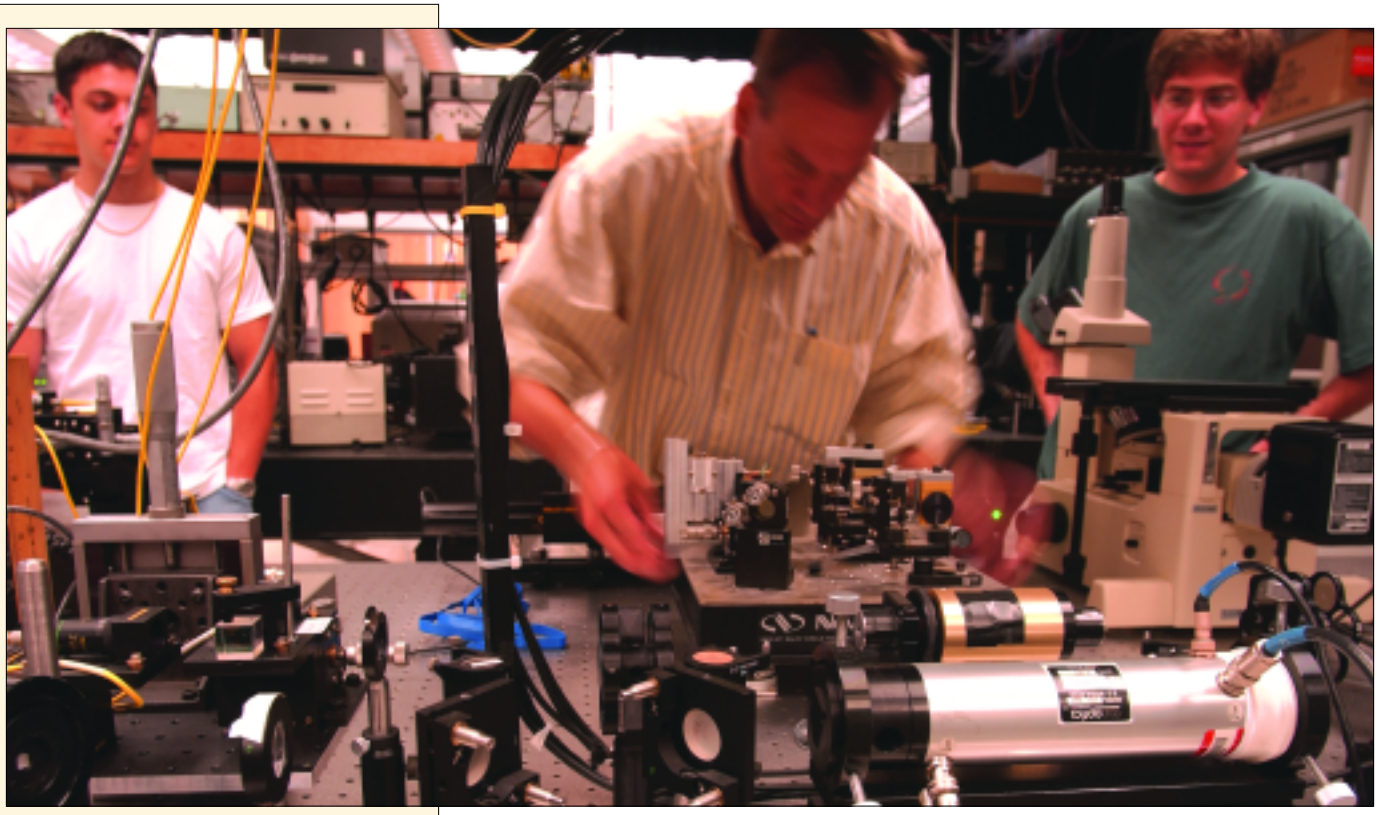

In 1928, two remarkable scientists, E.H. Synge[1] and C.V. Raman,[2] published separate papers on different topics. The two came from different worlds—one was Irish, the other Indian—and it is not known if they ever met. A characteristic they shared was a sense of supreme confidence in their own ideas, even in the face of intense skepticism. Nearly 70 years later, after both were long dead, the ideas published in the two papers were fused. The technique that emerged, evanescent wave vibrational microscopy, is an important analytical arrow in the quiver of today's nanotechnologists and biological physicists.

