The information content in single-molecule Raman nanoscopy

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ABSTRACT
It is now possible to establish the chemical identity of a substance at the ultimate detection limit of a single molecule, i.e. the sensitivity required to probe 1.66 yoctomoles (1/NA), using surface-enhanced Raman scattering (SERS). It is also possible to image within an individual molecule, all while retaining chemical selectivity, using tip-enhanced Raman scattering (TERS). The potential applications of ultrasensitive SERS and TERS in chemical and biological detection and imaging are evident, and have attracted significant attention over the past decade. Rather than focusing on conventional single/few-molecule SERS and TERS experiments, where the objective is ultrasensitive spectroscopy and nanoscale chemical imaging, herein we consider greatly informative, yet significantly underrated, signatures of single molecules. Namely, we review recent efforts ultimately aimed at probing different aspects of a molecule's local environment through a detailed analysis of its SERS and TERS spectra and images. Particular attention is devoted to local electric field imaging using TERS; we describe how the vector components and absolute magnitude of the local electric field may be inferred from molecular Raman signatures. We also propose experiments that can potentially be used to cross-check the insights gained from the described SERS and TERS measurements. The ultimate goal of this review is to demonstrate that there is much more to single-molecule Raman scattering than mere ultrasensitive chemical analysis.

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1. Introduction

1.1. Brief overview of the scope of this review

The dynamic interaction between radiation fields and molecular polarizabilities leads to Raman scattering (RS). Surface-enhanced Raman scattering (SERS) [1–4] is a variant of RS in which the driving fields are typically light waves trapped at the surface of metal nanostructures in the form of localized surface plasmons (LSPs). The effect is particularly evident in interacting plasmonic nanostructures, where the plasmonic modes of individual particles hybridize to form new collective modes in the assembly [5–7]. Confined and enhanced local electric fields at plasmonic nanojunctions formed between interacting metal nanostructures rendered SERS particularly popular, ever since the possibility of single molecule detection sensitivity in such constructs was demonstrated [8–11]. A closely related technique is tip-enhanced Raman scattering (TERS) [11–14]. TERS is made possible through a combination of scanning probe microscopy and RS. As such, this technique inherently possesses the spatial resolution attainable using scanning probe techniques (herein using atomic force microscopy, AFM) as well as the chemical selectivity and sensitivity of LSP-enhanced RS. The strong localization of optical fields at the apices of metallic tips [15,16] affords single molecule sensitivity [17, 18], but now with spatial resolutions conventionally on the order 10–20 nm, significantly finer than the diffraction limit of light. Important milestones in the field of ultrasensitive nanoscopy have been reached in the past few years, including (i) imaging within a single molecule (sub-nm resolution) using TERS,[19] and (ii) the first real-time observation of vibrational wavepacket motion on single molecules through surface-enhanced coherent anti-Stokes RS [20]. These significant advances, however, will not be discussed herein. Rather,
we will be mostly concerned with ongoing efforts ultimately aimed at using the Raman signatures of a single molecule to probe various aspects of its unique local environment.

### 1.2. A few remarks on accessing the single-molecule SERS and TERS regime

Pioneering SERS works were based on the ultralow concentration approach to explore the single molecule realm [8,9]. Namely, the samples used featured 1 molecular reporter (or less, on average) per effective SERS probing volume, defined by a high numerical aperture microscope objective. This approach was contested over the years, particularly for colloidal measurements, where several factors come into play in the interpretation of fluctuating Raman signatures [10]. Even in the case of solid or dried plasmonic substrates, well characterized using high-resolution microscopic techniques such as transmission electron microscopy (TEM), typical sample preparation methods introduce further complexity to the problem. Specifically, SERS and TERS samples are conventionally prepared by (i) mixing plasmonic particles with dilute solutions of molecular reporters or analytes, (ii) drop/spin casting dilute analyte solutions onto solid/dried plasmonic substrates, or (iii) incubating plasmonic substrates in dilute analyte solutions and allowing time for adsorption/chemisorption to take place. In all (i)–(iii), the exact location and number of probed molecule(s) in the effective SERS (and TERS) sampling area vary for different plasmonic substrates/molecular SERS reporters, and thus, are overall ill determined. For instance, factors such as preferable adsorption/chemisorption of analytes at specific sites of metal substrates are not easily discernible. In this regard, isolated engineered prototypical dumbbell antennae, defined by an aromatic dithiol at the junction formed between two plasmonic nanospheres and prepared through (i) are informative [21,22]. Indeed, such constructs have been recently used to explore some of the underlying physics in single-molecule Raman spectroscopy, based on prominent spectral fluctuations in Raman spectral sequences, *vide infra*. In the following sections, we highlight a promising alternative for substrate preparation for single-molecule SERS and TERS experiments.

