Tip-Enhanced Raman Spectroscopy: Near-Fields Acting on a Few Molecules

Bruno Pettinger, Philip Schambach, Carlos J. Villagómez, and Nicola Scott

Department of Physical Chemistry, Fritz Haber Institute of the Max Planck Society, 14195 Berlin, Germany; email: pettinger@fhi-berlin.mpg.de

Abstract

Tip-enhanced Raman spectroscopy (TERS) is a very powerful variant of surface-enhanced Raman spectroscopy (SERS). In a sense, TERS overcomes most of the drawbacks of SERS but keeps its advantages, such as its high sensitivity. TERS offers the additional advantages of high spatial resolution, much beyond the Abbe limit, and the possibility to correlate TERS and other scanning probe microscope images, i.e., to correlate topographic and chemical data. TERS finds application in a number of fields, such as surface science, material science, and biology. Single-molecule TERS has been observed even for TERS enhancements of “only” $10^6$–$10^7$. In this review, TERS enhancements are discussed in some detail, including a condensed overview of measured contrasts and estimated total enhancements. Finally, recent developments for TERS under ultrahigh vacuum conditions are presented, including TERS on a C$_{60}$ island with a diameter of a few tens of nanometers, deposited on a smooth Au(111) surface.

Keywords

field enhancement, surface plasmons, TERS, beyond diffraction limitation, single molecule
1. INTRODUCTION

Tip-enhanced Raman spectroscopy (TERS) (1–4) is a very powerful variant of surface-enhanced Raman spectroscopy (SERS). SERS, detected in the mid 1970s, has substantial drawbacks; for example, intense SERS occurs only for roughened substrates made of coinage metals or for clusters of nanoparticles, again made of coinage metals (5). In contrast to SERS, TERS operates on all adsorbate/substrate configurations, where the substrate may be rough or smooth (7), or even single crystalline (8), and can be either a metal (4), a semiconductor (9, 10), or an isolator (1) and where the adsorbate may or may not be in optical resonance with the exciting laser line (11). However, only a limited number of such systems have been investigated to date, but all results continue to indicate the very promising nature of TERS. Furthermore, due to the strong localization and height of the optical near-fields underneath the tip, TERS delivers with very high sensitivity, via Raman spectroscopy, chemical information on the nanometer scale; some reports already indicate that the single molecule detection level has been reached (12).

In this review, we begin with a short overview of SERS, focusing on its strengths and drawbacks, followed by an overview of TERS and its recent developments. We first address early TERS experiments, followed by a discussion on contrast and underlying enhancement and a specific, condensed overview of this topic. In the few-molecule TERS section, we address the requirements of few- or single-molecule TERS and present two examples in which UHV-TERS is employed.

2. SURFACE-ENHANCED RAMAN SPECTROSCOPY

Richard P. Van Duyne and coworkers (13) were the first to notice a very strong surface enhancement for Raman scattering performed on pyridine molecules adsorbed at silver (Ag) electrodes. Van Duyne repeated and extended the striking experiments of McQuillan et al. (14) and Fleischmann et al. (15), who observed strong Raman scattering for pyridine adsorbed at Ag electrodes that had been electrochemically roughened. Fleischmann et al. (14, 15) attributed the observability of a Raman signal to a substantial increase of adsorption sites for roughened Ag electrodes. Van Duyne, however, estimated an approximately $10^5$-$10^6$-fold increase of the Raman signal in comparison to the signal expected for a monolayer of pyridine adsorbed on a smooth Ag surface. Obviously, this almost millionfold increase of the Raman intensity upon roughening cannot be attributed to an increase in adsorption sites. Instead, the increase had to be attributed to a surface-enhancement process of an as yet unknown nature.2

Today’s consensus is that SERS involves two enhancement mechanisms, so-called chemical and electromagnetic (EM) enhancements, often occurring at quite different strengths.3 Chemical enhancement accounts for the chemical specificity of SERS (not all molecules will be enhanced equally),4 whereas EM enhancement is thought to operate on all adsorbates equally, with the exception of some polarization and molecular orientation effects that influence the (relative) Raman intensities to some extent. This latter enhancement mechanism is associated with the excitation of surface plasmons and the strength of their EM fields near the surface. These fields can be significantly larger than the incident fields. Together, chemical and EM enhancement may lead to an overall average enhancement of approximately $10^6$, as already reported by Van Duyne (13)

---

1Weak SERS for transition metal substrates was shown by Z.Q. Tian (6) in the late 1990s.
2A year later, Van Duyne et al. (16) presented an image field model that was soon disregarded in favor of surface plasmon-mediated enhancement (5).
3This concept of two cooperative surface enhancement mechanisms had already been proposed in the early 1980s (17–20).
4In the following, chemical enhancement will not be considered in more detail due to the specific theme of this review.
Hot spots: spatially narrow regions of strongly enhanced electromagnetic fields occurring, e.g., in narrow gaps between nanoparticles

Electromagnetic enhancement: the enhancement of Raman scattering related to the enhanced near-field of excited (localized) surface plasmons

for pyridine on Ag electrodes. Surprisingly, findings in the late 1990s disclosed huge variations of surface enhancement along a rough surface or over colloidal systems (21–26). Obviously, within such structures, so-called hot spots occur that can provide extremely high enhancements locally. Theory and experiments indicate that a few hot spots may make up most of the enhanced Raman signal, in contrast to the vast majority of sites that do not contribute significantly. The hot spots were believed to be specific interstitial sites between colloidal clusters or structural elements of roughened surfaces, where the field enhancement becomes extremely large (27). As such sites are in a sense hidden, and thus not accessible for detailed investigation, their precise nature is not yet experimentally clarified.

