Spiers Memorial Lecture

Surface-enhanced Raman spectroscopy: from single particle/molecule spectroscopy to ångstrom-scale spatial resolution and femtosecond time resolution

Anne-Isabelle Henry, Tyler W. Ueltschi, Michael O. McAnally and Richard P. Van Duyne

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Four decades on, surface-enhanced Raman spectroscopy (SERS) continues to be a vibrant field of research that is growing (approximately) exponentially in scope and applicability while pushing at the ultimate limits of sensitivity, spatial resolution, and time resolution. This introductory paper discusses some aspects related to all four of the themes for this Faraday Discussion. First, the wavelength-scanned SERS excitation spectroscopy (WS-SERES) of single nanosphere oligomers (viz., dimers, trimers, etc.), the distance dependence of SERS, the magnitude of the chemical enhancement mechanism, and the progress toward developing surface-enhanced femtosecond stimulated Raman spectroscopy (SE-FSRS) are discussed. Second, our efforts to develop a continuous, minimally invasive, in vivo glucose sensor based on SERS are highlighted. Third, some aspects of our recent work in single molecule SERS and the translation of that effort to ångstrom-scale spatial resolution in ultrahigh vacuum tip-enhanced Raman spectroscopy (UHV-TERS) and single molecule electrochemistry using electrochemical (EC)-TERS will be presented. Finally, we provide an overview of analytical SERS with our viewpoints on SERS substrates, approaches to address the analyte generality problem (i.e. target molecules that do not spontaneously adsorb and/or have Raman cross sections <10⁻²⁹ cm² sr⁻¹), SERS for catalysis, and deep UV-SERS.

1. Introduction

In the four decades since the publication of the seminal SERS papers and the 11 years since the last Faraday Discussion on SERS,¹ it is useful to reflect on how the field has evolved and matured. Clearly, there has been an enormous outpouring of results, both at the fundamental, basic science level (e.g. SMSERS, EC-SERS, TERS, EC-TERS) and at the applied science level (e.g. bioassays and diagnostic tools). This is also true in related areas such as localized surface plasmon
resonance (LSPR) biosensing and plasmonics in general. The timeline of
discovery and magnitude (presently ~8000 papers per year) of this activity has
been carefully reviewed. This introductory lecture discusses some aspects of all
four themes of the 2017 Faraday Discussions on SERS. A complete review citing all
the contributions from the community is not attempted. Rather, examples from
our own work are used to illustrate the main points. Fig. 1 provides a graphical
overview of the topics in SERS and TERS that we cover. Examples of the questions
that will be addressed include: what nanostructure has the largest enhancement
factor (EF)?; what is our current understanding of the magnitude of the chemical
(CHEM) mechanism of SERS?; what are the successes and limitations of SERS
biosensors?; and what progress has been made in pushing the limits of SERS and
TERS with respect to analyte generality, surface generality, spatial resolution, and
time resolution?

2. Theory of SERS enhancement

The central question of the origin of SERS has been a long-standing discussion in
the SERS community, since the early days of SERS in the late 1970s and early
1980s. The two components of the enhancement – electromagnetic and chemical – both contribute; but, are still not as well quantified as one would like. However,
significant progress has been made during the past 10 years in characterizing,
quantifying, and therefore solidifying our understanding of the mechanisms at
play in the enhancements. The following topics have been selected to illustrate
some important steps forward in understanding and broadening the theory of
SERS enhancement: (1) wavelength-scanned SERS excitation spectroscopy (WS-
SERES); (2) the distance dependence of SERS; (3) the magnitude of the chemical enhancement mechanism; and (4) ultrafast SERS.

2.1 WS-SERES of single Au nanosphere oligomers

The question of quantifying the enhancement factor and its relationship to nanoparticle structure and the LSPR spectrum has been addressed in a series of structure–activity relationship studies at the single ‘nanoparticle’ (or, more accurately, the single nanosphere oligomer) level. By taking advantage of the fact that metal nanoparticles strongly scatter light, so that they can be indexed when drop-cast at a relatively low concentration on a TEM grid, it becomes possible to do a spatially correlated LSPR-HRTEM-SERES study. In such studies the LSPR, SERS, and WS-SERES are measured for specific nanosphere oligomers of known structure – shape, size, number of cores and relative geometry in the case of multiple cores – by HRTEM.\(^5,^6\)

