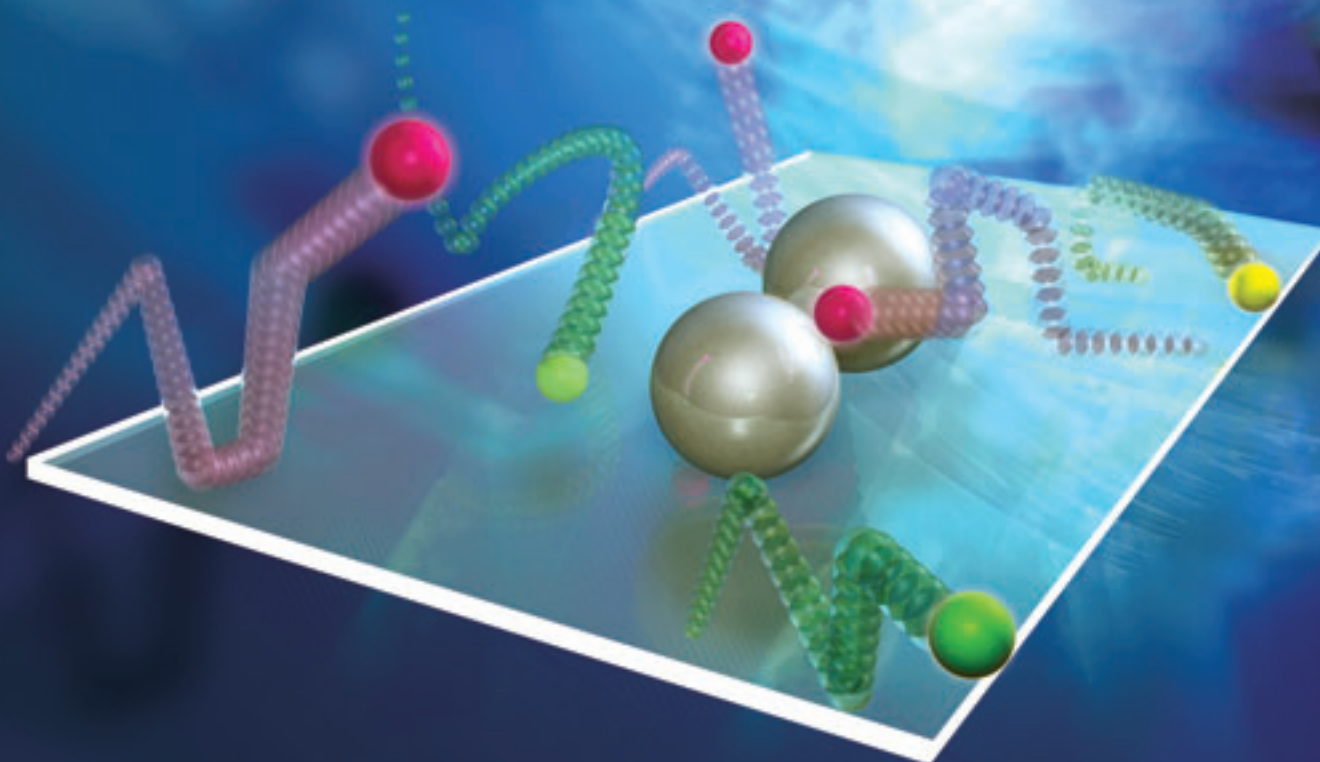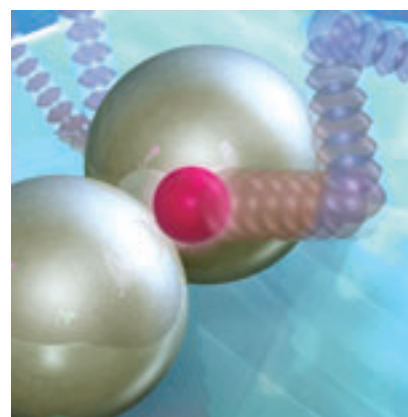**New substrates and single-molecule detection are just two of the advances that are fueling interest in SERS.**

Surface-Enhanced
RAMAN SPECTROSCOPY

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Smekal theoretically predicted inelastic light scattering in 1923 (*1*). Raman and Krishnan first experimentally observed the phenomenon and reported in their 1928 *Nature* paper that the inelastic scattering effect was characterized by "its feebleness in comparison with the ordinary scattering" (*2*). This "feeble" phenomenon is now known as Raman scattering. The change in wavelength that is observed when a photon undergoes Raman scattering is attributed to the excitation (or relaxation) of vibrational modes of a molecule. Because different functional groups have different characteristic vibrational energies, every molecule has a unique Raman spectrum. In accordance with the Raman selection rule, the molecular polarizability changes as the molecular vibrations displace the constituent atoms from their equilibrium positions. The intensity of Raman scattering is proportional to the magnitude of the change in molecular polarizability. Thus, aromatic molecules exhibit more intense Raman scattering than aliphatic molecules.

Even so, Raman scattering cross sections are typically 14 orders of magnitude smaller than those of fluorescence; therefore, the Raman signal is still several orders of magnitude weaker than the fluorescence emission in most cases. Because of the inherently small intensity of the Raman signal, the sensitivity limits of available detectors, and the intensity of the excitation sources, the applicability of Raman scattering was restricted for many years. However, its utility as an analytical technique improved with the advent of the laser and the evolution of photon detection technology.