Although much evidence supports it, the inference of single molecule sensitivity in ambient SERS and TERS is not trivial. The origin of spectral fluctuations in few/single-molecule Raman spectral sequences is still a subject of debate, four decades after the first observation of SERS [22,23]. Even the original observation [9] of quantized probability distributions corresponding to 0, 1, 2, and 3 molecules in the SERS probing volume was later challenged on the basis of inadequate statistical sampling [10]. More recent works [22,24,25] have associated intensity fluctuations in nominally *non-resonant* SERS and TERS trajectories with molecular reorientation dynamics at plasmonic nanojunctions. Namely, the removal of orientational averaging in the single molecule realm exposes the tensorial nature of RS. As such, the relative intensities of the observable vibrational Eigenstates
were used to gage the orientation of a single molecule relative to vector components of the local electric field at a given time [22,24,25]. In this context, the use of non-resonant SERS and TERS molecular reporters turns into a comparative advantage, as technical (photobleaching, photochemistry, resonant heating, etc.) and theoretical challenges [23] arise in interpreting surface- and tip-enhanced resonant RS. Tensorial RS from a single molecule constitutes the starting point of our discussion.

2. Single-molecule RS: theory

2.1. Theoretical framework

All of the calculations reported in this work were performed using the methodologies implemented in Gaussian 09 [26] and NWChem 6.5. [27] In general, using any quantum chemistry software package to compute the Raman spectrum of a molecule defaults the user to an orientationally averaged simulation. Namely, standard Euler-averaged RS activities are evaluated near the ground state minimum according to [28]

\[ S_n = 45\alpha^2_n + 7\gamma^2_n \]  

(1)

in which \( \alpha^2_n \) and \( \gamma^2_n \) are the spherical and anisotropic parts of the molecular polarizability derivative tensors given by

\[ \alpha^i_n = \frac{1}{3} \left( \frac{\partial \alpha_{xx}}{\partial Q_n} + \frac{\partial \alpha_{yy}}{\partial Q_n} + \frac{\partial \alpha_{zz}}{\partial Q_n} \right) \]  

(2)

\[ \gamma^2_n = \frac{1}{2} \left( \frac{\partial \alpha_{xx}}{\partial Q_n} - \frac{\partial \alpha_{yy}}{\partial Q_n} \right)^2 + \frac{1}{2} \left( \frac{\partial \alpha_{yy}}{\partial Q_n} - \frac{\partial \alpha_{zz}}{\partial Q_n} \right)^2 + \frac{1}{2} \left( \frac{\partial \alpha_{zz}}{\partial Q_n} - \frac{\partial \alpha_{xx}}{\partial Q_n} \right)^2 + 3 \left( \frac{\partial \alpha_{xy}}{\partial Q_n} \right)^2 + 3 \left( \frac{\partial \alpha_{yz}}{\partial Q_n} \right)^2 + 3 \left( \frac{\partial \alpha_{zx}}{\partial Q_n} \right)^2 \]  