Spatially correlated LSPR-HRTEM-SERS/SERES studies\(^5,^6\) have revealed several important new mechanistic insights about SERS. First, strongly coupled nanoparticles (i.e., 2 or more nanoparticles of the same or different structure containing a “hot spot”) behave in a fundamentally different manner than isolated nanoparticles.\(^7\) Whether in the single wavelength or the wavelength-scanned excitation studies on single nanoparticle oligomers, it was clearly observed that the wavelengths at which the enhancement factor maximum and the LSPR (i.e., extinction maximum) occur do not coincide. In other words, the LSPR scattering maximum could not predict the wavelength at which the maximum average EF occurs. Second, while the LSPR is extremely sensitive to very small structure changes, the SERS EF is more forgiving. Isolated Au nanospheres have maximum average EFs < 10^4; but, Au nanosphere oligomers have maximum average EFs of 1–4 \times 10^8 roughly independent of structure (e.g. dimers, trimers, tetramers, etc.) as long as they provide sub-1 nm crevices in or near the junction site.\(^5,^6\)

The practical implications of these studies are significant. First, they demonstrate that using the wavelength of the LSPR maximum to predict the wavelength corresponding to the maximum EF is not correct as it is in the isolated particle case. Second, single nanoparticle oligomer studies unambiguously show how simply changing the excitation wavelength increases the maximum average EF. This means that SERS substrates with strongly coupled nanostructures benefit from excitation wavelengths in the NIR.

2.2 The distance dependence of SERS

Gersten and Nitzan\(^8\) developed a model to describe the decay of the SERS intensity as a function of the molecule–metal distance for various plasmonic materials (e.g. Ag, Cu, Au) with spheroidal shapes. Experimental efforts followed to measure the SERS distance dependence using various molecular probes and dielectric spacer layers. Using a simplified sphere model and the \(|E|^4\) approximation, the distance dependence can be written as:

\[
I = \left(1 + \frac{r}{a}\right)^{-10}
\]

where \(I\) is the SERS intensity, \(a\) is the radius of curvature of the field enhancing nanostructure, and \(r\) is the distance between the adsorbate analyte and center of
the plasmonic sphere. One concludes that the maximum SERS intensity occurs when the adsorbate is in direct contact with the substrate and that the EM enhancement is a long-range effect that typically extends to distances ~10 nm away from the metallic surface.\(^9\) Kovacs et al. investigated the distance dependence of the SERS EF using Langmuir–Blodgett monolayers of arachidic acid on an island film substrate.\(^{10}\) This study concluded that the lack of direct contact with the tetra-t-butyl phthalocyanine probe molecule demonstrates that the long-range distance dependence observed can be attributed to the electromagnetic enhancement. Self-assembled monolayers (SAMs) of thiols have been widely used to probe the distance dependence of SERS. Here the thiol acted either as the spacer layer or as both the spacer layer and probe molecule. SAMs have shown fewer structural defects in the film than Langmuir–Blodgett spacer layers, and thus are viewed as more reliable and reproducible spacer layers.\(^{11-13}\) Van Duyne introduced the use of atomic layer deposition (ALD) to fabricate ultrathin, conformal dielectric spacers on SERS substrates.\(^4\) In this work, it was concluded that the SERS intensity decreased by a factor of 10 when the probe molecule, pyridine, was spaced 2.8 nm from the AgFON surface.

Recently, the Van Duyne group carried out a high-resolution distance dependence study by using atomically thin (~1.3 Å) layers of Al\(_2\)O\(_3\) deposited by ALD.\(^{14}\) In this study, a single Ag film-over-nanospheres (AgFON) substrate was used throughout the course of an ALD alumina deposition, using the methylalumina surface species as the probe molecule. As illustrated in Fig. 2A, we used two different vibrational modes (sym. C–H (2892 cm\(^{-1}\)) and sym. Al–CH\(_3\) (585 cm\(^{-1}\)) stretches) to monitor the decay of the SERS intensity with respect to the spacer layer thickness. Experimentally, the sharp decrease in SERS intensity as a function of spacer thickness is best fit using a two-term distance expression (see the bottom equation in Fig. 2) with a short-distance and a long-distance dependence. The relative SERS intensity decrease corresponds to a ~80% loss of signal intensity ~0.5 nm away from the surface and 90% loss ~2 nm away. In this modified Gersten and Nitzan model, \(a_1\) and \(a_2\) are the short- and long-range radii of curvature features, respectively. The different radii of curvature are associated with the small (<1 nm) and large (>10 nm) nanoparticle structures on the heterogeneous AgFON surface (Fig. 2D). Fig. 2B and C shows the near-field distance dependence of a AgFON calculated using FDTD. The cross-section of the near-field distribution (Fig. 2B) shows, as expected, a maximum value of \(|E/E_0|^4\) in the gap between two nanospheres. In some cases, one can also expect strong localized field hotspots at the top of the AgFON, in between pillars and irregularities. Fig. 2C shows how this local electric field varies when moving away from the metallic surface (red dashed line in panel B). The FDTD calculations clearly show that the field decreases to ~36% and ~10% of the initial intensity at distances of ~1 and ~3 nm from the surface, respectively. A fit of the FDTD data using the two-term Gersten and Nitzan model is in qualitative agreement with the experimental observations in Fig. 2A.

