Case Studies in the Application of Raman Microscopy

The authors discuss how the Raman microscope is being used successfully to characterize pharmaceuticals, analyze disease states, and to characterize semiconductors and nanotechnologies.

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The introduction of the Raman microprobe in the early- to mid-1970s (1) truly was an innovation in simplifying Raman sampling, which provided the additional advantages of confocality and fluorescence rejection. While its application to materials problems was instrumental in widening the use of Raman, the technique really didn’t become widely used until the reduction of the size of the instrumentation during the 1990s (2). This was due to the introduction of the holographic notch filter to separate the Raman signal from the unshifted laser radiation, air-cooled lasers, low noise, CCD detectors, and powerful desktop computers with mature software.

From the mid-1990s until the present the application of Raman instrumentation to ever-expanding materials studies has created a ground-swell of interest. During this period, the availability of Fourier-transform (FT) Raman instrumentation in the analytical community gave the technology credibility that continued to fuel this growth. However, the application of FT-Raman spectroscopy to problem solving apparently has peaked, and now is in decline. But in its wake the reluctance of the analytical community to employ Raman spectroscopy to solve its problems has been removed (3). Because of this enthusiasm, the equipment manufacturers have provided more and more specialized developments for targeted real-world applications. The remainder of this article will discuss some of these applications.

Characterization of Pharmaceuticals

With the large amount of time required between identifying a pharmaceutical need and introducing a drug to market, and the associated costs for all steps of this process, any technologies that can assist at any step can be of interest. Raman is being explored early in the process as a means of understanding the disease process at the molecular level (see the next section). But it also is being exploited in characterizing materials at every step in drug development. Both small molecules and larger active agents

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motorized stage (x, y, and z control) and has software modified for automated crystal location and spectral acquisition. The Crystal iD (Jobin Yvon, Villeneuve d’Ascq, France) software includes pattern recognition of the optical images, incorporating algorithms developed, in collaboration with a major drug manufacturer, by Imaging Associates in the UK.

Molecular Characterization of Metabolic and Disease States

Molecular definition of foreign materials and/or metabolic accretions and creation of maps correlated to white light microscopy have been performed successfully for a number of years (4). Because molecular changes precede visible histological changes in diseased tissue, numerous workers have been utilizing Raman microscopy to determine if disease state markers can be identified (5, 6). In our own laboratory we have collaborated with Rousseau and Yeh at the Albert Einstein School of Medicine on an apoE knock-out mouse model of human atherosclerosis (7). In addition to similar observations to what other workers are reporting for this disease process, we observed free fatty acid inclusions in the knock out mouse, and calcification in the form of calcite (CaCO$_3$) in a very old normal (wild type) mouse. One might assume that the first observation is relevant to the disease process itself. The second may be related to reduced availability or activity of carbonic anhydrase in the aged animal.

In Figure 2, the strong band at 1650 cm$^{-1}$ is assigned to the >C=C< unsaturation bond of the lipids. When esterified the >C=O group has a well-defined band at about 1750 cm$^{-1}$. The bottom spectrum is a typical protein spectrum and shows a fairly broad amide I band near 1650 cm$^{-1}$ (not be to confused with the sharper carbon double bond) and a sharp aromatic band at 1500 cm$^{-1}$. The middle spectrum is the anomaly. The 1650 cm$^{-1}$ band is too sharp for a protein suggesting that it could arise from the unsaturated lipid. But the carbonyl band at 1750 cm$^{-1}$ is lacking, suggesting that the carboxylate group is unesterified.

Another area of development is the definition enzyme/protein allotrope for selection of optimized drug for disease...
enables electrical isolation of the surface devices from the substrate, which reduces the capacitance of the devices and the possibilities for shorts. Si on SiGe produces stress in silicon, which increases the electronic mobility in a controlled manner. And there even is development of a combination of the two technologies. Raman has been shown to be a useful tool for measuring stress in situ, non-destructively with microscopic spatial resolution.

There is a concern, however, how to separate the shift of the Raman line due to stress and to Ge content. This has been worked out by Tsang and involves making simultaneous measurements of the Si–Si line (500–520 cm\(^{-1}\)) and the SiGe line (ca. 400 cm\(^{-1}\)) and solving an equation that appears in his publication (9). In addition, when there is a silicon layer overcoating a SiGe layer, it is possible to separate the contributions of the various layers by varying the excitation wavelength, which controls the depth of penetration of the laser beam.

Figure 4 shows the spectra of a sample of SiGe deposited on Si (the opposite of what is described above) when excitation wavelengths of 351 nm (left), 532 nm (middle) and 633 nm (right). The rectangle on the far left indicates schematically the depth to which the laser excitation can penetrate (not to scale).

Characterization of Nanotubes and Measurement of Stress in Integrated Circuits

Current developments in the design of integrated circuits are targeted on increasing the integration scale and speed of devices. Silicon-on-insulator enables electrical isolation of the surface devices from the substrate, which reduces the capacitance of the devices and the possibilities for shorts. Si on SiGe produces stress in silicon, which increases the electronic mobility in a controlled manner. And there even is development of a combination of the two technologies. Raman has been shown to be a useful tool for measuring stress in situ, non-destructively with microscopic spatial resolution. There is a concern, however, how to separate the shift of the Raman line due to stress and to Ge content. This has been worked out by Tsang and involves making simultaneous measurements of the Si–Si line (500–520 cm\(^{-1}\)) and the SiGe line (ca. 400 cm\(^{-1}\)) and solving an equation that appears in his publication (9). In addition, when there is a silicon layer overcoating a SiGe layer, it is possible to separate the contributions of the various layers by varying the excitation wavelength, which controls the depth of penetration of the laser beam.

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Raman maps of (left) the intensities of the silicon line from the substrate (light) vs. the SiGe cap layer (dark) and (right) the frequency of the Si–Si line in the SiGe layer.

**Figure 5.** Raman maps of (left) the intensities of the silicon line from the substrate (light) vs. the SiGe cap layer (dark) and (right) the frequency of the Si–Si line in the SiGe layer.

One line in the region of the radial breathing mode implies that sensitivity is adequate to detect one tube.

**Summary**

It is clear that Raman microscopes have an important role to play in the evolution of new state-of-the-art technologies. The examples described above were selected for the high profile that these developments currently have.

**References**