In 1977, Jeanmaire and Van Duyne demonstrated that the magnitude of the Raman scattering signal can be greatly enhanced when the scatterer is placed on or near a roughened noble-metal substrate (*3*). Strong electromagnetic fields are generated when the localized surface plasmon resonance (LSPR) of nanoscale roughness features on a silver, gold, or copper substrate is excited by visible light. When the Raman scatterer is subjected to these intensified electromagnetic fields, the magnitude of the induced dipole increases, and accordingly, the intensity of the inelastic scattering increases. This enhanced scattering process is known as surface-enhanced Raman (SER) scattering—a term that emphasizes the key role of the noble-metal substrate in this phenomenon.
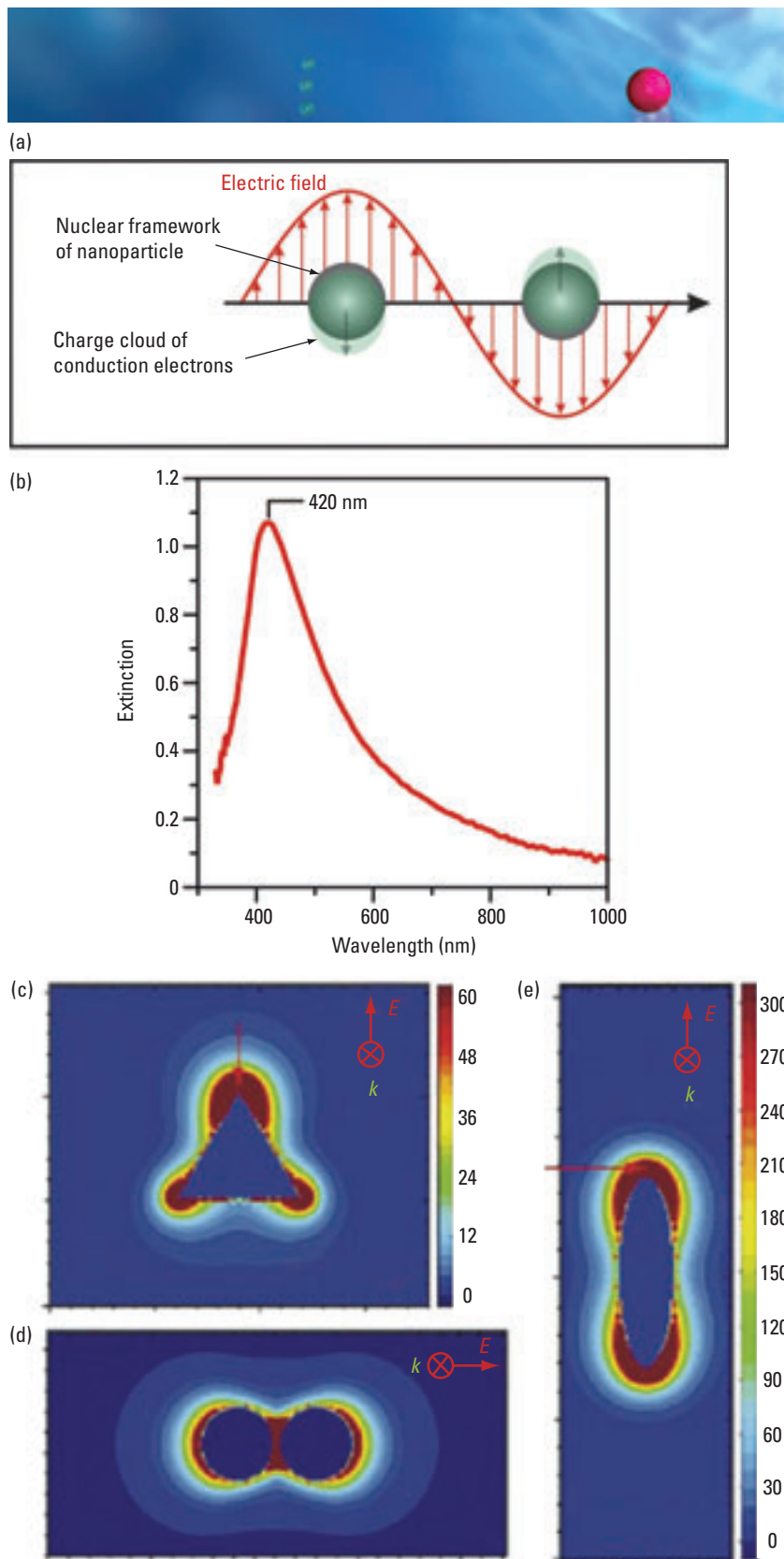
SER spectroscopy (SERS) can be exploited for sensitive and selective molecular identification. Recently, SERS has been used extensively as a signal transduction mechanism in biological and chemical sensing. Examples are trace analysis of pesticides (4), anthrax (5), prostate-specific antigen (6), glucose (7, 8), and nuclear waste (9). SERS has also been implemented for identification of bacteria (10), genetic diagnostics (11), and immunoassay labeling (12–14). A miniaturized, inexpensive, and portable SERS instrument makes the technique practical for trace analysis in clinics, the field, and urban settings (15).

In the 28 years since the discovery of SERS, the technique has progressed from model system studies of pyridine on a roughened silver electrode to state-of-the-art surface science and real-world sensor applications. SERS research currently produces an average of 200 papers per year, and the technique is also cited in many other manuscripts as an application for emerging nanotechnology. Over the past decade, the development of spectroscopic instrumentation, nanofabrication methods, theoretical modeling, and novel detection schemes has spawned widespread interest in the use of SERS and has established the technique as a powerful analytical tool.

Fundamental theory

The mechanism of SERS enhancement remains an active research topic. Since the discovery of the phenomenon, two major factions have disagreed on the relative significance of the proposed mechanisms, chemical enhancement, and electromagnetic enhancement. In the chemical mechanism, which is now thought to contribute an average enhancement factor of 100, a charge-transfer state is created between the metal and adsorbate molecules (16). In a manner analogous to that observed in resonance Raman spectroscopy, the existence of this charge-transfer state increases the probability of a Raman transition by providing a pathway for resonant excitation. This mechanism is site-specific and analyte-dependent. The molecule must be directly adsorbed to the roughened surface to experience the chemical enhancement.

