

Why Would the Owner of a Micro Raman System Want to Place a Nanonics NSOM/AFM-100 Confocal™ on his Micro Raman?

Introduction

Today Raman scattering is undergoing a renaissance but Raman scattering remains separate and removed from the proliferation of insights that the scanned probe microscopies can give.

In general investigating a sample with scanned probe microscopies requires removing the sample from the micro Raman spectrometer. This means that the exact region that was being interrogated by Raman would, for all practical purposes, not be found again for imaging with the chosen scanned probe microscopic technique. Direct correlation with an SPM technique with Raman scattering was a dream and far from reality.

Today, with the Nanonics NSOM/AFM 100 Confocal™, a new world has dawned for the micro Raman spectroscopist:

Direct On-line Simultaneous Correlated Raman and SPM Imaging

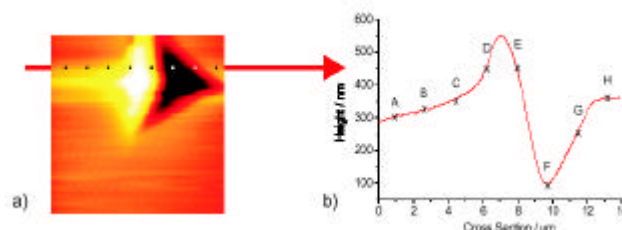
First, this system can be directly integrated into any of the standard micro Raman spectrometers, in particular from Renishaw or any homebuilt system. The commercial instruments usually employ upright optical microscopes and the Nanonics NSOM/AFM 100 Confocal™ is readily placed on the sample stage of any optical microscope (see below).



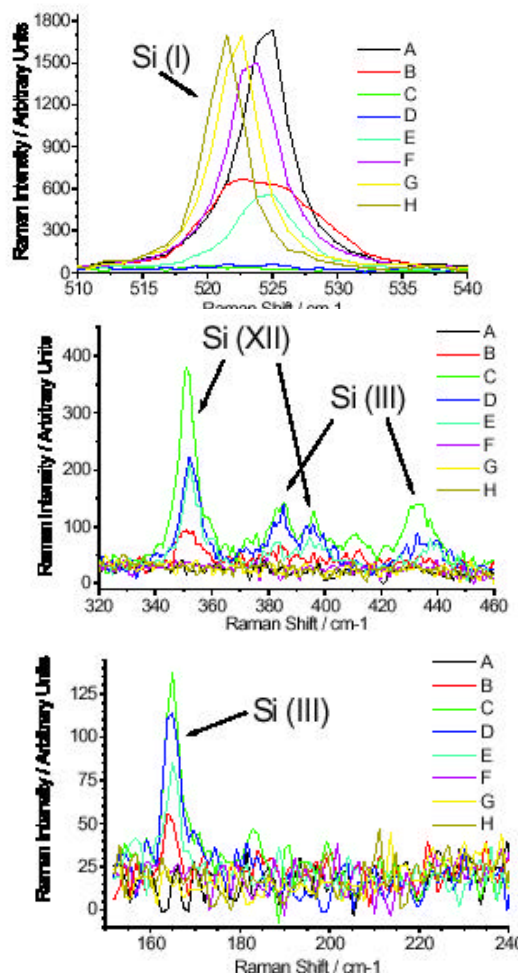
Also the Nanonics patented cantilevered optical fibers are held between the lens and the sample without obstructing any aspect of the far-field conventional microscope. The tip in these fibers is exposed and this allows for illumination by the lens of the microscope at the tip, the exact region where the scanned probe information is being collected. This is impossible in a standard AFM cantilever in which the tip is under an opaque silicon nitride region obscured from the lens of an upright microscope.