(3)

where \( \alpha_{ij} \) are diagonal \((i = j)\) and off-diagonal \((i \neq j)\) molecular polarizability derivative tensor elements and \(Q_n\) are the coordinates for the \(n\)th vibrational mode. Note that equation (1) assumes that (i) the measurement is performed using the right-angle scattering geometry, (ii) the polarization axis of the incident radiation is perpendicular to the scattering plane, and (iii) the scattered light is collected without an analyser. To compare the computed RS activities with experimental intensities, a useful quantity is the differential RS cross-section, \( \frac{d\sigma}{d\Omega} \), defined in terms of \( S_n \) as [29]

\[ \frac{d\sigma}{d\Omega} = \frac{(2\pi)^4}{45} \frac{\hbar c^4}{8\pi^2 c_0^3} \frac{S_n}{1 - e^{-\left( \frac{\hbar \omega_n}{kT} \right)}} \]  

(4)
in which \( \omega_s \) is the scattered frequency in \( \text{cm}^{-1} \), \( \omega_n \) is the frequency of the \( n \)th vibrational mode in \( \text{cm}^{-1} \), and the calculated \( S_i \) is typically in \( \text{A}^4 \text{amu}^{-1} \), governed by Equations (1)–(3). In the realm of single molecules, orientational averaging according to Equations (1)–(3) is no longer appropriate [22, 24, 25, 30]. In this case, the scattering tensor that governs Raman activity may be expressed as

\[
S_n^2 = \sum_{n} \left| E_{i}^{L} \alpha'_n (\Omega) E_{i}^{S} \right|^2
\]

(5)

where \( E_{i}^{L} \) are the enhanced incident and scattered local electric fields, \( \alpha'_n \) is the molecular polarizability derivative tensor of the \( n \)th vibrational Eigenstate, and \( \Omega = \alpha, \beta, \gamma \) are the Euler angles which determine molecular orientation relative to the local electric fields. This formalism stresses that a single molecule assumes a unique orientation with respect to vector components of the local electric field. In other words, removal of orientational averaging yields a different set of relative intensities for every unique orientation of a single molecule with respect to \( E_{i}^{L} \). If the field components are known (ideally measured), the relative intensities of the observable vibrational states may be used to deduce the 3D orientation of a single molecule [22, 24, 25]. This requires knowledge of \( \alpha'_n \), which can be somewhat reliably computed – at least in the limit of weak molecule–(plasmonic) metal interactions, \textit{vide infra}. Alternatively, if the molecular orientation is known, the vector components of the localized electric field can be inferred from single-molecule SERS and TERS spectra. Overall, this framework is flexible enough to allow for (i) the inclusion of meaningful electric field components, e.g. from finite-difference time domain (FDTD) simulations, (ii) the expansion of molecular polarizability and the inclusion of higher order terms, e.g. at plasmonic nanojunctions where electric field gradients are operative and may lead to optical activity [22], and (iii) the use of molecular polarizabilities of a molecule chemisorbed/physisorbed onto silver nanoclusters/slabs, and simulated using the electronic structure method of choice [24, 25, 30]. That said, there are limitations to this formalism. For instance, the assumption that molecular polarizabilities which govern RS activities are invariant to molecular (re)orientation with respect to a metal substrate does not hold in some cases, two of which have been noted in recent reports from our group [31, 32].

### 2.2. A numerical example

Figure 1 shows a numerical example that illustrates the concept of tensorial RS from a single molecular reporter [24]. Note that the model system used herein, 1,3-propanedithiol (PDT), has been carefully selected for illustrative purposes. Typically, the large polarizability associated with \( \pi \)-conjugated electrons in SERS and TERS reporters (i.e. functionalized aromatic molecules) results in \( \alpha'_{xx} \) components which largely predominate over \( \alpha'_{yy}, \alpha'_{zz}, \) and \( \alpha'_{ij} (i \neq j) \). In this case study,
however, elements of the spherical part of the computed $\alpha'_n$ tensor for PDT are comparable in magnitude. As such, PDT is a relatively fine gage of the incident and scattered electric fields; small rotations of $\alpha'_n$ result in significant changes in the simulated single molecule spectrum. The standard orientationally averaged Raman spectrum for a solvated PDT molecule is simulated according to Equation (1) and shown in Figure 1(A). Molecular orientation-dependent Raman spectra for (i) incident and scattered electric fields in the x-direction in the laboratory frame (defined in the inset of Figure 1(D)), and (ii) molecular rotation about the z-axis of the same frame are shown in Figures 1(B) and (C). The stark contrast between the spectral cuts taken at three different rotation angles ($\theta_z = 0, 45, 90^\circ$) and the orientationally averaged spectrum (Figure 1(A)) is the principle concept behind this illustration. The same effect is noticeable in Figure 1(D), where the relative...
The Raman spectra of an oriented single molecule are markedly different from their ensemble averaged analogues. In the following, we demonstrate that in practice, spectral evolution both in terms of changes in the relative intensities of the observable states and the gradual appearance/disappearance of the signatures of different vibrational eigenstates throughout the course of SERS and TERS spectral sequences may be rationalized on this basis.