To understand the electromagnetic enhancement, one must consider the size, shape, and material of the nanoscale roughness features. These characteristics determine the resonant frequency of the conduction electrons in a metallic nanostructure. When electromagnetic radiation with the same frequency is incident upon the nanostructure, the electric field of the radiation drives the conduction electrons into collective oscillation (Figures 1a and 1b). Excitation of the LSPR has two consequences: selective absorption and

scattering of the resonant electromagnetic radiation, and generation of large electromagnetic fields at the surface of the roughness feature (Figures 1c–1e). Electromagnetic enhancement relies on

FIGURE 1. SERS phenomenon.

(a) Illustration of the excitation of the LSPRs of a spherical nanoparticle by incident electromagnetic radiation. (b) Extinction spectrum of citrate-reduced silver colloids. The peak indicates the excitation of the LSPR. Theoretical simulations of the electromagnetic field enhancement around silver nanoparticles of (c) a triangular nanoparticle (700 nm), (d) a dimer of spherical nanoparticles (520 nm), and (e) an ellipsoidal nanoparticle (695 nm). The intensity scale for (c) also applies to (d). (Adapted with permission from Ref. 23.)

Raman-active molecules being confined within these electromagnetic fields (*17*) and contributes an average enhancement factor of ≥10,000.

Extensive theoretical modeling has been performed to provide insight into electromagnetic enhancement by noble-metal nanoparticles (*18*, *19*). The simple example of an isolated sphere, with a quasistatic description of the incident electromagnetic field, has been used to derive the proportionality

$$E^2 \propto E_0^2 \left| \frac{\varepsilon_m - \varepsilon_0}{\varepsilon_m - 2\varepsilon_0} \right|^2$$

in which E is the electric field magnitude at the surface of the sphere, E_0 is the incident field magnitude, ε_m is the wavelength-dependent dielectric constant of the metal composing the sphere, and ε_0 is the dielectric constant of the local environment around the sphere (*17*). This relation reveals that when $\varepsilon_m = -2\varepsilon_0$, which can be achieved for silver and gold at certain wavelengths in the visible and near-IR, the magnitude of the electric field at the surface of the sphere becomes very large. This field enhancement is induced by satisfying the LSPR condition. Similar relations can be derived for the extinction and scattering cross sections of the nanoparticle. Maximization of these cross sections at resonant wavelengths yields the spectroscopic signature of exciting the LSPR.

To help us understand the spectral characteristics and field-enhancing capabilities of a wide range of experimental SERS substrates, theoretical modeling has evolved to incorporate techniques for studying complex nanoparticle geometries. In this body of work, field enhancement has been studied as a function of nanoparticle aspect ratio and shape (*20*, *21*), extremely large SERS enhancement has been predicted in small nanoparticle assemblies (*22*, *23*), and interesting optical phenomena that occur in extended arrays of nanoparticles have been uncovered (*24*, *25*).

SERS substrates

The most critical aspect of performing a SERS experiment is the choice and/or fabrication of the noble-metal substrates. Because the SERS intensity depends on the excitation of the LSPR, it is important to control all of the factors influencing the LSPR to maximize signal strength and ensure reproducibility (*26*). These factors—which include the size, shape, and interparticle spacing of the material as well as the dielectric environment—must be chosen carefully to ensure that the incident laser light excites the LSPR. Traditional SERS substrates, chosen because they provide the desired optical properties, include electrodes roughened by the oxidation–reduction cycle (ORC), island films, colloidal nanoparticles, and surface-confined nanostructures (Figure 2). ORC-roughened electrodes provide reproducible, in situ SERS substrates with moderate (~10^6) enhancement factors. These substrates were exploited in the initial SERS studies of pyridine (*3*) and are used to study catalytic reactions on transition-metal-coated gold electrodes as well as other electrochemically active systems (*27*).
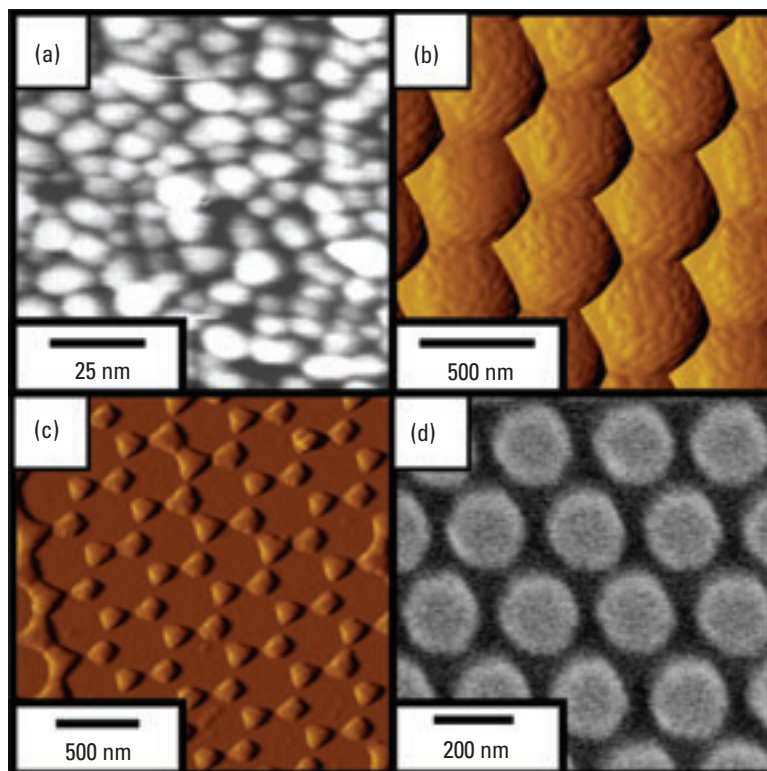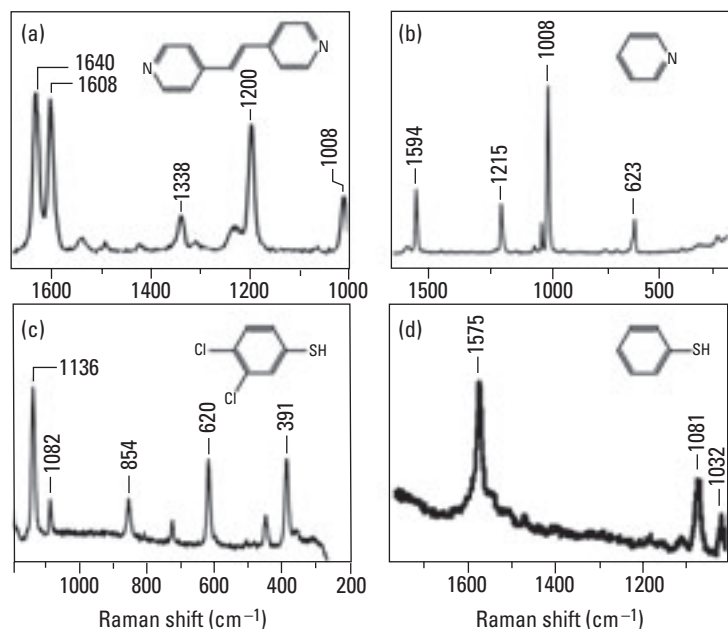