Theory has shown, however, that if a dimer of two suitable nanoparticles is illuminated, a strong enhancement of the EM field can occur in the narrow space between the two particles (28–31). Let us describe the field enhancement for the incident EM wave by a factor $g_i$, and the enhancement of the scattered field by $g_{sc}$. A hundredfold increase of the EM field relative to the incident one (i.e., $g_i = 100$) would result in a local 10,000-fold intensity ($I_{loc} = g_i^2 I_0$), and the total surface enhancement would rise to $10^8$ locally, assuming the $g^4$ law to be valid. Clear proof for the hot spot character of SERS originated from single-molecule SERS experiments, which could succeed only if a single molecule, located in the hot spot zone, were selectively exposed to a significant enhancement, whereas nearby molecules experienced only a minor enhancement. Kneipp et al. (33) and Nie & Emory (34) claimed enhancement factors of $10^{14}$ or higher (32–35). Although such large enhancement factors have recently been disregarded, the hot spot scenario remains valid. The maximal enhancement for SERS appears to be in the range of $10^{10}$–$10^{11}$ (36, 37).

In spite of the success of SERS, in terms of its outstanding sensitivity, conventional SERS has a number of drawbacks, such as the following:

1. The total degree of surface enhancement depends on the nature of the molecules.
2. SERS is mainly limited to substrates made of coinage metals such as copper, silver, and gold.
3. The hot spot scenario means that atypical/unknown sites for adsorption/chemisorption contribute the most to the surface enhancement; thus, SERS spectra may not be representative for the majority of sites.
4. EM and chemical enhancements can be inseparably intertwined.6

These drawbacks clearly indicate that SERS cannot be used as routine spectroscopy in general.7 In spite of SERS’ success, the old dream to perform Raman spectroscopy at smooth or even single crystalline interfaces remained a dream for a long time. Of course, several attempts were made in that direction, but success was limited: On smooth or single crystalline surfaces, surface plasmons cannot be excited by direct illumination; thus, electromagnetic enhancement is basically absent, and only chemical enhancement can prevail, if this enhancement is possible at all (41–45).

The question arises: Is it possible to overcome the above-mentioned limitations of SERS? The answer comes primarily from the theoretical concept of enhanced near-fields: By the 1980s, a concept had already been proposed that would permit the creation of strongly enhanced EM

---

1For $g \approx g_{sc} \approx g_i$, the EM part of the enhancement is simplified to $F_{EM} = g_i^2 g_{sc}^2 \approx g^4$.

2Even if the chemical enhancement is weak (~10–100-fold), it may alter Raman frequencies, relative intensities, and if symmetry changes occur, may also cause the appearance or disappearance of (new) Raman bands.

3This is due to point 4, which does not hold for TERS. The many attempts to overcome this problem, e.g., to functionalize the surfaces (38–40), are based on a strategy being analogous to that of TERS: the target molecules are separated from the surfaces (in the case of TERS they are separated from the tip); then, only the EM enhancement is operating. Under such or analogous conditions, the prevailing electromagnetic enhancement mechanism acts equally on all molecules located in the field-enhancement zone and, then, SERS may become an analytical tool as well.
fields in the vicinity of a smooth surface by placing spheres or even a single sphere next to a flat metallic or semiconducting surface (46–49). Illumination from above should thereby create electromagnetic fields in the gap between the sphere and the flat surface that would be stronger than those on the sphere alone.

At approximately the same time, 1985, Wessel (50) proposed a similar but extended concept: combining the use of a single metal nanoparticle with a scanning tunneling microscope (STM) that could be rastered over a surface (Figure 1). The nanoparticle acts as an antenna for incident and reradiated fields. Wessel noted that his approach might be suitable for enhanced Raman, two-photon or second-harmonic spectroscopy, to detect even single molecules (50). Approximately 15 years later, Wessel’s idea was realized by the Zenobi group (1), but using an atomic force microscope (AFM) and a cantilever tip covered with an approximately 50-nm-thick Ag film; the authors showed TERS for C₆₀ and brilliant cresyl blue (BCB) molecules with a spatial resolution of <50 nm (1).

3. TIP-ENHANCED RAMAN SPECTROSCOPY

The scheme of TERS is straightforward. The crucial part is the combination of a scanning probe device with a Raman spectrograph, where the scanning probe device can be an AFM, a shear force microscope, or an STM. The experimental arrangement of the two parts permits illumination of tip and sample by an incident laser beam and recording of Raman scattering from the focal region. Figure 2 illustrates this scheme, depicting the excitation of surface plasmons that produce enhanced near-fields in the vicinity of the tip apex. If the tip is located close to the sample, the enhanced near-fields cause an enhanced Raman scattering. In effect, the metalized tip acts as an optical antenna that enhances both the incident and the emitted fields. Suitable tips are quite important: either an AFM tip covered with a thin layer of Ag or Au or an STM tip, usually made of a thin Ag or Au wire having a sharpened end. The shape and curvature of the tip end determines both the enhancement and the spatial resolution of TERS.

It was evident right from the start that TERS not only overcomes the above described limitations of SERS but also offers the possibility of moving toward single molecule detection, as predicted by Wessel (50). In fact, TERS—representing a single hot spot—exhibits an extreme sensitivity as well as a high spatial resolution, down to a few nanometers, thus permitting the
Figure 2
Tip-enhanced Raman spectroscopy. A laser light is focused on the scanning probe microscope (SPM) tip and sample. The minimum size of the focus is $\sim\lambda/2$. The near-field created in the vicinity of the tip causes the enhanced Raman scattering from a few molecules underneath the tip. The diameter of the near-field scattering zone is $\sim R_{\text{tip}}/2$, where $R_{\text{tip}}$ is the curvature of the tip end.

correlation of scanning probe microscope (SPM) images with TER images i.e., of topographic with chemical data. This advantage of TERS led to its widespread application in the fields of biology (51–56) and physical chemistry, including surface science and materials science (9, 57–65).

3.1. Early TERS Experiments

In the first four papers reporting on TERS, all four groups used a similar inverted microscope approach; three employed an AFM or shear force technique, the fourth an STM.