3. Single–few molecule RS: practical examples

3.1. Single-molecule SERS spectroscopy

We recently designed and characterized a large-scale SERS substrate [33], consisting of a self-assembled 2D array of Ag nanospheres, with an average particle
diameter/interparticle separation distance of 9/3.7 nm. The structures of the individual particles and their assemblies were characterized using TEM, see Figure 2A. The plasmonic response of the nanoparticle network was gaged using multi-photon photoemission electron microscopy (MP-PEEM). TEM and MP-PEEM statistics revealed the topography and plasmonic response of the network to be homogeneous on a length scale of tens of microns. This translated into a uniform SERS response from biphenyl-4,4′-dithiol (BPDT) molecules adsorbed onto different sites of the nanoparticle network. FDTD simulations revealed that SERS enhancement factors exceeding $10^6$ are attainable at the nanogaps formed between the Ag nanospheres in the 2D array. Combined with modest chemical enhancement factors attainable to aromatic dithiols at plasmonic nanojunctions [21, 22], this uniform substrate is potentially capable of reproducibly broadcasting the Raman signatures of individual molecules.

In a follow-up report,[34] we do not rely on mixing, spin/drop casting, or incubation to functionalize the above-described plasmonic substrate with SERS reporters, for the reasons outlined in Section 1.2. Rather, we chemically functionalize the 2D array of silver nanospheres with 4,4′-dimercaptostilbene (DMS) using ambient electrospray deposition (ESD).[35–37] We find that ESD produces a uniform distribution of intact DMS molecules and affords superior control over the number of molecules within the effective SERS probing area. Specifically, molecular coverage is systematically varied from $3.8 \times 10^3$ to $3.8 \times 10^5 \times 0.38$ molecules/μm$^2$ using ESD of ethanolic DMS solutions. When compared to the ensemble averaged measurements, hundreds of spectra recorded from different regions of the substrate containing less than one DMS molecule per effective SERS probing area (on average) exhibit similar trends: (i) increased ratios of the predominant aromatic C=C stretching (1575 cm$^{-1}$) to the vinyl C=C stretching (1625 cm$^{-1}$) vibrations, and (ii) increased ratios of the C–S stretching (1085 cm$^{-1}$) to the aromatic C–H in-plane rocking (1186 cm$^{-1}$) vibrations, see Figures 2(B) and (D). Whereas the simulated orientationally averaged Raman spectrum reproduces the features observed in the measured ensemble averaged spectra, both are distinct from the recorded single-molecule SERS spectra, see Figure 2(E). Using the relative intensities of the vibrational SERS signatures of a single DMS molecule, we derived a unique orientation of that molecule at a nanojunction formed between two silver nanospheres in the 2D array [34]. The molecule was found lying nearly flat near the surface of a spherical silver nanoparticle.