(*Above*) Boston University professor Bennett Goldberg, co-inventor of the numerical aperture increasing lens, demonstrates alignment of an optical setup for students Raviv Perahia (*right*) and Steven Menn.

Evanescent Wave Vibrational Microscopy

Mi K. Hong, Anna Swan and Shyamsunder Erramilli

Edward Hutchinson Synge was a scientist with a penchant for off-the-wall ideas. At the time he wrote the article, it was understood that an optical microscope cannot resolve two structures closer than about half the wavelength of light. Nearly 50 years earlier, Ernst Abbe had shown that in a conventional microscope, two closely spaced objects each diffract the light that comes from an external illumination source. The diffraction pattern generated by each object is "filtered" by the finite diameter of the objective[3] (see Fig. 1). Each object thus gives rise to a diffraction pattern that can be recorded at the image plane by a camera or other means. According to the benchmark known today as the Rayleigh criterion, the objects are resolved if the two central diffraction spots are separated by their half-width. (Most readers of this article will have first encountered the Rayleigh criterion in a slightly different context in their freshman physics class: if you look at the headlights of a distant approaching vehicle at night, can you tell if it is a car or a motorcycle?) Using this criterion, Abbe showed that the smallest distance between objects that a microscope can resolve is $\sim 0.6\,\lambda/n \sin\theta$, where n is the refractive index of the objective lens used and θ is the half-angle subtended by the object at the lens.[3] The factor $n \sin\theta$ is the numerical aperture (NA). Even assuming an infinitely large glass lens, i.e., one with $\theta = \pi/2$, the best resolution one could hope to achieve using green light is about 200 nm. Coherence illumination increases the numerical factor of 0.6 slightly, and it remains true that a conventional optical microscope cannot resolve objects that are closer together than several hundred nanometers. Distant headlights are an example of an incoherent source, while illumination—

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not necessarily laser light but even the light from an incandescent bulb—in thin samples viewed through a microscope is much more coherent. Abbe conducted careful experiments to back up his theory of spatial resolution under coherent and incoherent illumination. Such a display of virtuosity is difficult to imagine in today's world of specialized theorists and experimentalists. Those who think that industry funding of university research is a modern phenomenon may also find it interesting that Abbe's work at the University of Jena was supported by Carl Zeiss. Abbe's insights provided the basis for revolutionary new microscope designs for Zeiss instruments. Zeiss went on to become a renowned maker of microscopes and optical instruments, a reputation the company continues to enjoy today.

Thanks to Abbe, image formation in microscopes using coherent or incoherent sources was already well understood by Synge's time. The resolution limit of several hundred nanometers achievable by optical microscopy appeared insurmountable. Nevertheless, Synge had begun to envision a way to break the diffraction limit. Figure 1 (b) shows the essence of his idea. A small aperture is placed very close to the sample so that only one of the two closely spaced objects is illuminated. Any light that emerges interacts only with that particular object before being detected. When the aperture is raster-scanned across the sample while proximity is maintained, a point-by-point image with a resolution limited not by the wavelength of light, but rather by the size of the aperture, can be reconstructed. Since the size of the aperture can be made much smaller than the wavelength, Synge argued, the diffraction limit could be broken.

The 1920s were years of great ferment in physics and engineering: a number of "crackpot" ideas were jostling for attention as an amazing new world was being revealed by researchers in quantum physics and relativity. An idea like Synge's might well have been lost in the shuffle had he not had the courage to send an outline to the most famous physicist in the world, Albert Einstein.* The exchange that ensued allowed Synge to refine and clarify his ideas.