### 2.3 Magnitude of the chemical enhancement mechanism

When the adsorbate is chemically bonded to the surface, the chemical (CHEM) mechanism contributes to the Raman signal enhancement in addition to the electromagnetic mechanism. This enhancement is primarily through charge
transfer mechanisms where the excitation wavelength is either resonant or non-resonant with the metal–molecule charge transfer electronic states.\textsuperscript{15} Jensen \textit{et al.} calculated theoretical CHEM enhancement factors as high as $7 \times 10^3$ for \textit{para}- and \textit{meta}-substituted pyridine on a silver cluster. We have investigated both experimentally and theoretically, in collaboration with the Schatz group at Northwestern, the origin and magnitude of the CHEM mechanism by investigating how the total enhancement varies with different substituents on a benzenethiol framework adsorbed on silver and gold.\textsuperscript{16} Experimentally, the total enhancement was measured to vary by a factor of 10 as a result of chemical substitution. In unpublished work, we have significantly extended the number of substituted benzenethiol systems to include more electron withdrawing and electron donating substituents. Experimentally, we can now report that the total enhancement varies by a factor of more than $2 \times 10^2$ over this data set.

$$I_{\text{SERS}} = C_1 \left(1 + \frac{r}{a_1}\right)^{-10} + C_2 \left(1 + \frac{r}{a_2}\right)^{-10}$$

Fig. 2 Distance dependence of SERS. (A) SERS intensity decay of sym. C–H (2892 cm\(^{-1}\)) and sym. Al–CH\(_3\) (585 cm\(^{-1}\)) stretches as a function of distance with a two-term fit (equation at bottom). (B) Spatial distribution of local electric field enhancement from FDTD calculations of simulated Ag FON surface. (C) Near-field distance dependence profile at gap of simulated Ag FON surface observed at the dashed red line in (B). (D) Side-view SEM micrograph of Ag FON substrate used. Reprinted and adapted with permission from ref. 14, copyright 2016 American Chemical Society.
Bromobenzenthiol shows the largest enhancement and 4-methylsulfanyl-benzenethiol the lowest.

2.4 Ultrafast SERS

In the spirit of pushing the boundaries of what is possible in the domain of SERS, the development of surface-enhanced femtosecond stimulated Raman spectroscopy (SE-FSRS) is an attractive research target. FSRS has been successfully used to monitor the vibrational signature of molecules with both a high temporal resolution (10–100 fs) and high spectral resolution (5–20 cm⁻¹). However, these experiments necessitate high analyte concentrations and large Raman cross sections. To overcome these challenges, Ploetz et al. attempted to combine the high enhancement factors of plasmonic substrates with FSRS using a 1 kHz repetition rate laser system. Unfortunately, they were not able to observe SE-FSRS signals. By using a 100 kHz repetition rate laser system with average powers high enough for signal generation, but low peak powers to avoid substrate damage, SERS was successfully combined with FSRS (Fig. 3A). This proof-of-principle paper presented ground-state SE-FSRS spectra with characteristic Fano-like line-shapes. The authors conservatively estimated time- and ensemble-averaged enhancement factors for SE-FSRS to be 10⁴ to 10⁶; however, this estimated EF was limited by sample degradation. While the dependence of SE-FSRS gain on Raman pump power was shown to be linear in extremely low power regimes, with increasing power sample degradation resulted in a signal decrease by a factor of 2 on a timescale of 30 minutes (Fig. 3D).

In order to improve the signal to noise ratio (SNR) of SE-FSRS both concentration and pathlength were varied to find the optimal balance between signal generation and probe extinction (i.e., absorption + scattering) (Fig. 3E). To further push the limits of SE-FSRS, a femtosecond stimulated Raman spectrometer was built around a tunable 1 MHz laser system to decrease peak powers while increasing the number of events probed to maximize the SNR (Fig. 3C). The sample damage problem has now been eliminated over the 30 minute data acquisition period required for a three-pulse time-resolved SE-FSRS experiment (Fig. 3D).