FIGURE 2. Sample substrates.

(a) Metal island film, (b) metal film over nanospheres, (c) triangular nanoparticle array fabricated with nanosphere lithography, and (d) cylindrical nanoparticle array fabricated with electron-beam lithography.

Metal island film substrates are easy to fabricate, and the LSPR wavelength can be tuned by varying the film's thickness and confluence. However, the enhancement factors achieved with these films are generally smaller (~10^4–10^5) than those observed with other SERS substrates. Recent work with silver island films has investigated the non-ensemble-averaged behavior of dye molecules in a Langmuir–Blodgett film (*28*). Colloidal nanoparticle substrates do not require sophisticated instrumentation for fabrication and are well suited to solution-phase SERS studies. The study of laser dye molecules adsorbed on silver colloids conducted by Hildebrandt and Stockburger laid the groundwork for the first single-molecule SERS experiments, which were performed in 1997 (*29–31*).

Surface-confined nanostructures can be produced by myriad fabrication schemes, including electron-beam lithography, colloid immobilization, and soft lithography. Recently, electron-beam lithography substrates have been used to explore the magnitude of the enhancement factor while the interparticle spacing is varied; enhancement factors as large as 10^8 have been achieved (*32*, *33*). Immobilized colloidal nanoparticles have been used as a SERS substrate for the analysis of the eluent in the CE separation of small molecules, such as amino acids (*34*). A soft technique known as nanosphere lithography has been used to fabricate SERS substrates for studying atomic clusters, biomolecules, redox couples, and other small molecules in ultrahigh vacuum (UHV), electrochemically controlled, and ambient environments (*35–38*). Researchers continue to develop novel SERS substrates to prolong substrate lifetime, provide stable and optimized enhancement factors, and permit SERS studies in diverse environments.

FIGURE 3. SER spectra of molecular adsorbates on the substrates shown in Figure 2.

(a) *trans*-1,2-bis-(4-pyridyl)ethylene on a metal island film, (b) pyridine on a metal film over nanospheres, (c) 3,4-dichlorobenzenethiol on a triangular nanoparticle array, and (d) benzenethiol on electron-beam-fabricated cylindrical nanoparticles. [(a) and (d) adapted with permission from Refs. 40 and 32, respectively; (b) and (c) adapted from Refs. 36 and 38, respectively.]

SERS analytes

A wide variety of molecular adsorbates ranging from diatomic molecules to enzymes have been studied by analytical SERS (Figure 3). The only requirement is that the molecule must reside within the enhanced electromagnetic fields that are generated upon excitation of the LSPR. Although it is difficult to determine the extent of the enhanced fields from the nanostructured surface, theoretical calculations for well-characterized nanoparticles suggest that these fields degrade exponentially with a characteristic decay length of ~2 nm (*17*). The three major strategies for confining a SERS analyte within the electromagnetic fields are physisorption, chemisorption, and partitioning via a self-assembled monolayer. Physisorption and chemisorption rely upon the natural adhesive forces between the analyte and the noble-metal surface. Partition layers have recently been exploited for quantitative sensing of glucose in physiologically relevant conditions and concentration ranges (*7, 8*).

It is also important to consider the spectroscopic characteristics of the SERS analyte, because electronic absorption bands with frequencies near those of the laser excitation will influence a SERS experiment. Surface-enhanced resonance Raman scattering (SERRS) occurs when the laser excitation wavelength overlaps with an electronic absorption band, thereby amplifying the Raman scattering intensities of the totally symmetric vibrational modes of the chromophore. This can be advantageous when investigating specific chemical moieties within a SERS analyte, such as the porphyrin in hemoglobin. However, the SERRS spectrum will be dominated by the resonantly enhanced modes. Accordingly, studies of nonresonantly enhanced regions of the SERS analyte require laser excitation outside of the electronic absorption band.

Instrumentation

Gas, dye, and solid-state lasers are all suitable excitation sources for SERS experiments. UV lasers have limited applicability in SERS because the dielectric properties of noble metals restrict LSPR excitation above a certain frequency threshold. Tunable lasers provide flexibility because it is possible to match the excitation frequency to the LSPR frequency of the substrate. However, complications arise when one attempts to prevent Rayleigh-scattered photons from reaching the detection components. For pulsed laser systems, peak power and bandwidth must be considered. High peak powers can damage the analyte or substrate, and pulse widths >5 ps are required to achieve a spectral resolution of 1 cm^{-1}. The excitation and collection optics are the same as those used for normal Raman experiments. For example, large camera lenses are needed for maximal efficiency of light collection, or optical microscopes for probing small sample areas.