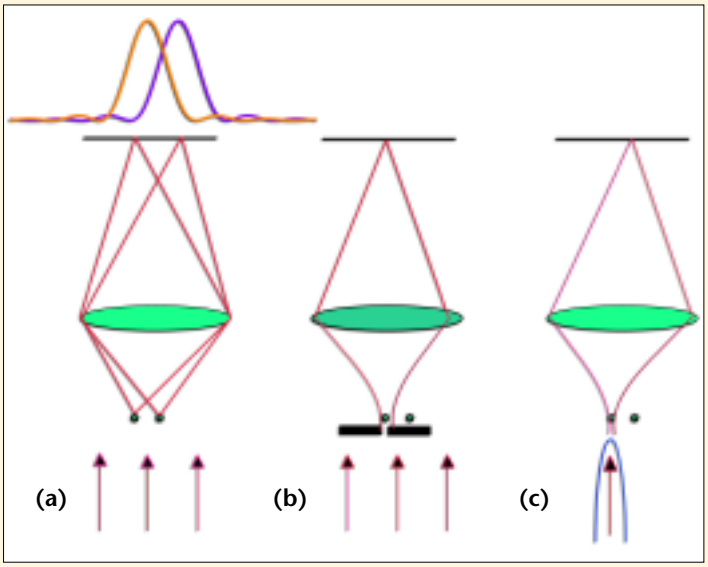


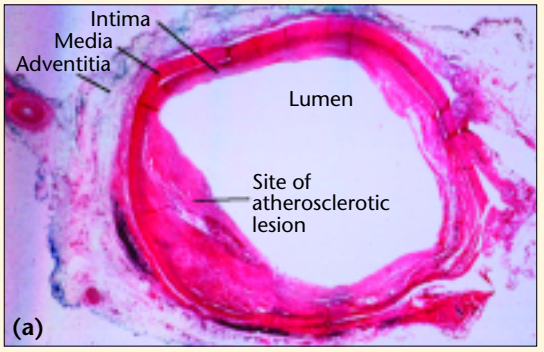
Figure 1. (a) Abbe's diffraction limit for resolving closely spaced objects in a microscope. (b) Synge's idea for breaking the diffraction limit using evanescent fields from a small aperture. (c) Aperture replaced by a tapered optical fiber in modern near-field microscopes.

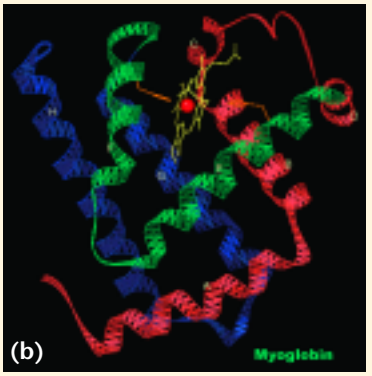
Encouraged by the correspondence with Einstein, Synge published his paper. He had conceived of a way to improve the spatial resolution of optical microscopes to 10 nm. There was, however, a problem: his microscope was impossible to build. Synge's design required in fact that a 10-nm hole be drilled in a metal plate. He needed a way to move the sample mechanically, with step-by-step increments on the nanometer scale, while the separation between the aperture and the sample was maintained to within a few nanometers. No researcher of the era knew how to achieve such a result. But Synge was undaunted; over the next decade, he proposed a number of ingenious solutions. He devised gears that could provide the tiny motions needed. Presciently, he proposed that piezoelectric positioners could be used to provide the nanoscale precision movement. But he was decades too early: technology had a long way to go before his vision could be realized. The first experimental confirmation of his ideas was made only in 1970, by Ash and Nichols,[4] who used microwaves. No one knew how the microwave studies could be extended to the visible region. Synge's ideas (along with Synge himself) eventually passed into obscurity.

Obscurity would never be Raman's fate. In 1928, he was already a member of the Royal Society of London and famous for his work on acoustics. While investigating the scattering from a clear liquid of monochromatic light provided by a mercury arc lamp, he and his students in Calcutta noticed new spectral lines, faint but discrete. Some observers mistakenly believed the lines to be a manifestation of fluorescence, or perhaps of the recently discovered Compton effect—the scattering of light by free electrons. Raman, convinced that the sharpness of the lines showed that what he and his students had observed was a new effect, published the idea within a month of the discovery. He correctly identified the new lines as having been caused by the inelastic scattering from vibrational normal modes of the molecules in the liquid. A visible photon can scatter off a molecule and lose some of its energy as it excites an oscillatory mode in the molecule. In 1930, for this discovery, he received the Nobel Prize in physics, the first to be awarded to an Asian scientist. Such was Raman's confidence that he had booked steamship tickets for the long voyage from India to Sweden a month before the names of the winners of the Nobel Prizes were announced. He was proud of the fact that he had done all his work in India, and he became a hero to his countrymen.

As a practical tool, however, Raman spectroscopy proved too difficult to use.