In addition to optimizing the SNR of SE-FSRS, significant effort has been put towards uncovering the origin of the observed Fano-like line-shapes arising from these colloidal nanoparticle–molecular systems from both experimental and theoretical perspectives (Fig. 3B). Experimentally, Frontiera et al. used nanosphere oligomer substrates consisting of aggregated 90 or 60 nm Au spheres coated with trans-1,2-bis(4-pyridyl)-ethylene (BPE) encapsulated in a silica shell to change the energy of the plasmon resonances of the substrate with respect to the wavelengths of the Raman pump and stimulating probe pulses. They observed that the phases of the dispersive line-shapes change as the plasmon resonance is varied. By fitting to extract the line width and coupling information they observed that the molecule–plasmon coupling has no observable effect on the vibrational coherence lifetime of the molecule. With the use of a tunable 1 MHz system, we have been able to follow up this report with a more in-depth wavelength dependent study. Using the same nanoparticle substrates, Buchanan et al. varied the wavelength of the Raman pump pulse and probed both the stimulated Raman gain (SRG) and stimulated Raman loss (SRL) activities (Fig. 3F). This study
demonstrated the wide variety of lineshapes attainable in SE-FSRS, as well as lineshape dependence that is not predicted by current SE-FSRS theories.\textsuperscript{25,26} This suggests that current theories are incomplete as demonstrated by the incorrectly predicted amounts of dispersive character either near an LSPR\textsuperscript{25} or at wavelengths to the red of the LSPR.\textsuperscript{26} Thus, further theoretical efforts are needed and should be aided by the collection of additional experimental data over wider ranges of excitation wavelengths. Both theoretical models were applied to single oligomers;\textsuperscript{25,26} the presence of dispersive peaks in both sets of calculated SE-FSRS spectra suggests that the complex lineshapes observed experimentally are not simply an artifact of the ensemble measurement. However, to better correlate with theoretical results, experimental studies of individual oligomers should be made in order to remove inherent ensemble averaging. Such measurements would also eliminate any possibility that the broadband probe pulse is being

Fig. 3 Ultrafast SERS. (A) SERS spectrum and SE-FSRS spectrum of trans-1,2-bis(4-pyridyl)ethylene (BPE)-functionalized Au nanosphere assemblies (inset in (D)). Both spectra have high S/N. The SE-FSRS spectrum shows dispersive lineshapes due to resonance effects from the plasmon. Image adapted from ref. 20 with permission, copyright 2011 American Chemical Society. (B) Comparison of SE-FSRS experiment and classical coupled wave theory to understand the dispersive lineshapes. Image adapted from ref. 25 with permission, copyright 2017 American Physical Society. (C) SE-FSRS spectra of BPE functionalized Au nanosphere assemblies obtained with 50 pJ per pulse of Raman pump at 100 kHz (red) and 1 MHz (blue). Both repetition rates yield similar SE-FSRS signal strengths; but, the spectra obtained at 100 kHz exhibit noise in the spectral region of 1000–1200 cm\(^{-1}\) that is 10\(\times\) greater than that obtained at 1 MHz. (D) Sample damage over 30 min. Data acquisition time has been eliminated at 1 MHz repetition rate. Images (C) and (D) adapted from ref. 22 with permission, copyright 2017 American Chemical Society. (E) To achieve maximum S/N in transmission SE-FSRS experiments requires balancing scattering and enhancement by optimizing nanoparticle concentration and pathlength. Image (E) adapted from ref. 77 with permission, copyright 2016 American Chemical Society. (F) The first multi-wavelength and anti-Stokes SE-FSRS is reported. The SE-FSRS lineshapes vary dramatically as a function of excitation wavelength. Image (F) adapted from ref. 24 with permission, copyright 2017 American Chemical Society.
chirped by propagation through the colloidal sample, potentially creating a phase shift in the stimulating field that could add an additional dispersive element to the measured lineshapes.

As single oligomer-SE-FSRS is experimentally realized, some questions will be answered, but new ones will arise. In particular, there are outstanding questions dealing with the self-heterodyne nature of the SE-FSRS signal that isn’t present in similar experiments, such as SE-CARS. Reducing the dimensionality of the experiment by probing individual oligomers will help, but conceptually an understanding of how a self-heterodyned signal field is plasmonically-enhanced is crucial to a deeper knowledge of SE-FSRS. In the same vein, understanding the plasmonic enhancement of a self-heterodyned signal may lead to clues as to why single-oligomer SECARS experiments have not shown dispersive lineshapes in comparison to bulk SECARS and solvent experiments.

3. SERS in biology and biomedical SERS

One of the most successful translations of SERS out of the analytical laboratory lies in its use to probe living systems – from cells to whole bodies – to assist biomedical research from fundamental mechanistic studies up to diagnostics. Owing to the formidable diversity of substrates available (e.g., nanostars, nanoparticles with or without magnetic cores, immunoassays, plasmonic paper, plain nanostructured metal films and more), the choice of plasmonic substrate can now be driven by the application itself rather than dictated by technical limitations regarding the incident wavelength or other factors. The substrate can also be adapted to the experiment format: e.g., microfluidic channels, or solution.

Probably the most impressive results towards in vivo, multiplexed diagnosis come from SERS nanotags that are both sensitive and stable SERS probes with bright, reliable signals in time even through tissue or bone. Typically, SERS nanotags consist of Au or Ag nanospheres on which a Raman reporter molecule is adsorbed, and the whole is coated with a protective silica or polymer shell. These nanotags target pre-determined analytes, e.g. biomarkers, through immuno-targeting. Measuring the dye spectrum indirectly proves the presence of the targeted biomarkers, without the need of aggregating metal colloids that inevitably complicate the data analysis. Finally, using different tags with a single measurement – so-called multiplexing – in an imaging detection mode dramatically increases the throughput of one single measurement, making SERS ideal to be used for clinical diagnostics, tumor detection and tumor margin monitoring. An additional factor that arises and is quite critical to the translation of Raman and SERS to non-experts and outside the analytical laboratory is the development of miniaturized Raman spectrometers about the size of a smartphone.