### 3.2. Few molecule TERS spectral sequences

In Figure 3, we illustrate how time- and frequency-dependent intensity variations in sequentially recorded TERS point spectra can be attributed to slow molecular reorientation dynamics [25]. A schematic diagram of our experimental setup is shown in Figure 3(A). The TERS spectra shown in Figures 3(B) and (C) were recorded by taking advantage of electric field enhancement at a nanojunction.
formed between a silver AFM tip and a corrugated silver surface coated with BPDT molecules. The temporal evolution of TERS intensities at select vibrational resonances of BPDT are shown in Figure 3(C). Of several TERS spectral sequences analyzed in the original report, we select the one shown in Figure 3(C) – which is strongly suggestive of signal loss-inducing processes – to illustrate the principle. Recognizing that the observed temporal intensity changes in the recorded TERS spectra are vibrational state specific is key to accounting for these observables. Notice that although intensity loss at 1585 cm$^{-1}$ in Figure 3(C) – in itself - might suggest desorption, diffusion, photobleaching, and/or photochemistry at the nanojunction; the distinct temporal evolution of the 1204 and 1017 cm$^{-1}$ modes of BPDT throughout the same trajectory indicates that this is not the case. With the experimental geometry in mind, we adopt incident and scattered local fields with $x$, $y$, and $z$ components ($E_{ld}^{i} = [0.33, 0.33, 0.33]$), to simultaneously satisfy the requirements for TERS enhancement and detection in the forward and reverse directions. We follow the treatment outlined in Section 2 and carry out a
complete search in Euler space to determine molecular orientation relative to the assumed local field components. We first match the relative intensities observed in the first ~100 spectra along the TERS trajectory, which established the initial orientation of the scatterer relative to the local electric fields, see Figure 3(D). Next, the oriented molecular polarizability tensors are further rotated in the $xy$, $xz$, and $yz$ planes ($R_{x,y,z}^T \alpha_n' (\Omega) R_{x,y,z}$). This yields rotation angle-dependent relative intensity profiles that closely match the temporal evolution of the relative TERS intensities recorded at 1585, 1284, 1204, and 1017 cm$^{-1}$ for a total of a 25° rotation of the molecule in the $yz$ plane, see Figure 3(D). It is important to stress that this result does not necessarily imply that photobleaching, photochemistry, diffusion, and desorption do not take place at plasmonic nanojunctions. Rather, a careful analysis of the recorded spectra is warranted, ultimately uncovering details about the interplay between a single molecule and its unique local environment. In this study, the improved sensitivity of our TERS measurements allowed us to integrate the TERS signal for relatively short time intervals (0.1 s), and as a result, to expose

**Figure 4.** Hyperspectral TERS image of a laser-irradiated region of a DMS-coated corrugated silver substrate. Note that the 4 images shown in panels A–D were simultaneously recorded throughout the same pass of the AFM probe through the laser-illuminated region of the substrate. They each represent a different vibrational resonance of DMS. The 2D contours have a color bar starting at white (brightest signals), passing through yellow to black (lowest signals). The reader is referred to reference [30] for more details.
what appears to be continuous molecular reorientation of a single molecule at a plasmonic nanojunction.[25]

### 3.3. Few-molecule TERS nanoscopy

Using the same experimental scheme illustrated in Figure 3(A), scanning the metallic AFM probe allows mapping TERS across the diffraction-limited laser-illuminated area of the substrate [38]. In Figure 4, TERS images of DMS molecules adsorbed on a thin silver film reveal that different vibrational resonances ($\nu_1 - \nu_4$) of the probed molecules are optimally enhanced at different sites of the metal surface. Note that the representative images shown were obtained from a single pass of the AFM probe through the laser-illuminated region of the substrate; together, they comprise a single simultaneously recorded frequency-resolved TERS image. We found that the predominant $\alpha'_{xx}$ element of the molecular polarizability derivative tensor of the brightest 1580 cm$^{-1}$ mode (Figure 4(B)) effectively maps the laser spot and suggests that the DMS molecules are uniformly distributed across the probed area. Namely, irrespective of the fine details of the enhanced local electric fields, there are always projections of the predominant $\alpha'_{xx}$ tensor element of the 1580 cm$^{-1}$ vibrational mode onto the local electric fields. This is contrasted with the 1188 and 1087 cm$^{-1}$ images plotted in Figures 4C and 4D, where the TERS response is more pronounced at 1.25 and 0.95 μm, respectively. This is because the 1188 and 1087 cm$^{-1}$ vibrational modes feature several $\alpha'_{n}$ elements that are comparable in magnitude. As such, these two modes are finer gages of the local electric field. Combined with the analysis outlined in Section 2, this observation suggests that normal modes featuring comparable magnitudes of their diagonal and/or off-diagonal polarizability tensor elements serve as better gages of the local electric field, and hence, result in a more spatially defined (localized) TERS response. In this regard, there is an obvious need for novel anisotropic SERS/TERS-active molecular reporters.