The first published paper on TERS was that of Stöckle et al. (1), who studied C$_{60}$ molecules and BCB molecules deposited as a thin layer on a glass support. The case of BCB is reproduced in Figure 3 showing two experimental conditions (insets) for the two Raman spectra A and B. In the first experiment (inset a), the glass support with the thin dye layer is illuminated via an inverted microscope (not shown), whereas the silverized AFM tip is kept in a retracted position. Raman scattering was recorded from dye molecules in the focal regime and plotted as spectrum A. The AFM tip was then moved into contact with the dye layer and into the center of the focus (inset b). The illumination not only caused the Raman scattering, as seen before, but also an enhanced Raman scattering arising from the few molecules that were underneath the tip and exposed to the enhanced near-field of the illuminated tip. For the enhanced Raman process, the authors estimated an enhancement factor of approximately $10^4$ (1).

Anderson (2) described a similar approach, using a Au-coated AFM cantilever tip to achieve enhanced Raman scattering from thin films of sulfur deposited on a quartz slide. A strong enhancement was seen only from the region of the illuminated tip (Figure 4, line A), whereas from a region 25 μm away, no Raman signal was detectable (Figure 4, line B). For the enhancement, Anderson gave a lower boundary of $10^4$ (2).
Figure 3
Tip-enhanced Raman spectra of brilliant cresyl blue dispersed on a glass support and measured with a silver-coated atomic force microscope probe. The two Raman spectra were measured with the tip retracted from the sample (inset a, line A) and the tip in contact with the sample (inset b, line B). Reprinted with permission from Reference 1. Copyright 2000 Elsevier Science B.V.

Hayazawa et al. (3) reported near-field Raman spectroscopy on rhodamine 6g (Rh6G) deposited at a Ag-island film, also using the inverted microscope approach and an AFM with a silicon cantilever covered with a 40-nm-thick Ag film. The thickness of the Ag-island film was varied, and the highest SERS signals were achieved for a film thickness between 8 and 10 nm. The preparation of the Rh6G led to Rh6G crystals of approximately 50-nm diameter on the Ag surface (3). In Figure 5, the far- and near-field spectra of Rh6G on the Ag-island film are reproduced, where the so-called far-field spectrum obviously represents the sum of far-field plus surface-enhanced Raman spectra (the underlying Ag film was rough). Also noteworthy is the fact that Hayazawa

Figure 4
Raman spectrum demonstrating gold-coated atomic force microscope tip causing a local surface-enhanced Raman effect (line A) on a sulfur film. When the beam is focused away from the tip on the film, the Raman signal (line B) is undetectable using the same microprobe parameters. Reprinted with permission from Reference 2. Copyright 2000 American Institute of Physics.
et al. (3) illuminated the sample prior to the experiment with an argon ion laser line of 488 nm, in order “to minimize photobleaching effects.” For these conditions, the authors estimated a 40-fold enhancement for TERS (3). However, they did not address the fact that observed SERS occurs in numerous so-called hot spots located anywhere in the focal area, but most likely not near or underneath the tip. Nevertheless, this comparison reveals an interesting circumstance: The single hot spot made by the tip-sample configuration is nearly as efficient in enhanced Raman scattering as the sum of all near-field configurations producing the SERS signal!

The approach of the Pettinger group (4) is similar to that of the first three papers as the authors also use the inverted microscope approach, but it differs in both the scanning-probe microscopy and the tip and sample preparation: An STM is combined with a Raman microscope, the tip is a Ag wire with a sharp end, and the sample is a monolayer of BCB molecules adsorbed on a very thin, smooth Au film evaporated on a glass slide. This metal film is only 12 nm thick and thus permits the inverted microscope approach by transmission of incident photons toward the tip and of the scattered photons back to the microscope. Figure 6 depicts this specific approach, whereas Figure 7 reproduces two spectra, one for the tip in retracted position and another for the tip in tunneling position. Again, these two curves and the corresponding insets refer to so-called far- or near-field spectra, which we have already encountered in the papers of Stockle et al. (1), Anderson (2), and Hayazawa et al. (3). Pettinger et al. (4) used a low incident laser power of 0.05 mW (633 nm) to prevent fast photobleaching and observed a contrast of 15, i.e., a 15-fold larger TERS intensity than resonance Raman scattering (RRS) intensity in the far-field spectrum.

3.2. On TERS Enhancements

To estimate the EM part of the enhancement in TERS, one needs the values of the following variables: (a) the intensity of the TERS peak (the near-field intensity produced by the molecules underneath the tip), (b) the far-field intensity, (c) the area and depth of the focus, and (d) the area and depth of the near-field contributing to TERS.

In general, the TERS enhancement reads as

$$F_{EM} = \left( \frac{I_{nf} + I_{ff}}{I_{ff}} - 1 \right) \frac{V_{ff}}{V_{nf}}.$$
Figure 6
Tip-enhancement of Raman scattering. A scanning tunneling microscope (STM) is used; the substrate for a monolayer of brilliant cresyl blue molecules is a thin, smooth gold layer with an approximately 12 nm thickness deposited on a glass slide. For the inverted microscope approach, the thin metal layer is sufficiently transparent for the incident and emitted light; in addition, it is conductive enough to be employed within an STM. The tip is thin silver wire etched to a sharp point. Reprinted with permission from Reference 4. Copyright 2000 Electrochemical Society of Japan. Abbreviation: TERS, tip-enhanced Raman microscopy.

where the first term in the bracket represents the (measurable) contrast, and \( V_{ff} \) and \( V_{nf} \) are the volumes probed by the far- and near-fields, respectively. When the tip is retracted from the sample, the measured Raman intensity is the so-called far-field intensity, \( I_{ff} \), arising from the focal volume \( V_f = \frac{R_{focus}^2}{4} \pi b_{ff} \), where \( R_{focus} \) and \( b_{ff} \) are the focal radius and the effective depth of the focus (from which Raman scattering is collected), respectively. On the other hand, when the tip is in proximity to the sample, the measured Raman intensity includes both the far-field and the near-field contributions, denoted as \( I_{nf} + I_{ff} \), where the near-field contributions arise from a rather small volume denoted as the TERS volume \( \left( \frac{1}{2} R_{tip} \right)^2 \pi b_{nf} \), with \( R_{tip} \) and \( b_{nf} \) being the tip radius and the effective height of the near-field, respectively. The factor 1/2 in front of \( R_{tip} \) is based on the approximation of \( R_{TERS} \approx \frac{1}{2} R_{tip} \) (66). In many experimental cases, the angle of incidence of the beam is nonzero (relative to the normal of the surface); thus, an elliptic shape of the focus has to be accounted for, i.e., with the term \( \cos \alpha \) because the elliptic shape affects the focal intensity acting on the tip, but it does not affect the recorded far-field intensity, because the lower intensity is compensated for by the larger number of molecules in the elliptic-shaped focus.