Spectrometers with long focal lengths ($\geq$500 mm) are generally used in SERS experiments to resolve a vibrational spectrum. In a single-stage spectrometer, the Rayleigh-scattered light must be efficiently rejected before the scattered photons are coupled into the spectrometer. This step is usually accomplished with notch filters that have high optical density over a narrow bandwidth centered on the laser excitation wavelength. Although notch filters are readily available for specific laser wavelengths, many filters would be required to cover the entire wavelength range of a tunable laser system. In this case, double- and triple-stage spectrometers are preferred to achieve sufficient Rayleigh light rejection. Although these multistage spectrometers eliminate the need for Rayleigh-rejection filters, they exhibit much lower throughput than single-stage instruments (*39*). The detectors commonly used for SERS include photomultiplier tubes, photodiodes, and CCDs. Though far less common, FT-Raman instruments have also been used to obtain SER spectra.

Pros and cons

The SERS technique has many advantages. Because it is a vibrational spectroscopy technique, a SER spectrum provides much more information about molecular structure and the local environment in condensed phases than an electronic spectroscopy technique, such as fluorescence. Because the SERS analyte must be on or near a noble metal, which provides nonradiative pathways for the decay of excited states, fluorescence interference is strongly quenched. The Raman selection rules are complementary to the IR absorption selection rules; hence, the information obtained from Raman spectra is unique. Also, minor changes in the orientation of an adsorbate can be discerned, because slight variations yield measurable shifts in the locations of SERS spectral peaks. With the appropriate instrumentation, low-frequency vibrational modes beyond the range of IR absorption spectroscopy can be observed with SERS.

Furthermore, the abrupt decay of the electromagnetic fields ensures that only adsorbate molecules on or near the noble-metal substrate (within 4 nm) are probed. This quality makes SERS an
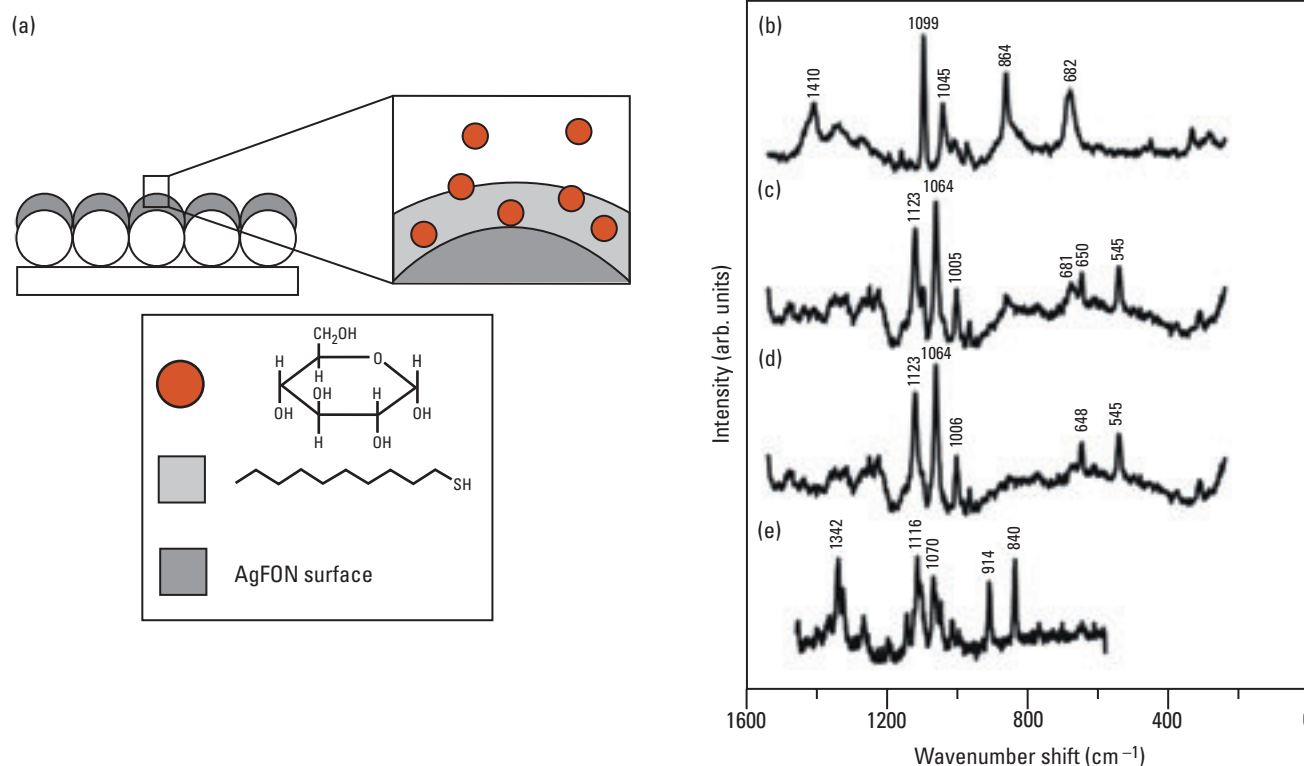
FIGURE 4. Prototype of an implantable glucose sensor.