With the Nanonics NSOM/AFM 100 Confocal™ and our cantilevered optical fibers, the Raman spectroscopist can now record, in parallel with Raman, the wide variety of scanned probe imaging modalities. For example, while the Si Raman peak of a microcircuit is being monitored to detect stress in the silicon, the Raman spectroscopist can measure simultaneously the electrical properties including dopant concentration and using the metal coated, cantilevered fiber probe to measure the micro topography with AFM and the micro reflectivity with NSOM. In addition Nanonics provides software that can display all these images at once for direct and simultaneous comparison and analysis.

To illustrate this combination of the worlds of AFM and Raman spectroscopy actual data has been obtained on the Si stress problem mentioned above. A 14 micron x 14 micron AFM height image of a nanoindentation in Si is shown in the Figure below (part a) with a line scan (part b) through a region of this AFM image.



The points on the AFM cross section are points at which Raman microscope spectra were collected. As a result of the nanoindentation it can be seen that the silicon has been displaced. The question is whether these regions correspond to different phases of the silicon that can be correlated with the AFM measurements. Only Raman microprobe spectroscopy can give this information. The Raman spectra that were obtained simultaneously with the positions on the AFM line scan are shown in the following three spectra below that are color coded with the letters on the line scan in part b in the above Figure.



Clearly it can be seen that the position of the Raman peaks change with the different positions in the AFM line scan. On the spectra the different phases of As another example, take the case of polymers, in this area the Raman spectroscopist armed with the Nanonics NSOM/AFM 100 Confocal™ can investigate the molecular structure of a polymer film and correlate it with the NSOM and AFM data. It has been shown recently that the NSOM measures the degree of crosslinking in the polymer by monitoring, with linearly polarized light, micro domains in polymer films such as polyethylene or polybutadiene [1]. On-line Raman will allow correlation of this microstructure with such important molecular parameters. Conversely, the AFM can monitor the elasticity of the polymer film by using the high resonance frequencies of the glass cantilevered tips and the intermittent contact mode of the NSOM/AFM 100 Confocal™. This can now be directly, correlated on-line with the Raman image and software that Nanonics can provide to display simultaneously multiple images including the Raman for direct comparison.

The NSOM/AFM 100 Confocal™ can work in such an intermittent contact mode even in liquids and Raman is ideal for working in aqueous media. Thus, the whole world of NSOM/AFM imaging of biological materials in physiological media can now be directly and on-line correlated with Raman.

Work on biological structures also extends to such important Raman developments as the investigation of dentine, calcified tissue from teeth. The microstructure and the reflectivity of dentine in microdomains are also critical parameters to know and to be correlated with the simultaneous Raman imaging.

Finally, among many forensic applications the microstructure of forged handwriting from NSOM reflectivity can now be directly correlated with the chemistry of the ink that was used. Such measurements can also be extended to dyes on paper where the microstructure of the paper with AFM and the micro-reflectivity and micro fluorescence of the paper with NSOM can be directly correlated with the Raman data.

Conventional Raman NSOM

The resolution of Raman today is 1 micron. The hope for near-field optics is that it will permit Raman resolution to be improved. Near-field optics is performed through a fiber which is tapered by glass pulling technology [2] to form a subwavelength aperture and the sample is scanned relative to this aperture in the near-field. From a practical point of view such a geometry can be used to either illuminate or to collect light from a sample. Two problems are encountered. The first is the Raman from the fiber. This can be essentially minimized or eliminated by many experimental manipulations including simply collecting, rather than illuminating with the fiber. The second problem is the extremely low amount of light that is transmitted through an aperture that is less than the wavelength. It does not matter how the aperture is prepared although in the literature there is a great deal of noise on this issue and the word noise is chosen judiciously. The fact is that even with the best aperture having the highest efficiency (and these can be made by the standard pulling method) there have been many valiant attempts [3-13] but no real breakthrough in the incredibly long acquisition times required. Each attempt is heralded as such a breakthrough but this is probably not the way to do near-field optical Raman.