For a sufficiently thin layer of adsorbed/deposited species, one can approximate \( b_{ff} \approx b_{nf} \) and, thus, \( \frac{V_{ff}}{V_{nf}} \approx \frac{R_{focus}^2}{\frac{1}{2} R_{tip}^2} \). The TERS enhancement is then

\[
F_{EM} = \left( \frac{I_{nf} + I_{ff}}{I_{ff}} - 1 \right) \left( \frac{R_{focus}}{\frac{1}{2} R_{tip}} \right)^2 \cos \alpha.
\]

\(^8\)The underlying assumption is equal density and composition of matter in both cases.
For example, assuming the parameter values reported in (8): For a contrast of \((I_{fg} + I_{ff})/I_{ff} = 10,000\), \(R_{\text{far}} = 1,000\) nm, \(R_{\text{sp}} = 90\) nm, \(\alpha = 60\), the second term becomes \(\sim 500\), and the third is 1/2. In total, the overall TERS enhancement is then \(F_{\text{TERS}} \sim 2.5 \times 10^6\).³

Steidtner & Pettinger (10) reported large contrasts for the case of a 1/2 monolayer of BCB adsorbed at a single crystalline surface of Au and platinum; for the former, with BCB at Au(111), the reported contrast is 3,900; the radii of the Au tip and the circular focus were 15 nm and 150 nm, respectively; due to the circular shape of the focus, \(\cos \alpha = 1\); the overall TERS enhancement becomes \(F_{\text{TERS}} \sim 1.6 \times 10^6\). If the tip radius were slightly larger, say 20 nm, the TERS enhancement would drop to \(F_{\text{TERS}} \sim 8.8 \times 10^5\). For the second case, with BCB on Pt(111), the reported contrast is 1,390, and with the other terms as given above, the TERS enhancement is \(F_{\text{TERS}} \sim 5.6 \times 10^5\).

Obviously, the estimation of the overall TERS enhancement also depends on parameters that are not precisely measurable, e.g., tip radius, focus radius, depth of focus and of near-field, and the angle of incidence. Even small errors of 20–50% in these values can result in substantial over- or underestimation of the TERS enhancement. Even greater error can arise from inhomogeneities in composition, density, and thickness of molecular films in the focal region; the far-field spectrum

³In the paper cited, the third term was not considered, and \(R_{\text{TERS}} = R_{\text{sp}}\) was used instead of \(R_{\text{TERS}} = \frac{1}{2} R_{\text{sp}}\); thus, the TERS enhancement was estimated at \(F_{\text{TERS}} \sim 10^6\) (8).
represents an average over all local variations, whereas the near-field of the tip probes only a local element of the film.

**Figure 8** compares overall TERS enhancements together with the respective contrast reported in more than 20 papers. To facilitate comparison, the data (bottom and top axis) are presented in a logarithmic scale, and the results are color coded for TERS, contrast, and whether an AFM or STM was used. As mentioned above, contrast is the ratio of the TERS intensity and the (unenhanced) Raman intensity ($I_{TERS}/I_{Raman}$). The reported contrast ranges between 1.5 and approximately 10,000, whereas the underlying total enhancement can be several orders of magnitude larger. This comparison shows that AFM-based TERS often yields rather low contrasts of $<100$, which usually means a TERS enhancement of $F_{TERS} \leq 10^4$. Exceptions are few and owe chiefly to a large $V_{ff}/V_{nf}$. On the other hand, high contrasts in the range of 1,000–10,000 are reported only for STM-based TERS. For $V_{ff}/V_{nf}$ between 100–10,000, the underlying TERS enhancements span a range of $10^4 \sim 10^7$. For TERS enhancements of $\sim 10^6$ and higher, single-molecule measurements are feasible (12, 77).

### 3.3. Few-Molecule TERS

Large enhancement is a prerequisite for few- or single-molecule measurements and, consequently, a rather high stability of the sample/tip system is required under locally extreme incident intensities. For a better understanding of this issue, let us consider the case of an incident laser power of 1 mW and a sharp focus of approximately 300-nm diameter. For these parameters, the average intensity in the focal area becomes more than $1.4 \times 10^6$ W cm$^{-2}$. Reported TERS enhancements range from $\sim 10^1$ to $5 \times 10^6$. This corresponds to a local field enhancement of $g \sim 10$ to 250 and means the local intensity underneath the tip will rise by a factor of $\sim 100$ to $6 \times 10^3$; for the latter value, the local intensity may reach an incredible level of $>8 \times 10^{10}$ W cm$^{-2}$. Such high intensities provide continuously oscillating field strengths on the order of $>2.8$ V nm$^{-1}$. Can any molecule withstand such extreme field strengths?11

Evidently, extreme local intensities upon enhancement may become counterproductive; to account for associated disadvantages, one has to use a comparatively weak incident power of a few $\mu$W cm$^{-2}$ (or a sufficiently large focus or short acquisition time) to avoid photobleaching, photochemical processes, sample heating, or desorption of molecules.