(a) Schematic illustrating the use of a partition layer for detecting glucose. SER spectra of (b) a 1-decanethiol monolayer on a silver film over a nanosphere substrate and (c) the substrate during exposure to a 100-mM glucose solution. (d) The residual spectrum of glucose obtained by subtracting (b) from (c). (e) The normal Raman spectrum of crystalline glucose, for comparison. (Adapted from Ref. 7.)

ideal tool for surface studies, trace analyses, or biomolecular interactions. In fact, SERS experiments are routinely used to characterize sub-monolayer coverage of surface-bound molecules; detection of pico- to zeptomolar adsorbate concentrations can also be achieved. The flexibility of the optical arrangement promotes the application of SERS in diverse instrumental configurations, such as UHV chambers and liquid flow cells. Another distinct advantage, especially because biological studies are becoming increasingly important, is that the technique is well suited for analyses performed on molecules in aqueous environments. Water exhibits extremely weak SERS signal intensities.

The inherent limitations of the technique must also be considered. The most apparent constraint lies in the limited substrate choices that exhibit LSPR behavior. The most commonly used substrates are made of silver, gold, and copper. Experimental and theoretical work has also demonstrated the SERS phenomenon on aluminum, cesium, gallium, indium, lithium, platinum, potassium, rhodium, rubidium, and sodium substrates; however, other materials are not usable unless they are applied as thin coatings on SERS-active materials. (For a comprehensive list of substrates, see Ref. 40.)

In addition, the need for a roughened surface still poses some experimental challenges. For experimental and theoretical SERS investigations to be correlated, the substrates' roughness features must be well characterized by atomic force microscopy, scanning electron microscopy, or transmission electron microscopy. This step is often tedious to perform on randomly roughened surfaces. Furthermore, many roughened surfaces have limited stability and reproducibility. For example, with time, colloids tend to aggregate, and changes in molecular surface coverage

occur. Variation in the fabrication processes used to produce SERS-active substrates leads to inconsistent optical properties and, accordingly, discrepant enhancement factors. Because most of the current nanofabrication techniques implemented to create SERS-active substrates exhibit some degree of irreproducibility, enhancement factors can fluctuate by up to an order of magnitude for substrates fabricated with seemingly identical methodology.

Although SERS is very effective as a surface-science technique, it has limited applicability when the molecule of interest is not adsorbed directly onto the substrate. Creative schemes, such as using a partition layer to concentrate the molecule of interest within the zone of electromagnetic enhancement, must be implemented to study molecules that do not naturally chemisorb or physisorb onto the noble-metal surface. Finally, a high background continuum is often evident in SER spectra. Although the source of the high background is under debate, the most significant ramification is that the high background can obscure low-intensity SERS signals and limit the dynamic range of measurement.

Applications in surface science

Comprehensive reviews have been written outlining SERS applications in electrochemistry and UHV surface science (*41–43*). Because of the widespread use of SERS in surface science, compiling a brief summary of all the important contributions in this field is impossible. Instead, we will discuss a few examples to emphasize the unique advantages of the technique, highlight interesting applications, and dispel common misconceptions.

Campion and co-workers have performed UHV studies to investigate the chemical enhancement mechanism on atomically flat single crystals. In their studies,
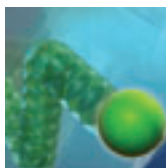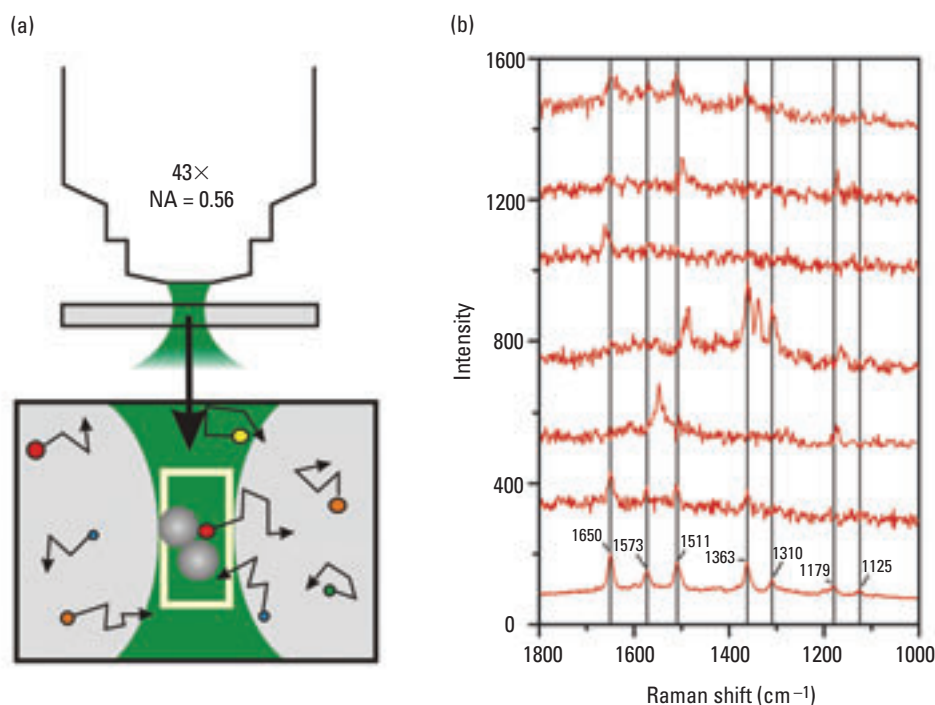
FIGURE 5. Confocal single-molecule SERS experiment.

(a) Schematic showing R6G molecules diffusing in and out of the confocal probe volume to interact with a compact aggregate of silver nanoparticles. (b) Six representative single-molecule SER spectra of 10 pM R6G in the presence of citrate-reduced silver colloidal nanoparticles and 750 μM NaCl, collected at 1-s intervals (top to bottom) with laser excitation at 514.5 nm and 3 mW of laser power. The labeled, ensemble-averaged spectrum of R6G is at the bottom for comparison.