### 4. Other information about the local environment from SERS and TERS

#### 4.1. Vibrational stark shifts in SERS and TERS: mapping the magnitude of the local electric field

The vibrational Stark effect is a change in a vibrational frequency as a result of a perturbation to the electronic environment of a bond [37]. DC fields arising from plasmonic excitations have been found to induce Stark shifts in molecules coaxed into plasmonic nanojunctions [22,39–41]. As such, vibrational Stark shifts observed in both SERS and TERS experiments provide a measure of the local electric field magnitude around plasmonic nanostructures [22,41,42]. The electric field of a prototypical nanoparticle dimer was recently gaged using the vibrational Stark effect of a CO molecule adsorbed in the interparticle nanogap [22]. Soon
after, vibrational Stark shifts observed in SERS from mercaptoalkyl monolayers on plasmonic nanostructures were also found to provide a direct measurement of the magnitudes of the operative local electric fields [42]. The latter experiment involved adlayers of CN−, p-mercaptobenzonitrile, and n-mercaptobutylnitrile, adsorbed to a rough silver substrate. The characteristic CN vibrational frequency shifts were correlated with the recorded SERS intensities. The observation was attributed to vibrational Stark shifts arising from optical rectification at plasmonic nanojunctions [42,43]. In this context, there is an obvious need for novel SERS-active molecular reporters featuring enhanced Stark coefficients.

4.2. Hyperspectral TERS: mapping topography and local electric fields

We recently reported tip-enhanced Raman imaging experiments in which information on sample topography and local electric fields was simultaneously

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**Figure 5.** (A) Left panel: Hyperspectral TERS cross-section of three adjacent 20 nm wide slits lithographically etched in a silver thin film using helium ion lithography. Middle panel: Helium ion microscopy image of the three milled slits. Right panel: Selected area TEM image illustrating that well-defined sub-10 nm slits with rather sharp edges can be fashioned using helium ion lithography. (B) The recorded intensities at 1628, 1580, and 1087 cm⁻¹ (molecular signals) as the AFM tip is scanned across the three slits. (C) A reflected low-frequency signal is compared to the FDTD simulated local electric fields ($E_{\text{total}}/E_0$) sustained near isolated 15 and 20-nm wide slits following focused (40 X objective, 0.7 NA) 514 nm irradiation. Note that the FDTD simulations assume perfect surface and slit structures. The reader is referred to reference [44] for more details.
obtained [44]. The results are illustrated in Figure 5. We employed a Raman-active 4,4′-dimercaptostilbene (DMS)-coated gold tip of an atomic force microscope to simultaneously map the topography and image the electric fields localized at nanometric (20 and 5 nm-wide) slits lithographically etched in silver, all using optical signals. Bi-modal imaging was feasible by virtue of the frequency-resolved optical response of the functionalized metal probe. Namely, the probe position-dependent signal was subdivided into two components, see Figure 5(A). The first is a 500–2250 cm −1 Raman-shifted signal, characteristic of the tip-bound DMS molecules. The molecules report on topography through the intensity contrast observed as the tip scans across the nanoscale features, see Figure 5(B). The variation in molecular Raman activity is a consequence of the absence/formation of a plasmonic junction between the scanning probe and patterned silver surface, which translates into dimmed/enhanced Raman signatures of DMS. Using these signals, we demonstrated that sub-15 nm spatial resolution is attainable using a DMS-coated gold tip with a 30 nm radius, see Figure 5B. The second response consisted of two correlated sub-500 cm −1 signals arising from mirror-like reflections of (i) the incident laser field, and (ii) the Raman scattered response of an underlying glass support (at 100–500 cm −1) off the gold tip. We illustrated that the reflected signals trace the local electric fields in the vicinity of the nanometric slits, see Figure 5(C). Ongoing works in our lab aim at nanoscale imaging of the vector components of the local electric field across various plasmonic substrates.