A clear signature of a high enhancement is a large contrast (TERS intensity/Raman intensity, or $T/R$), or if there is no Raman scattering detectable when the tip is retracted, a large TERS signal-to-noise ratio ($S/N$). Again, assuming adsorption of approximately a monolayer of large molecules such as dyes and a TERS radius of approximately 10 nm (the tip radius is then 20 nm), there are approximately 300 molecules located within the TERS radius, and they make up the total TERS signal. The height of this signal may already allow estimation of whether or not single-molecule detection is possible. In order to achieve single-molecule TERS, a significantly lower molecular coverage is required. Yet, a substantial $T/R$ and $S/N$ are needed, with values preferably $>4$, to facilitate the use of the fingerprint character of vibrational spectra. For practical reasons,

---

10From the literature, only papers that report contrast as well as the radii of tip and focus are considered; due to space constraints, only 21 papers could be considered; actually, among 220 TERS papers published to date, only 35 papers address the TERS enhancement and provide the required information.

11Enhancements of $10^{14}$ or larger are also in discussion; with the above parameters, the local intensity should reach levels of $>10^{11}$ W cm$^{-2}$ (corresponding to field strengths of $>25$ V nm$^{-1}$) continuously acting on the substrate, molecules, and tip. Recently, Etchegoin et al. (36) reported maximal SERS enhancements of approximately ten orders of magnitude; thus, for TERS, maximal enhancements by approximately nine orders of magnitude seem to be more realistic and are not even necessary for single-molecule detection (12, 77).
Figure 8
Reported overall tip-enhanced Raman spectroscopy (TERS) enhancements together with each contrast. For an easy comparison, the data are presented in a logarithmic scale (same scale for bottom and top axis), and the results are color coded for TERS, contrast, and whether an atomic force microscope (AFM) or scanning tunneling microscope (STM) was used.
there is an additional preference: the recording of TER spectra in (sub)second time intervals. Substantially extended acquisition times may not be a suitable solution to a low-signal problem, because, before the end of the measurement time, the single molecule (eventually observed by SPM imaging and then addressed by the illuminated tip) may disappear from the high-field zone by diffusion, desorption, photobleaching, photochemistry, and the like. Certainly, the above mentioned diffusion and desorption problems can be overcome by employing either low temperatures or by fixing the molecule to a specific adsorption site via strong chemical bonds.

The results published by Steidtner & Pettinger (10, 12) on the first extension of TERS to a UHV system using BCB as an adsorbate (Figure 9) are illustrative. This dye is in optical resonance with the exciting helium-neon laser line at 632.8 nm. Thus, upon excitation resonance, Raman processes take place, possibly in combination with tip-enhanced Raman processes. For the experiments described in (10), the preparation procedures led to a surface coverage of approximately 1/2 of a monolayer of BCB adsorbed at atomically flat Au(111) samples; here, Steidtner & Pettinger observed a strong band at 570 cm\(^{-1}\), showing a TER peak intensity of approximately 7,800 cps, but for approximately 90 molecules located in the enhanced field zone. Thus, each BCB molecule

![Figure 9](image_url)

**Figure 9**

Scanning tunneling microscope images of five BCB molecules (a) and a single BCB molecule (c) adsorbed on Au(111), and the resonance Raman and TER spectra (b,d). The spectra were recorded with an integration time of 1 s; all measurements occurred under ultrahigh vacuum conditions. Adapted with permission from Reference 12. Copyright 2008 The American Physical Society.
contributed approximately 87 cps to the total signal. For the experiments described in (12), the coverage of BCB was so small that STM images of surface sections of approximately $12 \times 12 \text{ nm}^2$ showed only a few, or even a single, BCB molecule; the molecules seem to be fixed on defect sites. For the tip centered over the molecular group or over the single molecule, the TER intensities amounted to 410 cps for five BCB molecules, and to approximately 90 cps for a single BCB molecule. In the latter case, the spectra reveal an S/N of $90/20 > 4$. Thus, STM images and the TER spectrum indicate remarkable evidence for single-molecule TERS.

The advantages of the UHV-TERS approach are manifold: (a) monitoring and control of adsorption, (b) high surface quality, (c) minimization of impurities, (d) SPM imaging of small sections of surfaces covered with either a monolayer or a submonolayer of adsorbates, or even with well-separated, individual molecules, (e) significantly lower photodegradation of adsorbed molecules under UHV conditions.

The experiment discussed below highlights these UHV-TERS advantages. Here, advantage is taken of a preparation chamber added to the UHV-TERS system. It permits the sputtering and annealing of the substrates as well as the evaporation of molecules. Again, Au(111) surfaces were used as substrates. The UHV-based preparation of the sample leads to atomically smooth Au(111), and STM images show large (111)-oriented, monoatomic terraces and three domains of the Herringbone reconstruction, indicating the smoothness of the sample and minimal impurities.

Fullerene C$_{60}$ molecules were chosen as adsorbates and were evaporated in low doses onto the smooth Au(111) surface. STM images reveal that the deposited C$_{60}$ molecules form small islands of approximately 40-nm diameter, preferentially aligned along monoatomic steps; whereas, the major part of the surface is free of C$_{60}$ and still exhibits the characteristic Herringbone reconstruction. In Figure 10, the inset exhibits a $15.5 \times 15.5 \text{ nm}^2$ region of such an island, where the C$_{60}$ molecules assume a hexagonal structure (84).

Most interesting are the TER spectra of such islands: A small island contains roughly 1,200 C$_{60}$ molecules; for a sharp tip ($R_{tip} \sim 20 \text{nm}$), the TERS region (i.e., the region of the enhanced field zone) has a diameter of $\sim 20 \text{ nm}$; therefore, within this region, only 300 C$_{60}$ molecules are present and only these contribute to TERS. After retraction of the tip, no Raman signal can be detected. Figure 10 shows TER spectra from a C$_{60}$ island. Because of the low incident laser power used ($P_L = 0.1 \text{ mW}$) and the short acquisition time (0.5 s), the TER signal is relatively low, and consequently, the spectral curves show substantial noise. However, as a spectrum is recorded every 0.5 s over a time interval of more than 50 s in this experiment, over 100 spectra are available for analysis. Instead of simple smoothing, eight groups of 16 successive spectra were averaged, where for each group the starting spectrum number is increased by ten; i.e., the first average spectrum covers the time range from the 0th second to the 8th second; the second average spectrum, from the 5th second to the 13th second; and so on. Although this procedure reduces the noise, it also averages away possible short time dependencies of the individual Raman bands, at least to some extent. Nevertheless, over the whole time period, each average spectrum deviates from the previous one in characteristic ways: The intensity drops in general; the relative intensities alter significantly; and frequencies shift to some extent. Nonetheless, all the individual >100 spectra and their various averages taken from this set of spectra display the general pattern of C$_{60}$ TERS.