Bio- and chemosensing

SERS holds great potential as a sensitive and selective signal transduction mechanism for the identification of biological or chemical agents. The narrow, well-resolved bands allow simultaneous detection of multiple analytes, and the low signal strength of water simplifies investigation of biological samples. Recently, SERS was applied as the signal transduction mechanism in a prototype for an implantable glucose sensor. Van Duyne and co-workers modified a roughened silver surface with a hydrophilic partition layer to facilitate the preconcentration of glucose and other small-molecule analytes within the zone of electromagnetic field enhancement (Figure 4). Glucose was detected and quantified in the physiological range with an accuracy approaching the requirements of a biomedical device (*7, 8*). SERS has also recently been applied in the detection of trace levels of chemical warfare agents. Langmuir–Blodgett-assembled silver nanowires have been used as the substrate to detect 2,4-dinitrotoluene, the most common chemical indicator of buried landmines and explosives, at a sensitivity of 0.7 pg (*48*).

In both the aforementioned glucose and 2,4-dinitrotoluene detection schemes, the SER spectrum of the target molecule was detected directly; in other recent work, SERS-active molecules have been implemented as labels on the analyte of interest. For example, Vo-Dinh and co-workers used Raman labels to identify cancer genes, thus avoiding the introduction of undesirable radioactive DNA labels. In this work, single-stranded (ss) DNA capture probes bearing a sequence from either the breast cancer susceptibility gene BRCA1 or the BAX gene, which encodes a protein that regulates apoptosis, were attached to a SER-active substrate. DNA probes bearing SER-active laser dyes were prepared and incubated with the capture-probe-modified substrate. If the labeled probe contained a sequence complementary to the capture probe, hybridization was successful, and a large SERS signal resulted (*49*).

In a similar experiment, Mirkin and co-workers used ssDNA coupled with a gold nanoparticle and a SERS label to detect DNA hybridization events. After the substrate had been exposed to the analyte solution of ssDNA, it was incubated in the SERS-labeled ssDNA, and then the gold nanoparticles were used as catalysts for the growth of an efficient silver SERS-active substrate. SERS detection of the Raman-active label revealed the identity of the target ssDNA. With this technique, they used multiplexed detection to distinguish hepatitis A, hepatitis B, HIV, Ebola, smallpox, and anthrax with a detection limit of 20 fM (*14*). A

they used electron-energy-loss spectroscopy to identify charge-transfer states that were created upon the adsorption of pyromellitic dianhydride on Cu(111) and Cu(100) (*44*). A resonant enhancement of ~100 was achieved by exciting the sample at wavelengths corresponding to the optical absorption of the charge-transfer state. Caldwell et al. have demonstrated SERS enhancement factors of ~10^3, attributed solely to electromagnetic enhancement, for self-assembled monolayers formed on gold grown epitaxially on mica (*45*). These experiments demonstrate that single crystals and epitaxially grown films can be used as SERS substrates. Even atomically flat metal island films can be used as SERS substrates if the excitation wavelength matches the SPR wavelength of the island film.

Weaver and co-workers developed a unique approach for studying heterogeneous catalysis on metals other than those traditionally used. Their technique involves creating nanoscale surface roughness features with a traditional SERS metal and subsequently electrodepositing a thin layer of catalytic metal on top of these features (*46*). These substrates provide the catalytic activity of the overlayer while maintaining the SERS activity of the underlying substrate. Systems studied with these substrates include methanol oxidation on rhodium and palladium as well as formic acid electrooxidation on platinum-group metals. Fokas and Deckert investigated similar substrates with near-field scanning optical microscopy. Their results demonstrate the ability to monitor single, catalytically active nanoparticles under reactive conditions (*47*). In addition to its high sensitivity, SERS provides an information-rich vibrational spectrum; this makes it an ideal tool for characterizing reactive intermediates involved in heterogeneous reactions. These characteristics are also exploited in sensing studies.
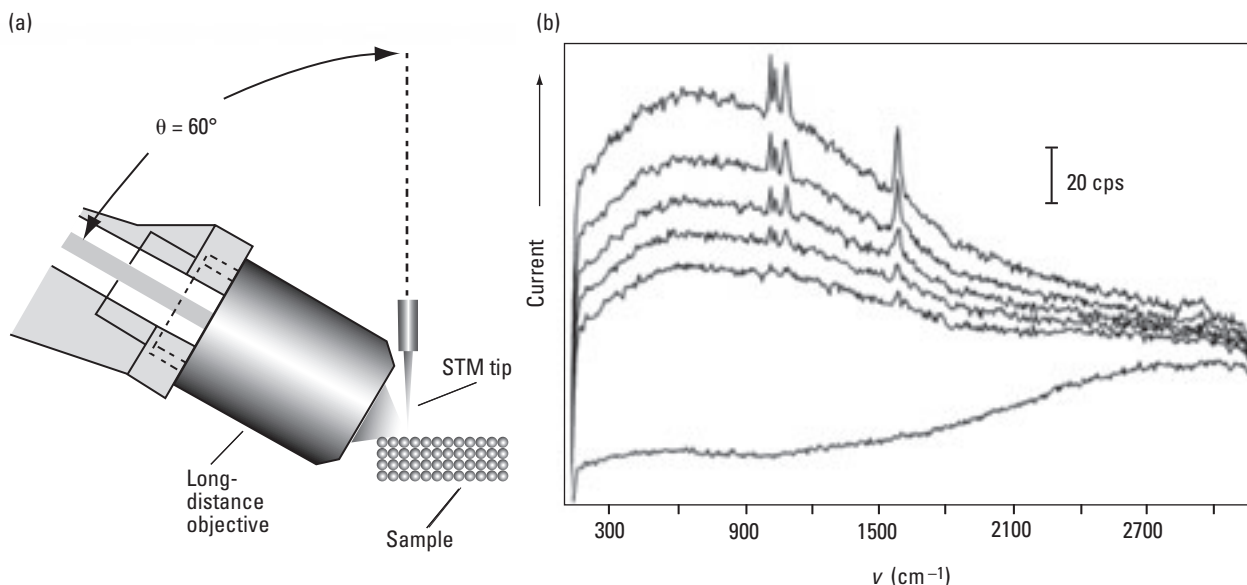
FIGURE 6. Experimental setup and spectra from a TERS experiment.