There is one caveat to this however. The z resolution of the near-field optical approach is much better than anything that can be obtained in micro Raman today which is done in confocal mode. Generously speaking the z resolution in such confocal

microscopes is 0.5 microns [14]. For a near-field aperture this z resolution is at most 0.25 microns for a 1 micron aperture. The near-field optical tip emits light with such extreme diffraction that it was shown in a 1986 analysis [15] that only spectral phenomena a few tens of nms from the tip is the dominant signal from conventional near-field optical tips and for the large tips being discussed here this z distance is somewhat larger.

Thus researchers, in biology for example, now can look at near membrane Raman to address the critical questions of near membrane molecular changes while using the simultaneous micro Raman to monitor deep alterations in the cell.

Additionally, in the vein of the discussion in the previous section of this review, the Nanonics NSOM/AFM 100 Confocal™ provides the researcher with on-line force sensing capabilities of the cantilevered optical tip with the optical capabilities of Raman. Thus, near-membrane Raman can now be correlated with the motion of the cell membrane with less than 0.5 Angstrom z resolution for monitoring mechanical or topographical changes with AFM. The resolution capability with cantilevered optical fiber is 3 orders of magnitude faster than silicon cantilevers in the microsecond time domain [16]. In addition, along these same lines the near-field optical capabilities can be used to trigger local uncaging of an effector molecule while the Raman and the AFM probe the surrounding molecular structure or the cellular motion.

Surface Enhanced Near-field Raman

The greatest hope for near-field Raman is the phenomenon of surface enhance Raman scattering. Based on the hard data that exists today there is little doubt that this will be realized and, as a result, the world of Raman scattering will never be the same.

Evidence dating back over 20 years suggests that the Raman signal can be magnified by many orders of magnitude when small metal particles are in proximity to molecular species [17]. The exact mechanism has been hotly debated over the past 20 years. Nonetheless, it is now accepted that metals such as silver, gold and copper that exhibit surface plasmon states which are highly polarizable by the light field have a considerable effect on neighboring molecular species. A pioneering paper that suggested that such an approach may work for near-field Raman scattering was by John Wessel [18]. Although this paper did not specifically relate to the linear phenomenon of Raman scattering, the paper did show that, if a tip was illuminated by an external light field then non-linear Raman signals from a surface that was in close proximity to such an isolated, illuminated nanoparticle with surface plasmon states, could be enhanced by as much as 8 orders of magnitude. This depended on the geometry of the nanoparticle and the near-field distance of this particle to the surface being probed. The paper was solely theoretical and gave little practical indication as to how one would approach such an experiment.

In spite of the fact that there were all sorts of attempts to externally illuminate near-field tips fluorescent [19] and otherwise [20] there was no real attempt at the Wessel proposal of an isolated metallic nanoparticle with surface plasmon states for nearly 11 years. The first such attempt was by Bouevitch et al. [21] and Peleg et. al. [22]. These workers focused on gold and silver nanoparticles and investigated a variety of non-linear optical generation from molecules associated with such isolated nanoparticles. Large enhancements were seen.

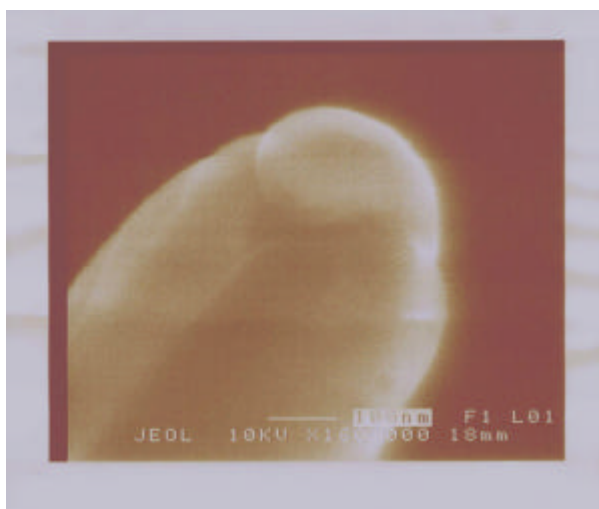
This work was followed by two studies by Nie and Emory and another by Kneipp et al [23-25]. These workers deposited molecules on a surface which also had silver nanoparticles. Thus, with some probability there was a likelihood of a silver