The above described spectral variations indicate that remarkable changes within the C$_{60}$ island occur during TERS measurements. Indeed, STM images before and after TERS measurement show the same island, but with altered composition. Possible causes are increased diffusion induced by the tip, which is common at room temperature; structural changes may also be caused by the enhanced incident field, photodesorption, photochemistry, or even photodegradation of the molecules. Connected with this are changes in the local adsorption sites and lateral interactions between the C$_{60}$ molecules, and all this may lead to the observed spectral variations in TERS.
Tip-enhanced Raman spectra on a C$_{60}$ island at Au(111). The spectra are subsequently recorded every 0.5 s and grouped into eight sets of averaged spectra (each over 16 original spectra) with a time delay for each group from the former of 5 s. Incident laser power is 0.1 mW, $\lambda_{ex} = 632.8$ nm. The color code for the eight spectra indicates time dependence. (Inset) Scanning tunneling microscope image of a C$_{60}$ island on a Au(111) surface across a monoatomic Au step. A scheme of (111)-oriented C$_{60}$ molecules is overlayed in the top region of the image analogous to Tang et al. (80, figure 7b). Abbreviation: TERS, tip-enhanced Raman spectroscopy.

In 2010, Luo et al. (81) published a paper concerning single-molecule SERS (SM-SERS) for C$_{60}$ fullerenes at Au nanoparticles. The authors observed spectral variations for distinct hot spots as well as spectral fluctuations with time and ascribed these effects to the single-molecule behavior; whereas, the appearance of new bands and band splitting were attributed to the symmetry reduction of C$_{60}$ upon adsorption. TERS measurements on C$_{60}$ islands, described here, show spectral patterns that closely resemble the SM-SERS spectra reported by Luo et al. (81).

Table 1 illustrates these findings, comparing data from Luo et al. (81) (columns 1–3) and data from Ikeda & Uosaki (83) (column 5), with UHV-TERS data (columns 6 and 7) and with frequencies and symmetry assignment from Menéndez & Page (82, table 2.4) (columns 8 and 9). Columns 1–3 look at SM-SERS on three different hot spots [note that our tip–C$_{60}$/Au(111) configuration (data are shown in column 7, whereas column 6 indicate whether a peak or a shoulder is observed] essentially represents a single hot spot]. Column 4 is the symmetry assignment given by Luo et al. Note that there are some discrepancies in the assignment, which will not be discussed.
### Table 1  Raman shifts for an island of fullerene $C_{60}$ molecules deposited on a Au(111) surface

<table>
<thead>
<tr>
<th>SM-SERS of $C_{60}$ at different hot spots$^a$</th>
<th>Resonant Raman at 785 nm$^b$</th>
<th>UHV-TERS at $C_{60}$ islands, 633 nm$^{c,e}$</th>
<th>Frequencies of isolated $C_{60}$ molecules$^d$</th>
<th>Symmetry$^d,f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>785 nm$^a$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>254</td>
<td>268</td>
<td>273</td>
<td>$H_g(1)$</td>
<td>$p$</td>
</tr>
<tr>
<td>269</td>
<td>309</td>
<td>308</td>
<td></td>
<td></td>
</tr>
<tr>
<td>344</td>
<td>341</td>
<td>347</td>
<td>$T_{2u}(1)$</td>
<td>$p$</td>
</tr>
<tr>
<td>381</td>
<td>388</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>402</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>425</td>
<td>425</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>491</td>
<td>492</td>
<td>495</td>
<td>$A_g(1)$</td>
<td>$p$</td>
</tr>
<tr>
<td>517</td>
<td>521</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>544</td>
<td>565</td>
<td>561</td>
<td>$T_{1u}(2)$</td>
<td>$p$</td>
</tr>
<tr>
<td>612</td>
<td>597</td>
<td>602</td>
<td></td>
<td></td>
</tr>
<tr>
<td>633</td>
<td></td>
<td>620</td>
<td></td>
<td></td>
</tr>
<tr>
<td>659</td>
<td>661</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>686</td>
<td>678</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>704</td>
<td>704</td>
<td>707</td>
<td></td>
<td></td>
</tr>
<tr>
<td>734</td>
<td>748</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>770</td>
<td>770</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>797</td>
<td>784</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>825</td>
<td>832</td>
<td>827</td>
<td></td>
<td></td>
</tr>
<tr>
<td>870</td>
<td>875</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>886</td>
<td>888</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>934</td>
<td>929</td>
<td>932</td>
<td>$T_{1g}(1)$</td>
<td></td>
</tr>
<tr>
<td>961</td>
<td>961</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>981</td>
<td>985</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>999</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,031</td>
<td>1,026</td>
<td>1,032</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,071</td>
<td>1,081</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,093</td>
<td>1,100</td>
<td>1,116</td>
<td></td>
<td>$p$</td>
</tr>
<tr>
<td>1,136</td>
<td>1,147</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,180</td>
<td>1,187</td>
<td>1,160</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,209</td>
<td>1,196</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Continued)
Table 1 (Continued)