(a) A long-working-distance objective collects Raman scattering from the tip–sample junction. (b) TERS for benzenethiol on Pt(110). Top 5 spectra were recorded with the tip in tunneling range; bottom spectrum was measured with the tip retracted. (cps, cycles per second; adapted with permission from Ref. 56.)

search of the recent literature reveals that bio- and chemosensor development is being heavily investigated. The SERS signal transduction mechanism, whether directly revealing the target analyte or indirectly detecting the fingerprint of a molecular label, offers a method for multicomponent or multiplexed detection of low-concentration analytes.

Single-molecule SERS

When researchers achieved the milestone of detecting a single molecule with SERS, they raised further questions about the enhancement mechanism(s). The Nie and Kneipp groups each achieved single-molecule detection independently and with different experimental conditions. The Nie study included a correlated topographical and optical characterization of unaggregated silver nanoparticles dosed with Rhodamine 6G (R6G) molecules. They hypothesized that only 1 of every 100–1000 nanoparticles is "optically hot" and that only 1 of every 10,000 surface sites on a "hot" particle has efficient enhancement (*31*). Accordingly, the single-molecule enhancement is 10^6–$10^7 \times$ larger than the population-averaged enhancement.

The Kneipp research group, on the other hand, probed small (100–150-nm) silver colloid aggregates dosed with crystal violet molecules. The large (10^{14}) single-molecule enhancement is hypothetically attributed to large electromagnetic fields generated by fractal-pattern clusters of silver colloid nanoparticles (*30*). Since these two pioneering experiments, SERS has been used to detect single molecules of biologically significant compounds, such as adenosine monophosphate (*50*) and hemoglobin (*51*). Although the entire SERS community is excited by the recent development of single-molecule SERS, a new controversy surrounds the huge enhancement factors.

Current hypotheses regarding the single-molecule enhancement mechanism center on the concept that SERS substrates have a small number of "hot spots", which are thought to occur at the junctions between two nanoparticles. Correlated single-molecule SERS and topographical studies have clearly demon-

strated that single-molecule SERS occurs only in compact aggregates of nanoparticles where these junctions may exist (*52*). The large electromagnetic fields at the junction act as an optical trap for the Raman-active molecule and yield large scattering signals as well as enhanced photochemistry. The single-molecule SER spectra fluctuate on the time scale of 1 s; this is a signature of the molecule moving in and out of the hot spot and changing its orientation (Figure 5). Theoretical modeling of the electromagnetic field between two nanoparticles separated by ≤1 nm reveals a surface junction excitation and the efficient interaction of the molecular wave function with the wave function of the excited metal surface (*53*).

The future

An encouraging method for generalizing SERS to a wide variety of substrates is the development of tip-enhanced Raman spectroscopy (TERS; *54*). In this technique, the electromagnetic field enhancement is provided by the excitation of the LSPR of a scanning probe. This eliminates the need to use noble-metal substrates to observe SERS. The probe can be a scanning tunneling microscopy probe, a metal-coated atomic force microscopy probe, a tapered optical fiber with a nanoparticle or thin metal film at the tip, or any other nanoscale-sharpened metallic object. Because the strongest field enhancement is localized to a small volume very close to the apex of the probe, TERS can potentially provide chemical information at a spatial resolution below the diffraction limit. Theoretical modeling of TERS has suggested that enhancement factors of 10^7 and a spatial resolution of <5 nm can be achieved (*55*).

In practice, the development of TERS has been hindered by irreversible adsorption of analyte onto the probe, the presence of amorphous carbon backgrounds, the inability to determine the optimal excitation wavelength for a given probe, the difficulty in fabricating reproducible tip geometries, and the observation of only moderate field enhancement. Nevertheless, significant progress has been made. Ren et al. have used TERS to study molec-

ular adsorbates on smooth single-crystal substrates (Figure 6; *56*). Anderson et al. have applied TERS to study single-walled carbon nanotubes with 14-nm spatial resolution (*57*). These experiments demonstrate the technique's salient features, which are the ability to probe molecules on non-SERS active substrates and high spatial resolution. Continual advances in nanofabrication technology and optical characterization techniques are expected to overcome the difficulties encountered in the development of TERS. Eventually, the technique should provide an invaluable tool for the study of catalysis, biological membranes, composite materials, and all other surface-science systems.

Another important concept in the continuing development of SERS is instrument miniaturization, which requires reducing the size of both the detection system and the source of laser excitation. Handheld, CCD-based spectrometers are supplied by various manufacturers for this purpose. In addition, vendors sell small laser systems that provide the narrow linewidths (<10 cm^{-1}) and sufficient powers (a few milliwatts) required to perform Raman spectroscopy. One major challenge is achieving the collection and detection efficiency necessary for the sensitivities of traditional SERS instrumentation to be attained. Another challenge is the fabrication of an optical probe that incorporates a SERS substrate. The modification of fiber-optic probes with metal films or nanoparticles provides a promising solution, but great care must be taken to ensure long-term stability of the metal substrate and to permit reuse of the probe. For field-portable devices, a stable power supply is required so that reproducible laser excitation powers can be achieved and precise signal intensities can be measured. If these requirements can be met, miniaturized SERS instruments should have a significant impact on homeland defense applications, point-of-care medical diagnostics, and environmental monitoring.

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