The work in the Van Duyne lab to develop an in vivo glucose sensor based on SERS is a good example of an important biosensing problem that requires chemical modification of the surface to effect glucose binding or close approach. While glucose is an important analyte, the Raman cross-section was only recently published by the Van Duyne group, who demonstrated the difficulties in glucose Raman scattering – necessitating the use of SERS. The first-generation sensor was based on mixed self-assembled monolayers that acted as partition layers for close glucose approach, but had no selectivity for glucose vs. fructose. The second
generation SERS glucose sensor improves the selectivity towards glucose. A novel bisboronic acid ligand with a tunable scaffold incorporating two mono-fluoro boronic acid motifs, known to bind saccharides and a thiol moiety.
enabling binding to metal surfaces has now been developed. By varying the distance between the amine and each boronic acid moiety, the binding constant for glucose can be tuned from 29 up to $167 \text{ M}^{-1}$. In addition, the ratio of the binding constants for glucose to fructose can be tuned, resulting in more favorable selectivity of glucose with respect to fructose. Spectroscopically, glucose is qualitatively and quantitatively detected via SERS difference spectroscopy, i.e. the subtraction of the ‘BBA only’ from the ‘BBA with glucose’ spectrum. The unique glucose vibrations ($840 \text{ cm}^{-1}$, $1095 \text{ cm}^{-1}$, $1342 \text{ cm}^{-1}$) and the distinctive derivative lineshape ($\sim 1620 \text{ cm}^{-1}$) that are observed attest to the effective binding of glucose to the immobilized ligands. Fig. 4A displays the quantitative relationship between the SERS signal and glucose concentration. Principal component analysis (PCA) shows that the concentration response of the sensor is clustered in distinct groups (Fig. 4B). Further, Fig. 4C shows that even greater distinction between spectra is found when Hierarchical Cluster Analysis (HCA) is coupled with PCA. HCA enables the classification of the sensor response as indicative of hypoglycemia ($1–3 \text{ mM}$), the normal glucose range ($4–8 \text{ mM}$), and hyperglycemia ($>8 \text{ mM}$).

4. Ultrasensitive and towards single molecule SERS

4.1 Single molecule SERS and TERS

SERS has the capability of detecting and identifying single molecules that are resonance Raman active. The strongest evidence to date for single molecule SERS (SMSERS) is the isotopologue proof (Fig. 5A–D). Silver nanoparticles are dosed with a 50 : 50 mixture of the two R6G isotopologues at a total concentration of $10^{-9} \text{ M}$ (Fig. 5B). Very high SNR single molecule SERS spectra are obtained on each nanoparticle that show isotopically sensitive vibrational modes at $601 \text{ cm}^{-1}$ and $611 \text{ cm}^{-1}$ (Fig. 5C). A histogram ($N = 50$) of such single molecule events is shown in Fig. 5D. The structures of the silver nanoparticles (Fig. 5E) that yielded single molecule SERS was obtained by the correlated LSPR-HRTEM-SMERS method. These results show that only nanoparticle assemblies yield SMSER spectra confirming the hypothesis and early results on the need for 'hot spots' in or near nanoparticles' junction(s). Further, it is demonstrated that fused nanoparticles of any shape yield high quality SMSERS and nanoparticle assemblies that have nanogaps are not required to observe SMSERS.

 Likewise, the isotopologue proof has been used to establish the existence of single molecule tip-enhanced Raman spectroscopy (SMTERS). A combination of experimental and theoretical studies provides a detailed view of the isotopic response of R6G-$d_0$ and R6G-$d_4$ in the 600–800 cm$^{-1}$ region. The single-molecule nature of the TERS experiment is confirmed through two lines of evidence. First, the vibrational signature of only one isotopologue at a time was observed from multiple TER spectra. Second, the spectral wandering of the $610 \text{ cm}^{-1}$ mode of R6G-$d_0$ was less than 4 cm$^{-1}$, which in turn is less than the 10 cm$^{-1}$ isotopic shift so that no confusion in assignment resulted. Consequently, the total TERS enhancement factor can now be accurately established as $E_{\text{TERS}} = 1.0 \times 10^{13}$ because only one molecule at a time is measured. Furthermore, $E_{\text{TERS}}$ can be
partitioned into an electromagnetic contribution of $10^6$ and a molecule-localized resonance Raman contribution of $10^7$.