<table>
<thead>
<tr>
<th>SM-SERS of C60 at different hot spots&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Resonant Raman at 785 nm&lt;sup&gt;b&lt;/sup&gt;</th>
<th>UHV-TERS at C60 islands, 633 nm&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Frequencies of isolated C60 molecules&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Symmetry&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,223</td>
<td>1,229</td>
<td>s</td>
<td>1,222</td>
<td>Hg(6)</td>
</tr>
<tr>
<td>1,244</td>
<td>1,250</td>
<td>p</td>
<td>1,240</td>
<td>Hg(6)</td>
</tr>
<tr>
<td>1,269</td>
<td>1,264</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,289</td>
<td>1,292</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,319</td>
<td>1,332</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,344</td>
<td>T&lt;sub&gt;1g&lt;/sub&gt;</td>
<td>1,332</td>
<td>Hg(6)</td>
</tr>
<tr>
<td></td>
<td>1,386</td>
<td></td>
<td>s</td>
<td>1,364</td>
</tr>
<tr>
<td>1,419</td>
<td>1,428</td>
<td>1,430</td>
<td>1,426</td>
<td>Hg(7)</td>
</tr>
<tr>
<td>1,419</td>
<td>1,428</td>
<td>1,430</td>
<td>T&lt;sub&gt;1u&lt;/sub&gt;(4)</td>
<td></td>
</tr>
<tr>
<td>1,464</td>
<td>1,462</td>
<td>A&lt;sub&gt;g&lt;/sub&gt;(2)</td>
<td>1,468</td>
<td></td>
</tr>
<tr>
<td>1,497</td>
<td>1,498</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,538</td>
<td>1,564</td>
<td>1,552</td>
<td>1,543</td>
<td>Hg(7)</td>
</tr>
<tr>
<td>1,585</td>
<td>1,604</td>
<td>1,582</td>
<td>s</td>
<td>1,574</td>
</tr>
<tr>
<td></td>
<td>1,574</td>
<td></td>
<td>p</td>
<td>1,562</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data adapted with permission from Reference 81. Copyright (2010) Wiley & Sons Ltd.

<sup>b</sup>Data adapted with permission from Reference 83. Copyright (2008) American Chemical Society.

<sup>c</sup>Data from this work.

<sup>d</sup>Data taken with kind permission from Reference 82. Copyright (2000) Springer Science+Business Media.

<sup>e</sup>Bold and nonbold items indicate clearly visible and very weak bands or shoulders, respectively.

<sup>f</sup>The ten Raman active modes are indicated by eight light-green (Hg symmetry) and two light-blue (A<sub>g</sub> symmetry) rows; the four infrared active modes (T<sub>1u</sub> symmetry) are indicated by four light-red rows. Assignment in the first through fifth column is according to Reference 81. Assignment in the sixth through ninth column is according to Reference 82.

Abbreviations: p, peak; s, shoulder; SM-SERS, single-molecule–surface-enhanced Raman spectroscopy; UHV-TERS, ultrahigh vacuum-tip-enhanced Raman spectroscopy.

Here, Column 5 shows the ten allowed Raman frequencies for a C<sub>60</sub> film observed with a 785-nm exciting line, reported by Ikeda & Uosaki (83). The UHV-TERS data (column 7) are taken with the 633-nm line of a helium-neon laser, using 0.1 mW incident power (84). Most bands differ in frequency by only a few wavenumbers from the results of Luo et al. as well as from Menéndez et al. Thus, for the assignment, we make use of the data of Menéndez et al., which are only strictly valid for isolated C<sub>60</sub> molecules having I<sub>h</sub> symmetry. For better readability, colors are added to 14 rows assigned either with Hg symmetry (8 green rows), A<sub>g</sub> symmetry (2 blue rows), or with T<sub>1u</sub> (4 light-red rows) indicating ten Raman active and 4 infrared active modes, respectively; the fact that more than 30 vibrational modes are seen in TERS of C<sub>60</sub> molecules indicates a significant reduction in symmetry due to the adsorption and formation of C<sub>60</sub> islands at an Au(111) surface. A detailed discussion of the spectral assignments and the influence of symmetry reduction (by adsorption and/or by formation of a two-dimensional hexagonal structure) is beyond the scope of this review. Further UHV-TER experiments on freshly prepared C<sub>60</sub> islands also yielded remarkable spectral fluctuations in time, showing slightly different spectral patterns. These experiments indicate a remarkable variability of C<sub>60</sub> islands in structure, adsorption, and lateral interactions (84).
Often, spectral fluctuations, similar to those obtained with UHV-TERS or even more severe, have been considered as the proof for single-molecule SERS or single-molecule TERS, whereas ensemble-averaged SERS or TERS is believed to show no spectral fluctuations. The above described experiment provides additional information: STM images from the investigated region showing hundreds of relatively ordered C$_{60}$ molecules in an island. Yet, such an ensemble of molecules also shows significant spectral fluctuations. These observations rule out the idea that spectral fluctuations necessarily point to single-molecule events. Without doubt, these preliminary results require further detailed investigation into the causes of the observed spectral variation. Corresponding experiments are on the way.

4. CONCLUSION

TERS overcomes most of the drawbacks of SERS but keeps its advantages, such as its high sensitivity. In addition, TERS provides a very high spatial resolution, much beyond the Abbe limit. TERS permits the correlation of topographic and chemical data, finding application in a number of fields, such as surface science, material science, and biology. Single-molecule TERS has been observed even for TER enhancements of “only” $10^6$ to $10^7$. A condensed overview concerning measured contrasts and estimated total enhancements and a discussion reveal that an extreme enhancement $\gg 10^7$ in combination with a high laser power is probably counterproductive as its associated local intensity may affect or even destroy adsorbed molecules. Finally, preliminary results of UHV-TERS on a C$_{60}$ island were presented; these islands have a diameter of a few tens of nanometers and are deposited on a smooth Au(111) surface. Most striking is TERS’ significant time dependence for C$_{60}$, not so much in frequencies but in general intensity and in relative band intensities as this can point to structural, adsorptive, and chemical changes within the C$_{60}$ adlayer. The remarkable variability of C$_{60}$ TER spectra makes this system an interesting laboratory for molecule-substrate and intermolecular interactions.