* All of Einstein's papers, formerly at Boston University, are archived at Caltech at http://www.einstein.caltech.edu. At the time of this writing, Synge's letters are not available online





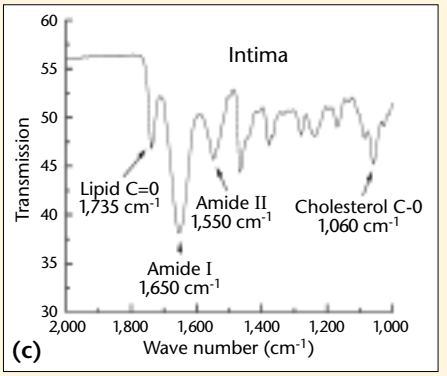


Figure 2. (a) Stained microscope image of diseased coronary artery showing lesion. (b) The protein myoglobin, showing an iron atom at the center of a hemegroup, as displayed in MAGE [Jane & David Richardson, Duke University]. (c) Infrared transmission from the intima, showing peaks caused by lipids (cholesterol) and protein.

The intrinsic cross-section for most molecules is very small. All other things being equal, the probability that a molecule would scatter a photon via the Raman effect is some 15 orders of magnitude smaller than the probability of exciting fluorescence. The sources of light available at the time were not bright, and the use of diffraction gratings to filter out the incident light meant that great skill was required to measure the spectra. Infrared spectroscopy, which detects vibrations by direct absorption and with cross-sections higher by 10 or more orders of magnitude, became vastly more popular. Over time, the technique Raman developed began to fade from use. It was the invention of the laser some 30 years later that rescued Raman spectroscopy from oblivion.

Synge, on the other hand, was lifted from the dust heap of history by the invention of the scanning tunneling microscope. In the modern version of Synge's microscope, the end of a tapered optical fiber is coated with metal to form the subwavelength aperture. Because the size of the aperture is smaller than the waveguide cutoff, the electromagnetic fields are said to be evanescent. Interaction with an object placed close to the aperture results in the evanescent wave being scattered and transformed into a traveling wave that can be detected. By use of techniques perfected in scanning probe microscopy (SPM), a near-field—or evanescent wave—image with a spatial resolution well below the diffraction limit can be provided by scanning the tapered fiber over the sample surface.[5] This technique is called scanning near-field optical microscopy (SNOM) on the eastern shores of the Atlantic Ocean and near-field scanning optical microscopy (NSOM) on the western shores of the Atlantic Ocean.

Vibrational spectroscopy is an exquisitely sensitive tool for identifying and characterizing molecular bonds. A molecule like myoglobin, an oxygen-binding protein present in red meat, is made up of thousands of atoms connected by spring-like bonds. As a result, the entire molecule can vibrate in a large number of normal modes. When a mode is associated with an electric dipole that depends on the lengths of atomic bonds—as, for example, the permanent dipole formed by bonds between dissimilar atoms like carbon and oxygen—the molecule can absorb infrared radiation the frequency of which matches the quantum vibrational energy of the normal mode. In the absence of a permanent dipole—as, for example, in the case of the bonds between identical atoms such as those that compose a carbon nanotube—a dipole can be induced by the external electromagnetic wave and the molecule exhibits the Raman effect. Because the modes are characteristic of bonds at the molecular level, the spectral region from about 3-12 μm is called the "fingerprint" region. [It is common practice to describe vibrational spectra in terms of wave numbers ($1/\lambda$), in cm^{-1}; 6 μm corresponds to about 1,667 cm^{-1}.] The sharp lines that Raman discovered were formed by just such well-defined modes.

Why would one want to combine vibrational spectroscopy with use of a microscope? Microscopy works only when there is contrast, and the vast majority of biomolecules are completely colorless. The only reason we can see the cross-section of a coronary artery in Fig. 2 is because the sample has been stained to provide contrast. Biological samples are most often stained with colored dyes, radioactive labels or fluorescent labels. There is always the worry that the stain itself is perturbing the system (and most fluorescent labels are, in fact, carcinogenic). Vibrational microscopy, in that it relies on the intrinsic normal modes of the molecules themselves, offers a nondestructive method of generating contrast without the use of stains or labels.

Infrared (IR) and Raman microspectroscopy, both of which are forms of vibrational microscopy, are used in areas as diverse as: forensics; archaeology; dating paintings from the pigments used; measurement of localized stress in diamond films and semiconductor microelectromechanical and Si integrated circuits; and carbon nanotube research.

