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Combine & Conquer

Confocal Raman microscopy teams high-resolution capabilities with powerful materials analysis.

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Materials research, biomedical research, semiconductor manufacturing, and medical imaging can all benefit from nondestructive, high-resolution analysis methods. By combining chemical analysis techniques like Raman spectroscopy with high-resolution imaging methods like confocal microscopy, scientists can attain powerful, flexible instruments for a range of applications.

The Raman effect refers to the interaction of electromagnetic waves (light) with matter in which a vibrational quantum is excited (Stokes Raman scattering) or annihilated (anti-Stokes Raman scattering). The incident light causes the molecules to vibrate, and the result is an energy shift between the excitation and the Raman-scattered photon. The energy shift is a function of the mass of the involved atoms and the binding strength and coordination, so every chemical species shows its own, distinct fingerprint. Raman spectroscopy offers a nondestructive and noninvasive technique for obtaining detailed chemical information on the species involved in the scattering process, without sample preparation or labeling.

Chandrasekhara Raman was awarded the Nobel Prize for his discovery in 1930.^{1,2} As the Raman effect is very weak (typically less than one in a million incident photons undergo Raman scattering), the technique requires a medium-power laser for excitation and a sensitive detector to obtain a good signal-to-noise ratio in a reasonably short time. A system with an excitation power of several hundred milliwatts typically requires integration times on the order of 1 to 10 min, even when incorporating a multichannel CCD detector.

In the analysis of inhomogeneous samples, the distribution of specific materials can be very important. With an excitation spot size on the order of 100 μm , however, most spectroscopy setups suffer from insufficient spatial resolution. Optical microscopy, on the other hand, is capable of providing a spatial resolution down to

200 nm using visible-light excitation. Combining microscopy and Raman spectroscopy yields a powerful tool for materials analysis.

routes to Raman

The first Raman microscope, built in 1975, combined an optical microscope with a Raman spectrometer to obtain Raman images.³ In a Raman microscope, 3-D information has to be measured with a 1-D (photomultiplier/avalanche photodiode (APD)) or 2-D (CCD camera) detector. This can be achieved by either direct imaging or image reconstruction. In the direct-imaging technique, the sample is homogeneously illuminated and the Raman signal of only one spectral component is detected through a narrowband filter (or monochromator).

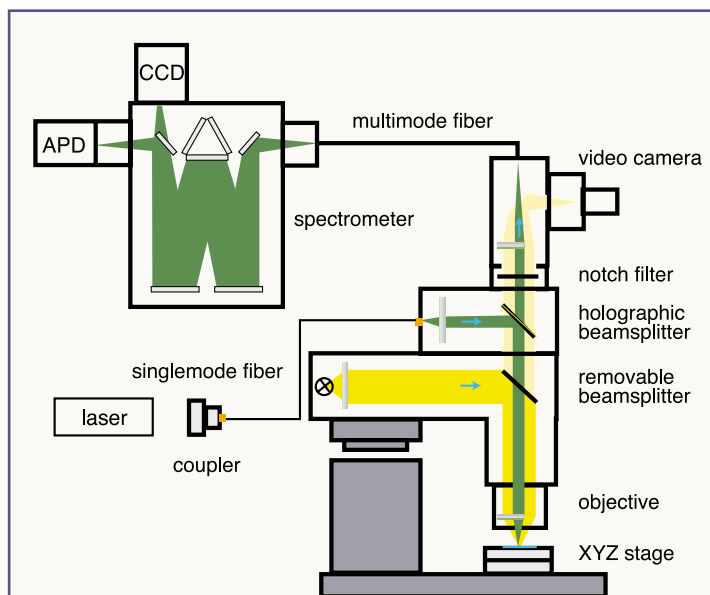


Figure 1 A confocal Raman microscope consists of a dual-output-port spectrometer integrated with a confocal microscope. A singlemode optical fiber provides point-source illumination and the core diameter of a multimode fiber provides the confocal pinhole in the focal plane.

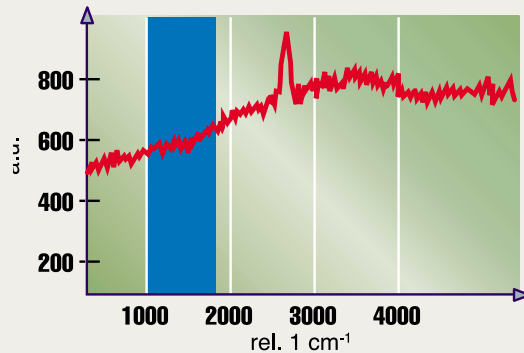
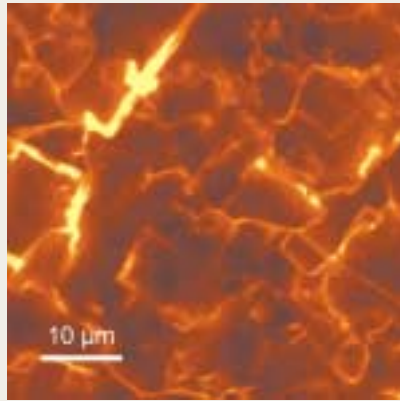


Figure 2 The fluorescence background in this image is quantified by integrating the signal over the marked area from 1000 to 1080 cm^{-1} ; this area does not contain the Raman signal. Note the concentration of fluorescence at the grain boundaries.