nanoparticle being in close proximity to a molecule. These workers showed that it was possible to obtain the surface enhanced Raman spectrum of a single molecule associated with a nanoparticle. All of the above papers noted that the enhancement from such particles was not uniform. Some particles were hotter than others and Nie and Emory obtained simultaneous AFM of images of the hot particles showing that there appeared to be preferred shapes for these particles, the more elongated the better. This work on Raman scattering has been extended in several papers with encouraging results [26-29].

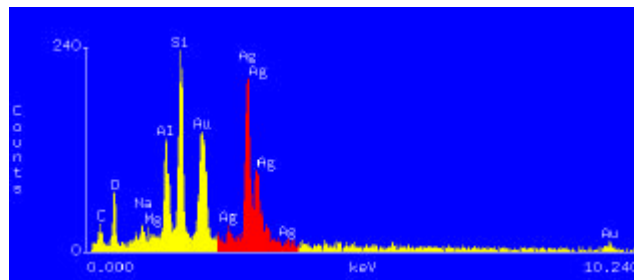
Recently Peleg et al [30] showed that a 1 nm gold particle when coupled to an antibody and directed to a specific site in a cell membrane that was stained with a highly dipolar dye molecule gave enough enhancement of the non-linear phenomenon of second harmonic generation to be able to detect a single molecule with this extremely weak phenomenon. This study emphasized the importance of coupling a highly polarizable metallic nanoparticle with a highly dipolar molecule in order to affect maximum enhancements. There is little doubt that the dipolar nature of the molecule will also be of considerable importance for Raman scattering. Previously this had not been emphasized.

In summary, all of these studies have clearly laid the ground work that shows that it is surely possible, from a fundamental point of view, to probe, with nanometer precision, the Raman spectra of surfaces. For a full demonstration, the open architecture and the confocal detection abilities of the Nanonics NSOM/AFM 100 Confocal™ will be crucial. The latter point is important in order to selectively detect, without high background, the scattering from the particle and not from the surrounding.

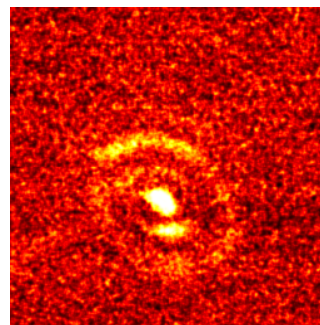
A crucial experimental step to achieve this goal is the appropriate placement of a single metal particle of gold or silver at the tip of a force sensing structure such as our cantilevered near-field optical elements. At Nanonics we have recently made considerable progress toward this goal and this is shown below.



The material composition through the line scan is indicated by electron induced x-ray emission. These measurements through the yellow line at the tip is shown as a graph of counts at various energies that correspond to the silver particle at the tip, the Si and Al of the glass and the overcoating of Au for the electron microscopy.



An example of one such application is demonstrated in the image that is shown below. In this image a gold-tipped AFM glass cantilever that was produced by Nanonics was brought in close proximity to a styryl dye with a large induced dipole. The tip with the 100 nm gold nanoparticle enhanced the signal of the molecules by at least three orders of magnitude. The extent of the enhancement is shown by the fact that the image around the gold tip indicates the presence of diffraction rings of intensity that resulted from the intense second harmonic generation that was induced.



With such tips and the Nanonics AFM/NSOM 100 Confocal System™ Nanonics is committed to exploiting the ultimate resolution achievable in Raman spectroscopy and in enhancement schemes using non-linear optics.

ACKNOWLEDGEMENTS

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