4.3. SERS and TERS at the quantum limit: probing local electric field gradients and charge shuttling across a plasmonic Gap

A recent SERS study [22] tracked the trajectories of both a single BPDT molecule and a single CO molecule that were co-adsorbed at a hot spot formed between two silver nanospheres. As discussed in the previous sections of this review, whereas

Figure 6. Time evolution of TERS intensity from biphenyl-4,4′-dithiol (BPDT, panel A) and biphenyl-4-thiol (BPT, panel B) coated silver substrates monitored at 1580 cm −1 (C=C stretch, TERS signal maximum in both cases). See reference [45] for more specifics.
the linear Stark shift of CO serves as an absolute gage of the local field, the polynatomic spectra of BPDT can be used to characterize its vector components. Notably, throughout the laser-induced fusion of the two spheres, the authors also observed sequential evolution of the enhanced spectra from dipole-coupled Raman, to quadrupole- and magnetic dipole-coupled Raman, followed by a transition from molecular line spectra to band spectra [22]. The observation of multipolar spectra necessitates that electric field gradients are operative. In this regard, local field gradients may be inferred from surface-enhanced multipolar Raman spectra. Moreover, the transition from molecular line spectra to band spectra was associated with the cross-over to the conduction limit of plasmons. In the prior work [22], this limit was reached when the junction gap was reduced to a few Angstroms, as the spheres fused.

Figure 7. (A) Schematic representation of the scanning and data acquisition in STEM-EELS. (B) Dark-field (Z-contrast) STEM image of an Ag nanorod (aspect ratio: 4), supported on a 5 nm thick amorphous silicon substrate. (C) Cropped high-resolution Z-contrast STEM image providing local structural and morphological information with atomic resolution. The scale bar is 5 nm. The inset shows the Fourier transform of the area indicated and corresponding to the [0 0 1] zone axis of Ag. (D–F) STEM-EELS spectral maps corresponding to the highlighted energy ranges. The modes spatially resolved in (D) and (E) correspond to the dipolar and quadrupolar longitudinal LSP resonances, respectively. (F) Transverse LSP resonance. The EELS spectrum was obtained by averaging individual EELS spectra at each pixel in the EELS hyperspectral map (65 × 125 pixels).
Figure 6 shows that intensity spikes in TERS can be similarly assigned to the shorting of a junction plasmon through conductive molecular bridges. The effect was demonstrated through Raman trajectories recorded at a plasmonic junction formed by a gold AFM tip in contact with a silver surface coated with BPDT (Figure 6(A)) [45]. When BPDT molecules were replaced with biphenyl-4-thiol (BPT, Figure 6(B)) molecules, which cannot chemisorb to the tip and surface simultaneously, neither spiking in TERS intensities, nor switching between line and band spectra was observed. This work clearly demonstrates the possibility of shorting the plasmonic junction through conductive chemical contacts. It also stresses that a single molecule can slide the charge transfer plasmon into resonance. Overall, not only did we demonstrate that charge shuttling across a plasmonic nanojunction can be essentially ‘chemically’ mediated and even controlled, but we also took advantage of the optical signatures of charge-transfer plasmons (CTPs) to monitor what appears to be the making and breaking of single chemical bonds.[45]

5. Cross-checking insights: direct measurements of the local electric fields

One of the ongoing challenges in the field of enhanced spectroscopy is to achieve beyond structural characterization of plasmonic constructs on a sub-nm length scale. The goal is to gain independent access of the local electric fields over the length scales of relevance to SERS and TERS. Even the most advanced optical techniques are generally limited to spatial resolutions on the order of tens of nanometers. The same resolution is typical of our microscopy technique of choice for mapping surface plasmons in plasmonic substrates, namely nonlinear PEEM [46,47] To cross-check the insights gained about the local electric fields from single-molecule Raman SERS and TERS experiments, correlated Raman/electron energy loss spectroscopy (EELS) experiments are warranted. Such experiments have not been performed to date, mainly because it wasn’t until very recently that the energy resolution in EELS was adequate for such studies.