### 4.2 2.6 ångstrom resolution UHV-TERS

UHV-TERS combines the ability of scanning probe microscopy (SPM) to resolve atomic-scale surface features with the single molecule chemical sensitivity of surface-enhanced Raman spectroscopy (SERS). The conformational dynamics of a free-base porphyrin, meso-tetrakis(3,5-ditertiarybutylphenyl)-porphyrin (H$_2$TBPP, Fig. 6B), adsorbed on a Cu(111) surface at room temperature are interrogated with UHV-TERS (Fig. 6A–E). The H$_2$TBPP/Cu(111) system has two metastable surface-mediated isomers (Fig. 6A). At room temperature, the barrier between the porphyrin ring buckled up and buckled down conformations is easily overcome and the two conformers randomly switch between these two states (Fig. 6C). Simultaneous measurement of STM (Fig. 6E middle) and TERS line scans (Fig. 6E bottom) across four H$_2$TBPP molecules (Fig. 6C) demonstrates that TERS has 2.6 Å lateral resolution under ultrahigh vacuum (UHV) conditions. This work presents the first UHV-TERS on Cu(111) and unambiguously demonstrates...
that TERS can distinguish the conformational differences between neighboring molecules with angstrom-scale spatial resolution.

4.3 Single molecule electrochemistry with EC-AFM-TERS

A nanoscale understanding of electrochemical processes, including heterogeneities in electrochemical behavior across an electrode surface, is critical to understanding electrocatalysis, biological electron transfer, energy production, and energy storage. TERS is an ideal tool for elucidating structure–activity relationships in electrochemical systems at the nanoscale. Electrochemical atomic force microscopy tip-enhanced Raman spectroscopy (EC-AFM-TERS) was first demonstrated by Kurouski et al. and electrochemical scanning tunneling microscopy tip-enhanced Raman spectroscopy (EC-STM-TERS) by Zeng et al. Kurouski et al. published the first TERS study of a redox reaction. Using an electrochemical AFM (EC-AFM) platform (Fig. 7A) the authors investigated the redox behavior of Nile Blue (NB) spontaneously adsorbed onto an indium-tin oxide (ITO) surface. TERS spectra were acquired with a Au tip (70 nm Au on Si) using 632.8 nm excitation. The spectrum of NB was monitored during cyclic voltammetry in Tris buffer (50 mM Tris + 50 mM NaCl at pH 7), scanning from 0 to −0.6 V versus Ag/AgCl and back with a scan rate of 10 mV s⁻¹ (Fig. 7B). As the potential was swept from 0 to −0.6 V, the resonant oxidized form (NB oxidation) was converted to the nonresonant reduced form (NB reduction) and the intensity of the 591 cm⁻¹ mode decreased (Fig. 7C). Upon scanning the potential back to 0 V versus Ag/AgCl, NB was oxidized back to NB oxidation and the signal increased back to its original intensity (Fig. 7C). TERS voltammograms were constructed by...
integrating the intensity of the 591 cm\(^{-1}\) mode. Remarkably, some TERS voltammograms, such as the one shown in Fig. 7D, exhibit steplike features indicative of few- or single-molecule behavior. That we are working in the few- or single-molecule regime is further corroborated by calculating the average number of molecules under the tip. For a tip radius of 20 nm and an average surface number density of \(2 \times 10^{12}\) molecules per cm\(^2\), the average number of molecules under the tip is \(\sim 6\). In other locations on the sample, the TERS voltammogram did not contain steps, demonstrating a nonuniform surface coverage of NB across the ITO surface. This work demonstrated the potential of TERS for studying redox reactions at the nanoscale, probing few- or single-molecule behavior across a nonuniform surface coverage inaccessible by SERS.

EC-AFM-TERS was employed for the first time to observe nanoscale spatial variations in the formal potential, \(E^0\), of a surface-bound redox couple.\(^{58}\) TERS cyclic voltammograms (TERS CVs) of single Nile Blue (NB) molecules were acquired at different locations spaced 5–10 nm apart on an indium tin oxide (ITO) electrode (Fig. 7E). Analysis of TERS CVs at different coverages was used to verify the observation of single-molecule electrochemistry. The resulting TERS CVs were fit to the Laviron model for surface-bound electroactive species to quantitatively extract the formal potential \(E^0\) at each spatial location. Histograms of the single-molecule \(E^0\) at each coverage indicate that the electrochemical behavior of the cationic oxidized species is less sensitive to the local environment than the neutral reduced species (Fig. 7F). This information is not accessible using purely electrochemical methods or ensemble spectroelectrochemical measurements. We anticipate that the
quantitative modeling and measurement of site-specific electrochemistry with EC-AFM-TERS will have a profound impact on our understanding of the role of nanoscale electrode heterogeneity in applications such as electrocatalysis.

The most recent EC-STM-TERS study examined adsorption geometry and chemical reactivity as a function of applied potential. Combined experimental and simulation data for the case of adenine/Au(111) demonstrated that protonated physisorbed adenine adopts a tilted orientation at low potentials while it is vertically adsorbed near the potential of zero charge. Further potential increase induces adenine deprotonation and reorientation to a planar configuration.