There are, however, some technical problems. The Abbe limit is a serious hindrance in infrared microscopy, where wavelengths are 10 times longer than in the visible region. At a 6-μm wavelength, even with the increased refractive index that semiconductor lenses can have in this region, the spatial resolution in a microscope image is theoretically limited to above 1 μm. Although with

synchrotron-based sources or infrared lasers it is possible to reach the diffraction limit, in practice, commercial infrared microscopes equipped with relatively weak Globar sources can rarely achieve resolution much better than 20 μm. That level is good enough for forensic use, where the goal may be, for example, to discover whether a fiber taken from a murder scene comes from the carpet or is a piece of the suspect's hair, but it is simply too coarse for imaging biological cells. With the development of scanning near-field infrared microscopy (SNIM),[6,7] however, the limitation has been overcome.

Two advances had to take place before this extension to the infrared of Synge's idea became practicable. One was the appearance of infrared transmitting fibers made of chalcogenide glasses, which were developed most notably for use in infrared thermal measurements and imaging in the first Gulf War. The second was the development of tunable mid-infrared laser sources—such as free electron lasers, ultrafast optical parametric amplifiers and quantum cascade lasers—that could hit the vibrational molecular resonances in the fingerprint region. By use of a very fine tapered chalcogenide fiber illuminated with a tunable infrared laser, SNIM provides sub-diffraction-limited spatial resolution.

Thanks to SNIM, researchers were able to: obtain the first underwater near-field infrared images of single living cells; acquire near-field infrared spectra from atherosclerotic tissue samples; and identify single cholesterol crystals by their characteristic IR signature (see Fig. 3; Ref. 7). When they obtained the image of the cell shown in Fig. 4, D. Palanker and collaborators at Stanford achieved a sensitivity at the femtogram level. Besides these and other applications in pathology, the application of evanescent infrared waves to detect the amide bands in proteins holds promise for researchers working in the field of proteomics (the large-scale study of proteins, usually by biochemical methods).

Fiber-based microscopy systems are very inefficient, primarily because as mentioned above, as the fiber is tapered, its diameter falls below the cutoff wavelength of the optical waveguide. The fields are strongly attenuated as the aperture size D at the end is reduced to subwavelength dimensions. The light throughput falls off as $\sim(D/\lambda)^4$ for apertures $D \ll \lambda$. Even in the best designs, only about one photon in 10^4 emerges from the end of the tapered fiber. Significantly improved spatial resolution has been reported by use of apertureless methods, where light is scattered off a sharp metal tip placed close to the sample. This technique successfully combines the light collection efficiency of far-field microscopy with the subwavelength resolution of near-field scanning optical microscopy. Keilmann[8] and co-workers used an AFM tip in scattering mode (see Fig. 5) to obtain spectrally sensitive images with a stated resolution of about 100 nm, obtaining infrared spectra from attogram quantities of sample.

Apertureless methods have proved useful in Raman microscopy as well, with one added benefit. As mentioned above, the application of Raman spectroscopy is hindered by the fact that the cross-sections of molecules are low. At the apex of the sharp metal tip, the optical field is strongly enhanced. The static field enhancement at a sharply pointed feature (the "lightning rod effect") is well known from field emission, where a larger curvature of the tip decreases the emission voltage for electrons. At certain incident laser wavelengths, localized surface plasmon resonances can further enhance the field localized at the tip. Only light that is polarized parallel to the tip axis contributes to the field enhancement. This is problematic in a conventional optical setup, since the tip coincides with the propagation direction of illumination and hence has only a very small component polarized along the tip axis. Lukas Novotny at Rochester and Achim Hartschuh at the University of Siegen ingeniously solved this problem by using a radially polarized beam which has a large perpendicular field component. Since the Raman signal scales as the product of incoming and scattered intensity, the enhancement factor $M \sim (E_{local}/E_{in})^4$ is about 2,400. A similar enhancement effect is encountered in surface enhanced Raman scattering (SERS) when there are nanometer-sized metal particles close to the molecules being studied. In SERS, enormous enhancement factors of 10^{15} have been observed, with the strongest fields being found at interstitial sites. For a single spherical particle, in agreement with the observed tip-enhanced signal increase, the local enhancement is expected to be 100 to 1,000 times lower.

