polymer film by using its intermittent contact mode. This information can now be directly correlated on-line with the Raman data.

Recently, work by Demangeot and co-workers (5) also has demonstrated the benefits of using AFM and Raman methods for studying single indium nitride dots grown by metal-organic vapor phase epitaxy on gallium nitride. In these studies the methods have been employed to investigate the morphology and internal strain within the dots. The shape of the dots was revealed using the AFM while the position of the Raman bands, in particular the E2 photon frequency at ~490  $\text{cm}^{-1}$ , allowed an estimate of the residual strain field to be made.

The previous shortcoming of available signal intensity in the combined NSOM-Raman experiment has been addressed in part by the use of apertureless tips which, when coated, generate field enhancements at the sample surface (6). These methods are at present still very much in their research phase and have yet to become routine and “user friendly.” However, it is anticipated that, given the successful outcome of this research, routinely obtaining high-quality data from 100-nm sample areas could become a reality.

### Conclusion

The combination of techniques is seen as an immense step forward in providing a more complete analysis of samples without the need to move them from instrument to instrument, thereby maintaining sample integrity.

The combination of Raman with SEM is a method in its infancy, but with potential to become the preferred detector on SEMs. The chemical and structural information contained within the Raman spectra offers significantly more to the analyst than that provided from EDS detection. Raman with AFM provides a unique method for acquiring spectroscopic information from highly specific locations on samples that are invisible to optical microscopy. This clearly has significant benefits in many nanotechnology applications. Raman-FT-IR microscopy as a combined method is a vibrational spectroscopist’s dream come true — a complete vibration analysis of a sample on one instrument.

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