4.4 Summary comments on ultrasensitive and single molecule SERS/TERS

Enormous progress has been made in the last 10 years in the area of ultrasensitive and single molecule SERS/TERS. SMSERS has been convincingly demonstrated by the isotopologue method; it requires “hot spots” but not necessarily nanogaps in nanoparticle assemblies, and the excitation of a molecular electronic resonance. SMSERS is beginning to move from proof-of-concept experiments to fundamental studies of molecular motion on nanoparticle surfaces using super-resolution microscopy approaches, monitoring catalytic processes, and studying chemical reactivity through single-site, single-molecule electrochemistry.

Likewise, SMTERS has been established by the isotopologue method and the tip or tip–sample junction is a scanning “hot spot” with extraordinary spatial resolution down to the few ångstrom level. TERS of adsorbed molecules that do not possess an electronic resonance at the laser excitation wavelength shows an electromagnetic enhancement of only 4–6 orders of magnitude. This is sufficient to observe a few hundred molecules under the tip at packing densities near full monolayer coverage; but, to achieve single molecule sensitivity, an additional resonance enhancement of 4–7 orders of magnitude is required.

Over the last decade, TERS and UHV-TERS in particular have been established as important techniques for the high-resolution functional imaging of surfaces. A notable example is the demonstration of the nanoscale chemical imaging of a dynamic molecular phase boundary. Other noteworthy outcomes include low-temperature (19 K) UHV-TERS, minimized tip and molecular degradation, and improved stability in the presence of ultrafast irradiation. Moving forward, TERS needs to demonstrate its generality with respect to substrates and adsorbates. While it is being used very successfully on Au, Ag, and Cu through the hybridization between the plasmon resonances of the tip and sample (gap-mode plasmons), the high resolution mapping of 1 nm or less has yet to be demonstrated on non-plasmonic substrates. However, one can point to the successful use of the ITO surface as the working electrode in the single molecule EC-AFM-TERS studies cited above. If current trends continue and TERS can be demonstrated under a wider range of experimental conditions, including ever more realistic in situ studies, it is destined to become a major analytical technique for surface science.

5. Analytical SERS

The analytical application of SERS is a broad and rapidly developing sub-field. No attempt is made here to present a comprehensive review, rather the following...
topics are presented to illustrate some key points relevant to: (1) SERS substrates; (2) the analyte generality problem; (3) *operando* SERS for catalysis; and (4) deep UV-SERS.

### 5.1 SERS substrates

Without delving into the broad topic of SERS substrates, recently reviewed else-where, a few comments are, nevertheless, in order. Many papers continue to be published on SERS substrates. A possible explanation for this circumstance is that SERS continues to be a favourite ‘application’ reported for virtually any plasmonic substrate, therefore not adding much more than noise to the SERS literature. The Van Duyne lab views the SERS substrate problem as one that is largely solved. The key rules for the rational design of SERS substrates have been established. We argue that new SERS substrates should not be designed based on solely the novelty of a nanoparticle shape or size, but driven more by experimental constraints or applications.

Taking the three high performance substrates (FONs, NSL nanotriangles, and nanosphere oligomers) used in the Van Duyne lab (Fig. 8A–C) as examples, all possess a minimum average EF $\sim 10^8$ at an excitation wavelength of 785 nm, have tuneable LSPR responses to achieve a maximum average EF at excitation wavelengths ranging from UV-NIR, are reproducible, and are uniform (<10% intensity.

![Fig. 8](image)

**Fig. 8** High-performance SERS substrates showing strong enhancement factors (EFs). (A) Silver film-over-nanospheres (AgFONs) exhibiting an immobilized nanopillar array (AgINRA) as seen by high-resolution SEM. (B) AgFON substrates with AgINRA features are reproducible on the macroscopic scale with near uniform EFs. Reprinted with permission from ref. 71, copyright 2013 American Chemical Society. (C) An extension of the AgFON–AgINRA substrates, periodic particle arrays (PPAs) designed by nanosphere lithography (NSL) techniques and mask-stripping. Reprinted with permission from ref. 78, copyright 2007 American Chemical Society. (D) To fully understand the surface-enhancement process from plasmonic substrates, fully correlated studies are performed on silica-encased colloidal SERS substrates. Reprinted with permission from ref. 5, copyright 2010 American Chemical Society.
variation across the surface). All can be fabricated at wafer scale given enough effort (Fig. 8D).\textsuperscript{24} To be sure, fabrication and information transfer challenges remain in order to achieve these metrics. That said, a reliable commercial source of SERS substrates with optimum metrics would be of significant benefit to the SERS field.