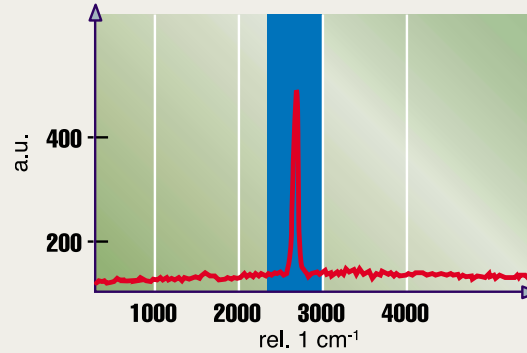
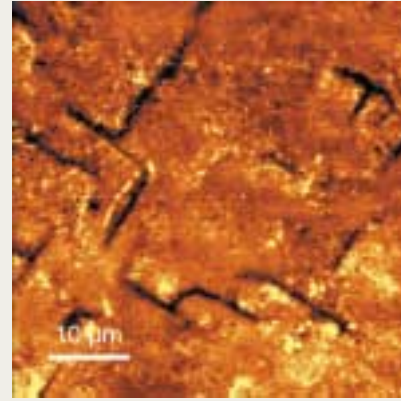


Figure 3 Raman signal of the diamond line obtained by integrating over the marked area. The fluorescence is not visible because the background was subtracted in each spectrum. Note that not all grain boundaries (dark lines in this image) show a strong fluorescence.

In the image-reconstruction technique, either a point or a line of the sample is illuminated and the full Raman spectrum captured. Images in all Raman bands can then be reconstructed from the measurements of separate points or lines.

A relatively new technique in optical microscopy is the confocal arrangement.⁴ In confocal microscopy, light from the sample is detected through a pinhole placed in the back focal plane of the microscope. This setup captures only light from the focal plane, while out-of-focus Raman light and fluorescence background is strongly rejected. The application of the confocal technique allows us to segment a specimen along the optical axis and even generate a depth profile or a 3-D image.

A disadvantage of this technique is that the image cannot be acquired as a whole but must be assembled point-by-point and line-by-line. This disadvantage is by far outweighed by the benefits of increased image contrast and signal-to-noise ratio.

combining Raman and confocal

No confocal direct-imaging Raman microscope can be produced, because in direct imaging, light is collected equally from each plane of the sample and no depth resolution is possible. By combining a research-grade confocal

optical microscope with extremely high optical throughput and a high sensitivity Raman spectrometer, however, we can not only obtain Raman spectra from extremely small sample volumes (down to $0.02 \mu\text{m}^3$), but can also collect high-resolution Raman images.

One approach, called Raman fast imaging, is to perform imaging in the light of a single Raman line. In Raman fast imaging, the spectrometer is used as a spectrograph and the light of a selected Raman line is directed to a high-sensitivity silicon photon-counting detector such as an APD. While scanning the sample, the image is obtained in the light of a single Raman line.

Another approach, called Raman spectral imaging, involves tuning the spectrometer to an area of interest and acquiring a complete spectrum at every image point with a CCD camera. The number of image points (spectra) is typically 65,536 for an image of 256 lines and 256 pixels/line, but can range higher, limited only by the memory of the computer. A multispectrum file of 65,536 spectra would be about 170 MB, which was considered to be overwhelming only a few years ago, but can be easily handled by today's computers.

engineering around challenges

Confocal Raman microscopy is rewarding but also challenging. To collect a Raman image in a reasonably short time,