The unique properties of single-walled nanotubes (SWNT), which were discovered in 1991, stem from the combination of the unusual properties of graphite and the one-dimensional (1D) character of the tubes[10] (Fig. 6). Nanotubes, which have become the poster child of nanoscience and nanotechnology, are the

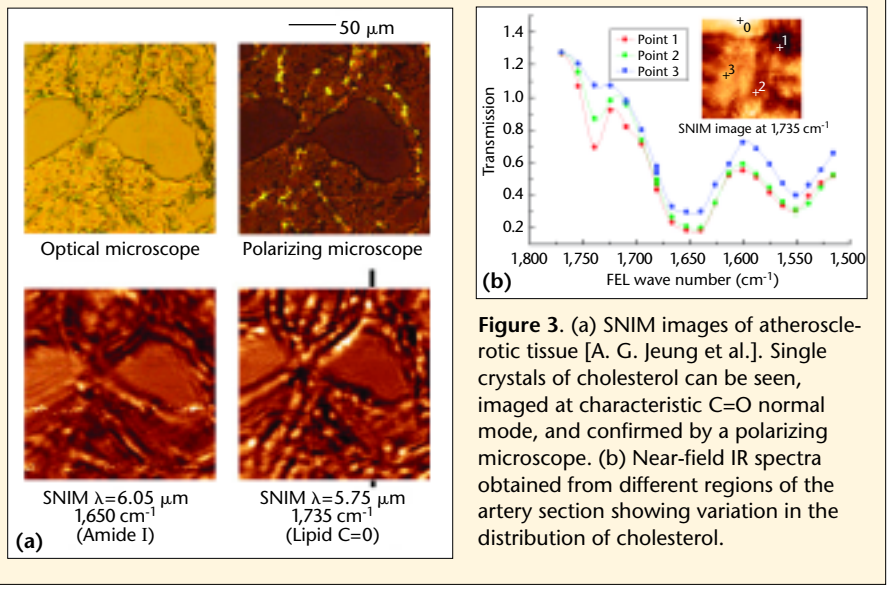


Figure 3. (a) SNIM images of atherosclerotic tissue [A. G. Jeung et al.]. Single crystals of cholesterol can be seen, imaged at characteristic C=O normal mode, and confirmed by a polarizing microscope. (b) Near-field IR spectra obtained from different regions of the artery section showing variation in the distribution of cholesterol.

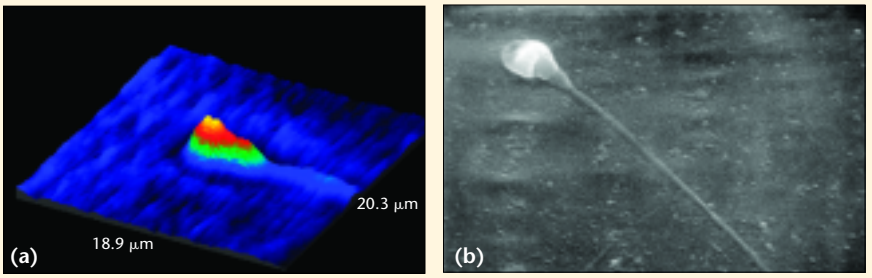


Figure 4. (a) SNIM Image of a single sperm cell at a wavelength of 5.5 μm. (b) Scanning electron microscope image of sperm cell. [Sample and image courtesy D. Palanker, Stanford University].

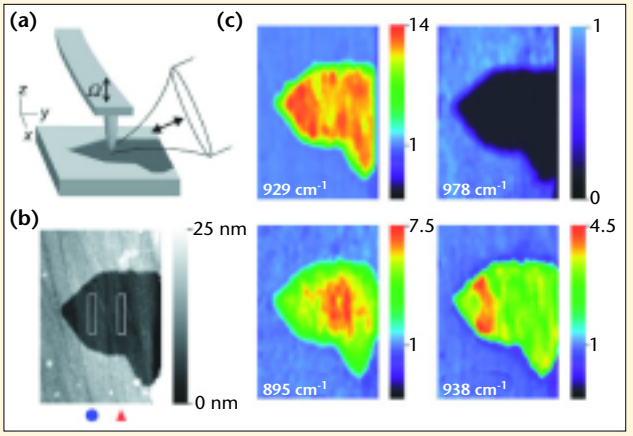


Figure 5. Experimental scheme (a) and images (b, c) taken with a scattering-type mid-infrared scanning near-field microscope (s-SNOM) (Keilmann & Knoll). The topography (b) shows a partially gold covered silicon carbide (SiC) sample (image size 1.6 x 2.3 μm^2). Two rectangles mark areas used for data extraction. The infrared near-field images (c) display the scattering amplitude *s* taken at different frequencies of illumination as indicated and are scaled so that Au appears blue. On phonon resonance, the SiC area appears much brighter than the surrounding Au (929 cm^{-1}). The contrast reverses at 978 cm^{-1}. Two examples taken on either side of the resonance (895 cm^{-1} and 938 cm^{-1}) indicate a systematic local variation in the infrared images.