The development of spherical aberration correction in scanning transmission electron microscopes (ac-STEM) has afforded remarkable advances in the spatial resolution and sensitivity of EELS measurements. Figure 7(A) shows a schematic of the data acquisition procedure in a STEM-EELS setup. With probe sizes typically less than 1 Å in diameter, the beam electrons can lose energy as they pass through a material system. In the context of this review, energy loss occurs as a result of the resonant interaction between the incident electrons and LSP resonances in plasmonic nanoparticles [48]. As such, the electric fields sustained around plasmonic nanostructures can be mapped [49]. Overall, EELS is the only technique that allows the correlation of atomically resolved local electric field maps with particle morphology, as elegantly described in a recent review [50]. Besides correlated topographic and electric field maps [49,51–54], EELS images
readily reveal hybrid plasmon resonances formed between interacting plasmonic particles [52,55–57], as well as higher order multipolar resonances that are characteristic of relatively large plasmonic structures [55,57,58].

Figure 7 shows an exemplary ac-STEM EELS spectrum acquired from a silver nanowire. These spectra are acquired in a serial fashion by scanning the probe across the sample pixel by pixel, and integrating the signal intensity at selected energy loss ranges. This produces hyperspectral maps, cross-sections of which are shown in Figures 7(D)–(F). Evidently, the recorded spatial patterns of the induced local electric fields vary with energy loss, revealing different LSP resonances. In this example, the dipolar response shown in Figure 7(D) is contrasted with the quadrupolar resonance of the silver nanowire shown in Figure 7(E) [55].

The detection of LSP resonances below 2 eV can now be routinely achieved using electron source monochromators [59], in STEM-EELS setups featuring energy resolutions as high as 60–90 meV [60–62]. Using monochromated EELS, CTP resonances of touching Ag nanosphere dimers were recently measured at ~1.75 eV [63]. CTP resonances have also been observed from individual dimers of metallic particles bridged by different molecules [64]. The observation of CTP resonances for gaps above 1 nm (as large as 1.3 nm) provided a direct experimental confirmation of otherwise purely theoretical predictions [65–67]. Monochromated EELS measurements have also been used to study the electron kinetics of single plasmon modes in individual nanoparticles. A resolution of 60 meV (at 2 eV) in the frequency domain was demonstrated in these measurements, which is equivalent to a temporal resolution of ~2 fs [68]. On a related note, spectral features at energy losses below 100 meV can already be detected using a new generation of monochromators affording a nominal energy resolution of about 10 meV [69–73]. By taking advantage of this threefold increase in resolution, correlated vibrational EELS and infrared spectroscopic measurements are now possible [74]. Performing EELS in allof-beam mode, namely placing the electron beam at a nanometric distance from the area of interest, provides the best signal-to-noise ratio for vibrational electron loss spectroscopy, all while preserving the molecules from radiation damage [72]. Overall, the spatial and energy resolution attainable in EELS combined with novel plasmonic fabrication and chemical functionalization methods offer exciting opportunities for probing the plasmonic response of chemically functionalized metal substrates [65,67]. Eventually, correlating EELS maps with SERS and TERS spectra and images ought to unlock the full potential of single-molecule Raman nanoscopic measurements.

6. Brief conclusions and outlook

There is much more to ultrasensitive Raman nanoscopy than the chemical detection and imaging of single molecules. The underrated phenomena addressed in this review are at least as informative. Through a few selected studies, we illustrated how various aspects of a single molecule’s local environment can potentially be
probed through a detailed analysis of its SERS and TERS spectra. On the theoretical end, a proper treatment of enhanced RS is still the subject of extensive ongoing research. Because of the nature of this problem, which lies at the interface of classical and quantum theories, this is a challenging task. Until the experiments discussed herein can be fully simulated in silico at reliable levels of theory, experiments which provide direct access to the localized and enhanced electromagnetic fields remain invaluable. In this regard, correlating Raman nanoscopic measurements with modern (beyond-topographic) microscopic tools, such as nonlinear PEEM and EELS, seems to be the most promising approach to unlocking the full potential of SERS and TERS.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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