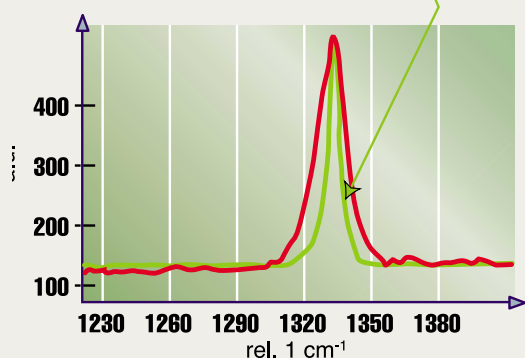
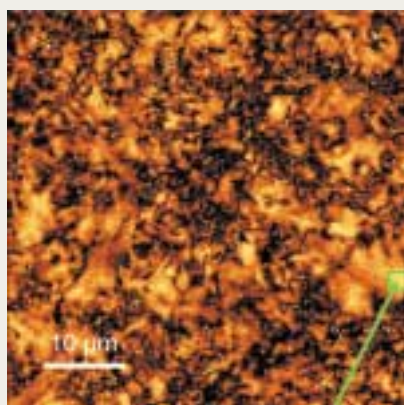


Figure 4 In this image, areas where the FWHM of the diamond line is below 5 cm^{-1} appear white, while the dark areas correspond to linewidths larger than 15 cm^{-1} . The FWHM in all spectra varies from about 5 cm^{-1} to more than 60 cm^{-1} . The green spectrum has a FWHM of 5.9 cm^{-1} and was obtained by integrating over all spectra in the green box, whereas the red spectrum is the average over all 65,536 spectra and has a FWHM of 15.7 cm^{-1} .

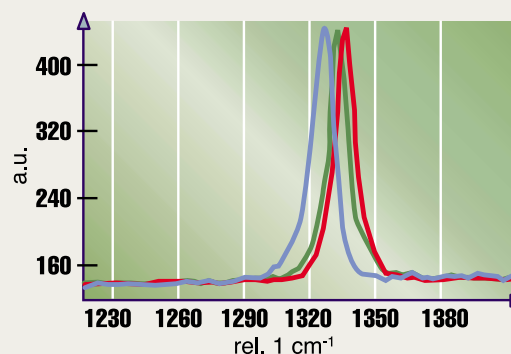
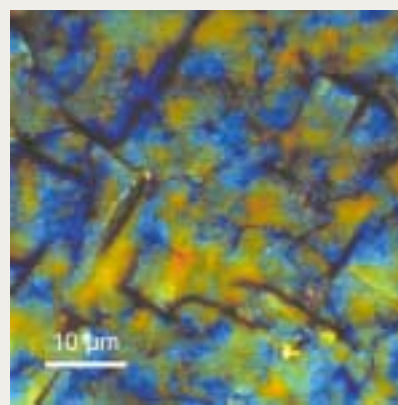


Figure 5 Stress in the diamond film, color coded from red (compressive strain, peak position above 1333 cm^{-1}) to blue (tensile strain, peak position below 1333 cm^{-1}).

special care must be taken to optimize the optical throughput and sensitivity of the setup. As a Raman image consists of several thousand pixels (spectra), the integration time per pixel (spectrum) must be as short as possible. Integration times above 1 s per spectrum are usually not acceptable.

A low-resolution Raman spectral image of 100 lines and 100 pixels per line contains 10,000 spectra. Even assuming a 1-s acquisition time per spectrum, the total image acquisition time is nearly 3 hrs. Although this might be acceptable in rare cases, it is hardly acceptable for routine work.

In most cases it is not possible to increase the exciting laser power above 10 to 30 mW. A laser power of only 1 mW, focused to a $0.5\text{-}\mu\text{m}$ spot, results in a power density of $4 \times 10^5\text{ W/cm}^2$. Compare this to the power density of a cooking plate of only 2 W/cm^2 !

Because the power density is strongly confined, however, heat dissipation can be very effective. A delicate polymer sample might easily dissipate 10 mW of power focused to a $0.5\text{-}\mu\text{m}$ spot, while it will be immediately destroyed by 1 W focused to a $100\text{-}\mu\text{m}^2$ area, although the latter represents a

400-fold lower power density than the former.

In many cases a fluorescence background can be very effectively bleached using high-power-density excitation and a confocal setup. The fluorescence will hardly be bleached using global illumination, which is moreover not compatible with a confocal detection scheme.

studying samples

Our group has developed a confocal Raman microscopy system that features an excitation laser coupled to a microscope with a singlemode optical fiber (see figure 1 on p. 17). A single-mode fiber provides a Gaussian beam profile that can be focused to a diffraction-limited spot size. The laser beam is reflected by a holographic beamsplitter and focused onto the sample with a microscope objective, while the sample is scanned with a piezoelectric scan table. The same objective collects the Raman-scattered light, and a holographic notch filter or an extremely steep edge filter suppresses reflected laser and Rayleigh-scattered light.