focus of intense interest among researchers. Potential applications range from use as field effect transistors, stable single molecule light sources and low voltage field emitters, to mention just a few. Carbon nanotubes have several significant Raman lines and the strongly peaked electronic density of states typical of 1D structures has made it possible to study individual tubes with resonant Raman scattering and deduce both the structural and electronic properties of individual tubes. Figure 6 (b) shows a high spatial resolution Raman image of single-walled carbon nanotubes dispersed on a glass substrate. Here the graphitic shear mode at ~1,600 cm^{-1} is used.

A problem of interpretation

It is clear that evanescent wave methods can enhance the spatial resolution of both infrared and Raman images. Are there disadvantages? One problem common to all scanning probe methods is that the speed of image acquisition is slow (in contrast to video microscopy, acquisition times in scanning probe methods are ~ 1-10 minutes per image). This is because the distance between the tip and the sample has to be maintained within close tolerances, and also because the feedback methods used limit the speed of scanning. The delicate nature of the tip also means great care must be taken to avoid damaging it. A more fundamental problem is that the interpretation of the images is not straightforward. A molecule in close proximity to a near-field probe does not experience the same electromagnetic field as a molecule in a conventional spectrometer.[11] The absorption cross-sections will not be the same. One way to appreciate this is to note that traveling electromagnetic waves in a spectrometer are transverse. But evanescent waves, like sound waves, can have a longitudinal component. Longitudinal components can transfer energy from the incident beam to the molecule and alter the absorption. Variations in height can alter the efficiency of coupling between the probe and the sample. The efficiency of this coupling is extremely sensitive to distance. Changes in coupling may lead to significant contributions to SNIM images from the topography of the sample, as in an atomic force microscope. C. Michaels, S. J. Stranick and co-workers at NIST have studied coupling between the infrared absorption magnitude and changes in tip-sample separation and concluded that near-field absorption imaging is relatively insensitive to topographic artifacts.[11] Images obtained at several wavelengths on and off the normal mode resonance allow the topographic contributions to be separated from the purely spectroscopic ones.

Comparisons with the topographic image in Fig. 6 (c) demonstrate that many of the nanotube features are more crisply rendered in the optical Raman image than in the shear mode topographical image. The sharpest image observed so far features an optical resolution of 12 nm, limited only by the tip diameter. With tip-enhanced Raman microscopy, the resolution is good enough to correlate variations in the Raman signature with localized defects. Furthermore, local variations can be correlated with other behavior, for example with transport properties which are very sensitive to local defects.

Work is now underway by the Rochester group and Goldberg, Ünlü and Mohanty at Boston University to combine tip-enhanced microscopy with solid immersion lens technology by inserting the tip into a numerical aperture increasing lens [(NAIL); see Fig. 7]. The combination of a higher index of refraction with the refraction at the hemispherical surface yields a higher numerical aperture, resulting in a more closely focused spot and higher signal gathering. The NAIL technique, developed by Ippolito, Goldberg and Ünlü at Boston University, has been used to

image integrated circuits from the backside of silicon chips since the many metallic layers prevent imaging through the top layers.[12] The NAIL is made of a material with the same refractive index as the sample, in this case silicon. The planar surface of the NAIL closely matches that of the sample so that light propagates from the object into the NAIL by evanescently coupling across the gap between them. After propagating through the NAIL, the light refracts at the spherical surface into air and is collected by a conventional optical microscope. Because of the refraction of the spherical surface of the NAIL, even with a moderate NA microscope objective, a large numerical aperture is achieved. Figure 7 shows a static random access memory (SRAM) chip from Intel with 0.18-μm lines, imaged with a state-of-the-art chip inspection μAnalysis System from Hamamatsu [Fig. 7(a)]. Using NAIL for the same area demonstrates the more than six-fold increase in resolution [see Fig. 7(b)]. When it is applied to infrared and Raman imaging, evanescent wave microscopy has great potential.