5.2 The analyte generality problem

When target analytes have high affinities for bare plasmonic surfaces, there is no problem getting high quality SERS data. However, many important target analytes do not bind to bare plasmonic surfaces. The glucose sensing problem, briefly described above, is a case in point. In such cases, it becomes evident that engineering plasmonic surfaces for all types of analytes is required to enable SERS to be a truly general analytical method. Further, all such surface engineering approaches must also satisfy the constraints imposed by the SERS distance dependence.

![Fig. 9 Strategies for substrate generality. (A) From left to right, schematic drawings illustrating: the adsorption of analyte-specific adsorbate layers aiming to capture small biological molecules (e.g., glucose), biomarkers, and oligonucleotides; thin (less than 1 nm thick) conformal layers made of alumina or titania deposited by atomic layer deposition (ALD), or thin polymer layers, enabling non-binding analytes to still lay within the sensing volume of the SERS substrate; porous layers such as metal–organic frameworks and mesoporous silica that can trap small non-binding molecules and thus give physical access to the SERS substrate. (B) Illustration of an analyte-specific capture layer for glucose, consisting of a fluorinated bisboronic acid analogue, whose perturbation of the ~1620 cm$^{-1}$ band informs the presence of glucose both qualitatively and quantitatively (direct correlation between glucose concentration and integrated intensity). Reprinted with permission from ref. 48, copyright 2016 American Chemical Society. (C) In operando SER spectra and corresponding molecular structures of methylalumina ALD species. Reprinted with permission from ref. 75, copyright 2017 American Chemical Society. (D) Scanning electron microscopy image of a MOF (ZIF-8)-protected AgFON substrate and corresponding SER spectra displaying growth of captured species. Reprinted with permission from ref. 73, copyright 2014 American Chemical Society.](image-url)
Fig. 9A schematically illustrates three surface engineering strategies that have been demonstrated to deal with target analytes that do not directly bind to plasmonic surfaces. All three are layered structures that: (1) specifically capture the target analytes; (2) are ultrathin (<1 nm) and conformal; and (3) have a tuneable pore size. An example of the short capture ligand strategy is the bisboronic acid SERS glucose sensor, further illustrated in Fig. 9B. ALD was used to implement the ultrathin conformal layer strategy to detect an anthrax biomarker (Fig. 9C). Kreno et al. (Fig. 9D) demonstrated the porous layer approach by trapping weakly binding analytes (volatile aromatic molecules: benzene and toluene) within metal–organic frameworks grafted on AgFON surfaces. Concentration dependence and time resolved measurements provided evidence for the hypothesis that these vapors were reversibly adsorbed on the surfaces of MOF nanocrystals exposed at grain boundaries. This work represents an interesting but nascent avenue that needs to be generalized.

5.3 Operando SERS for catalysis

Operando spectroscopy simultaneously studies the fundamental spectroscopic characterization of surface phenomena and catalytic performance. Operando measurements differ from in situ measurements by being performed under “true catalytic” conditions, where catalytic activity/selectivity measurements can be
made alongside structural measurements.\textsuperscript{74} \textit{In situ} SERS typically falls short of providing insight into the catalytic system of interest due to either the cell or the substrate being incapable of withstanding the high pressures and temperatures necessary for measuring the relevant kinetic information. Issues such as heat and mass transfer gradients can also arise from cell design and can complicate kinetics measurements. Over other techniques, \textit{operando} SERS has the advantage of being able to probe the lower wavenumber region during reaction-specific conditions as a means of acquiring structurally relevant information about the analyte species. By probing the low wavenumber region, where pertinent metal–carbon and metal–oxygen vibrations are located, one can determine the structures of transient species present during the reaction. This type of study was recently performed on the alumina ALD process to show previously unestablished transient dimeric surface species.\textsuperscript{75} Illustrated in Fig. 10 are the low wavenumber vibrational modes that were used to make structural determinations about the dimeric methylalumina species.

5.4 Deep UV SERS

Aluminum film over nanosphere (AlFON) substrates have been fabricated for UV-SERS at the deepest UV wavelength reported to date ($\lambda_{ex} = 229$ nm). The AlFONs were fabricated with two different support nanosphere sizes using localized surface plasmon resonance spectroscopy, electron microscopy, the SERS of adenine, tris-(2,2′-bipyridine)ruthenium(II) and trans-1,2-bis(4-pyridyl)-ethylene, the SERS of 6-mercapto-1-hexanol (as a nonresonant molecule), and dielectric function analysis. Overall, we found that AlFONs with a native Al$_2$O$_3$ oxide layer have plasmonic properties for deep UV-SERS that in combination with the resonance enhancement of the adsorbates can give average enhancement factors of the order of $10^6$. These experimental results are supported by theoretical analysis of the dielectric function.\textsuperscript{76}

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70 Over 6200 published paper found (~800 in 2016) when searching ‘SERS substrates’ in the Web of Science (Clarivate Analytics) database.