SUMMARY POINTS

1. SERS focuses on the concept of a few hot spots that make the most of the signal and that in a sense led to TERS.
2. TERS is based on a single hot spot, a narrow gap between the tip and sample, but avoids the drawbacks of SERS.
3. The presentation of some early TERS experiments shows TERS for a variety of systems and large variations in contrast and underlying enhancement.
4. A comparison of contrast and overall enhancement data for TERS based on AFM, shear force, and STM reveals large differences in measured contrast as well as in the estimation of underlying enhancements.
5. A moderate enhancement of “only” $\sim 10^6$ is sufficient to achieve few-molecule TERS. Significantly higher enhancements may cause photodegradation.
6. UHV-TERS combines the advantages of a UHV system with those of TERS. Vibrational spectroscopy studies on a single or a few molecules on otherwise clean surfaces become achievable. This high sensitivity of TERS may lead to its application in the field of heterogeneous catalysis.
DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Prof. M. Wolf for his scientific advice and generous support of this UHV-TERS project and Prof. H.J. Freund for his very valuable support as our host. The authors also gratefully acknowledge W. Mahdi for carefully reading the manuscript and S. Kubala, D. Bauer, S. Hagen, and K.P. Vogelsang for their substantial help in upgrading the UHV-TERS system.

LITERATURE CITED


36. Limitation of SERS enhancement factor to $<10^4$. 

22. First hot-spot concept for giant SERS.
Contents

Membrane Protein Structure and Dynamics from NMR Spectroscopy
   Mei Hong, Yuan Zhang, and Fanghao Hu ................................................................. 1

The Polymer/Colloid Duality of Microgel Suspensions
   L. Andrew Lyon and Alberto Fernandez-Nieves ....................................................... 25

Relativistic Effects in Chemistry: More Common Than You Thought
   Pekka Pyykkö ........................................................................................................... 45

Single-Molecule Surface-Enhanced Raman Spectroscopy
   Eric C. Le Ru and Pablo G. Etchegoin ................................................................. 65

Singlet Nuclear Magnetic Resonance
   Malcolm H. Levitt ..................................................................................................... 89

Environmental Chemistry at Vapor/Water Interfaces: Insights from
   Vibrational Sum Frequency Generation Spectroscopy
   Aaron M. Jubb, Wei Hua, and Heather C. Allen ...................................................... 107

Extensivity of Energy and Electronic and Vibrational Structure
   Methods for Crystals
   So Hirata, Murat Keçeli, Yu-ya Ohnishi, Olaseni Sode, and Kiyoshi Yagi ................. 131

The Physical Chemistry of Mass-Independent Isotope Effects and
   Their Observation in Nature
   Mark H. Thiemens, Subrata Chakraborty, and Gerardo Dominguez ...................... 155

Computational Studies of Pressure, Temperature, and Surface Effects
   on the Structure and Thermodynamics of Confined Water
   N. Giovambattista, P.J. Rossky, and P.G. Debenedetti ............................................ 179

Orthogonal Intermolecular Interactions of CO Molecules on a
   One-Dimensional Substrate
   Min Feng, Chungzei Lin, Jin Zhao, and Hrvoje Petek ............................................. 201

Visualizing Cell Architecture and Molecular Location Using Soft
   X-Ray Tomography and Correlated Cryo-Light Microscopy
   Gerry McDermott, Mark A. Le Gros, and Carolyn A. Larabell ............................. 225
Deterministic Assembly of Functional Nanostructures Using Nonuniform Electric Fields
Benjamin D. Smith, Theresa S. Mayer, and Christine D. Keating ........................................... 241

Model Catalysts: Simulating the Complexities of Heterogeneous Catalysts
Feng Gao and D. Wayne Goodman ................................................................. 265

Progress in Time-Dependent Density-Functional Theory
M. E. Casida and M. Huix-Rotllant ................................................................. 287

Role of Conical Intersections in Molecular Spectroscopy and Photoinduced Chemical Dynamics
Wolfgang Domcke and David R. Yarkony ..................................................... 325

Nonlinear Light Scattering and Spectroscopy of Particles and Droplets in Liquids
Sylvie Roke and Grazia Gonella ................................................................. 353

Tip-Enhanced Raman Spectroscopy: Near-Fields Acting on a Few Molecules
Bruno Pettinger, Philip Schambach, Carlos J. Villagómez, and Nicola Scott .............. 379

Progress in Modeling of Ion Effects at the Vapor/Water Interface
Roland R. Netz and Dominik Horinek ............................................................. 401

DEER Distance Measurements on Proteins
Gunnar Jeschke ................................................................. 419

Attosecond Science: Recent Highlights and Future Trends
Lukas Gallmann, Claudio Cirelli, and Ursula Keller .......................................... 447

Chemistry and Composition of Atmospheric Aerosol Particles
Charles E. Kolb and Douglas R. Worsnop ..................................................... 471

Advanced Nanoemulsions
Michael M. Fryd and Thomas G. Mason ..................................................... 493

Live-Cell Super-Resolution Imaging with Synthetic Fluorophores
Sebastian van de Linde, Mike Heilemann, and Markus Sauer .................................. 519

Photochemical and Photoelectrochemical Reduction of CO₂
Bhupendra Kumar, Mark Llorente, Jesse Froehlich, Tram Dang, Aaron Satbrum, and Clifford P. Kubiak .................................................. 541

Neurotrophin Signaling via Long-Distance Axonal Transport
Praveen D. Chowdary, Dung L. Che, and Bianxiao Cui ........................................... 571

Photophysics of Fluorescent Probes for Single-Molecule Biophysics and Super-Resolution Imaging
Taekjip Ha and Philip Tinnefeld ................................................................. 595
Ultrathin Oxide Films on Metal Supports:
Structure-Reactivity Relations
S. Shaikbutdinov and H.-J. Freund ........................................... 619

Free-Electron Lasers: New Avenues in Molecular Physics and
Photochemistry
Joachim Ullrich, Artem Rudenko, and Robert Moshammer .................. 635

Dipolar Recoupling in Magic Angle Spinning Solid-State Nuclear
Magnetic Resonance
Gaël De Paepe ................................................................. 661

Indexes
Cumulative Index of Contributing Authors, Volumes 59–63 ...................... 685
Cumulative Index of Chapter Titles, Volumes 59–63 ............................. 688

Errata
An online log of corrections to Annual Review of Physical Chemistry chapters (if any, 1997 to the present) may be found at http://physchem.AnnualReviews.org/errata.shtml