The outlook for near-field vibrational spectroscopy is very bright indeed, with recent technical advances in both resolution and throughput exceeding what Raman and Synge could have imagined. With such huge signal enhancements, resolution is now limited only by tip radius. The ability to obtain high resolution images without stains or labels and to perform bond-specific non-destructive testing at the nanometer scale suggest that vibrational evanescent wave microscopy will be a useful tool for nanotechnologists, proteomics researchers and biological physicists.

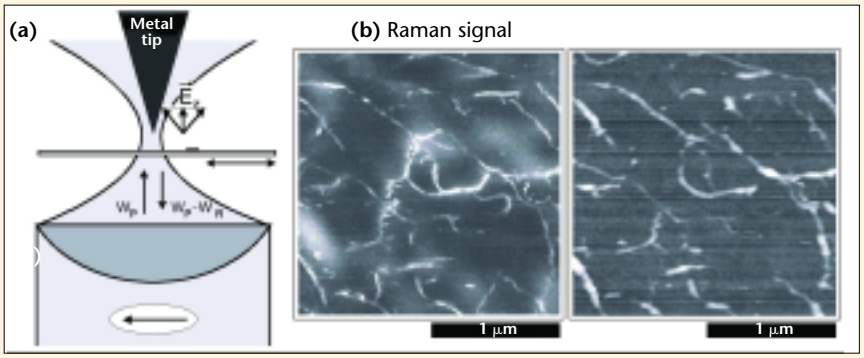


Figure 6. Tip-enhanced Raman scattering from carbon nanotubes. (a) A sharp metal tip is held in close proximity to the imaged sample. Laser light with radial polarization is focused onto the tip and sample. A large axial polarization component creates a local field enhancement at the apex of the tip. The signal is collected by the objective and the sample mapped through scanning the sample relative to the tip. (b) The Raman shear mode from carbon nanotubes at ~1,600 cm^{-1} is imaged with a higher resolution than the topographic image shown in (c). [Ref. A. Hartschuh, R. Beversluis, A. Bouhelier & L. Novotny, Phil. Trans. R. Soc. Lond. A 362, 807-19.]

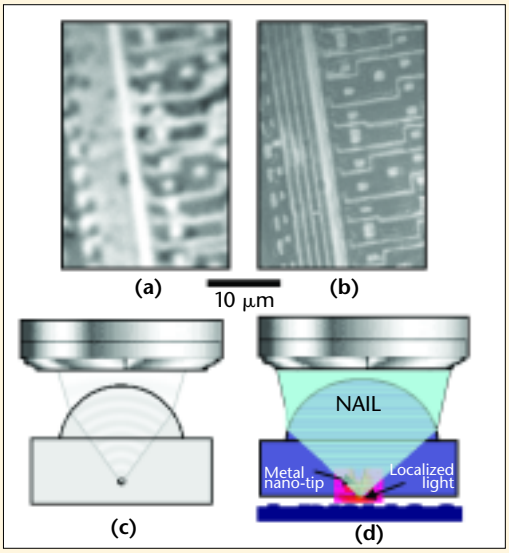


Figure 7. Images of an interconnect area of a 0.18-μm Si IC imaged using a Hamamatsu μAMOS-200 IC μAnalysis system. (a) A 100X objective lens (no NAIL) with effective NA=0.5 is used. (b) The same area imaged with a 20X objective and a NAIL with an effective NA=3.3. A 10-μm scale bar is shown. The resolution improvement is a factor of about 6.The NAIL is shown in (c). Note that both the added lens and the planar substrate are part of the NAIL.

Acknowledgments

Our intention has been to provide a brief tour of the fascinating field of evanescent wave vibrational microscopy. Editorial limits prevent us from fully acknowledging all the original references, and we apologize for any oversight. We thank our friends B. Goldberg, A. G. Jeung, T. I. Smith, D. Palanker, S. Ippolito, P. Huie, F. Keilmann, L. Novotny, S. Ünlü and A. Hartschuh. Support for this work has come from the National Science Foundation and the Department of Defense. We have taken the liberty of providing review articles or Web sites[13] where more information can be found, rather than citing the original literature.

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