The scattered light is then focused into a multimode optical fiber in which the fiber core ($100\text{ }\mu\text{m}$, $50\text{ }\mu\text{m}$, or $25\text{ }\mu\text{m}$) acts as a pinhole for confocal microscopy. The multimode fiber is coupled to a spectrometer with two output ports, one equipped with a CCD camera for Raman spectral imaging and the other with a photon-counting APD for Raman fast imaging. The core of the multimode fiber also acts as an entrance slit for the spectrometer.

many paths lead to a microscopy expert

Touted as an enabling technology, photonics provides the tools for technical disciplines from microscopy in life sciences to semiconductor inspection systems. With such a broad range of applications, it's probably no surprise that photonics attracts modern renaissance men, individuals who acquire knowledge in multiple fields, motivated as much by the wonders of science as the beauty of the natural world.



"Whether it was an engine or a bird, I was always interested in how things work," says Olaf Hollricher, managing director of research and development at WITec GmbH (Ulm, Germany).

"My father was a physicist, and while that wasn't the reason I eventually became a physicist, it was great how he could explain how things worked in an interesting way," remembers Hollricher. "All children are curious, but if you want to get a good answer about how things work, ask a physicist!"

A case of father-and-son affinity combined with a fascination for lasers led Hollricher to work with electron microscopy and superconductors at Julich Research Center (Julich, Germany), and near-field optics and microscopy at the University of Ulm (Ulm, Germany) during a post doctorate under Othmar Marti.

Under Marti's tutorship, Hollricher met a couple of like-minded fellows, Joachim Koenen and Klaus Weishaupt, each with his own expertise. After a few years developing near-field microscopes at the University of Ulm, the three men answered a commercial request for the same from a U.S. university, and started WITec Wissenschaftliche Instrumente und Technologie GmbH. Today, with 25 employees and offices in Champaign-Urbana, IL and Ulm, WITec continues to develop new hybrid systems, including its latest creation: a combination near-field optical, confocal, and atomic force microscope. —Winn Hardin

We used the system to investigate a diamond film deposited on silicon. Usually, the width of the Raman line at 1333 cm^{-1} is used to characterize the quality of these polycrystalline diamond films, but if the laser spot has a diameter of $100\text{ }\mu\text{m}$, for example, the width of the measured Raman line is an average over the laser spot diameter.

To investigate the sample in detail, we analyzed a $50\text{-}\mu\text{m} \times 50\text{-}\mu\text{m}$ area using 256×256 pixels (65,536 spectra) with an integration time of 50 ms per spectrum. For excitation and detection we used a 100X objective with a 0.9 numerical aperture (diffraction-limited spot size 360 nm, pixel size 195 nm) and 10 mW of power from a frequency-doubled

neodymium-doped yttrium aluminum garnet laser operating at 532 nm. A 300-mm imaging spectrometer equipped with a 1800-lines/mm grating and a back-illuminated CCD camera captured the spectra in under an hour; from this multispectrum file, we calculated the rest of the images shown in this article. Each image took only a few seconds to be calculated.

Fluorescence due to impurities is present in many parts of the sample, but strongest at the grain boundaries. To generate the image, we integrated the marked area from about 1000 cm^{-1} to 1080 cm^{-1} (see figure 2 on p. 3). Even if the diamond line is included in the integration area, however, the image looks pretty much the same, because fluorescence dominates the spectrum.

We can examine the quality of the diamond film by integrating over the diamond line in each spectrum, corrected for the fluorescence background (see figure 3 on p. 3). As one can see, the Raman intensity (sum of the Raman scattered signal) is relatively homogenous; background subtraction removes the fluorescence signal.

The width of the Raman line shows strong local variations (see figure 4 on p. 4). The bright areas correspond to areas with small linewidths, while the dark areas show broadened Raman lines. The color scale is chosen such that areas appear white where the FWHM of the diamond line is below 5 cm^{-1} , while the dark areas correspond to linewidths larger than 15 cm^{-1} . The FWHM in all spectra varies from about 5 cm^{-1} to more than 60 cm^{-1} . As can be seen, there are areas containing very sharp lines indicating pure diamond. The small spot size of the excitation laser allows us to detect these variations; a measurement with a laser having a $50\text{-}\mu\text{m}$ spot would deliver an inhomogeneous broadened spectrum.

Stress and strain in the material affects the Raman signal by shifting the peaks to lower wavenumbers (tensile strain) or higher wavenumbers (compressive strain; see figure 5 on p. 4). The corresponding spectra have been obtained by integrating over all spectra in the red, green, and blue areas, respectively. The blue spectrum shows a shift of 5.4 cm^{-1} to smaller wavenumbers, while the red spectrum is shifted by 4.2 cm^{-1} to larger wavenumbers.

Combining confocal microscopy with Raman spectroscopy techniques provides a powerful tool for materials analysis. The technique presents certain design challenges, but the capabilities of such a system outweigh the difficulties. **oe**

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