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Experimental correlation of electric fields and Raman signals in SERS and TERS

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Abstract

Enhanced Raman scattering from plasmonic nanostructures associated with surface enhanced (SERS) and tip enhanced (TERS) is seeing a dramatic increase in applications from bioimaging to chemical catalysis. The importance of gap-modes for high sensitivity indicates plasmon coupling between nanostructures plays an important role. However, the observed Raman scattering can change with different geometric arrangements of nanoparticles, excitation wavelengths, and chemical environments; suggesting differences in the local electric field. Our results indicate that molecules adsorbed to the nanostructures are selectively enhanced in the presence of competing molecules. This selective enhancement arises from controlled interactions between nanostructures, such as an isolated nanoparticle and a TERS tip. Complementary experiments suggest that shifts in the vibrational frequency of reporter molecules can be correlated to the electric field. Here we present a strategy that utilizes the controlled formation of coupled plasmonic structures to experimentally measure both the magnitude of the electric fields and the observed Raman scattering.

Keywords

Plasmonics; SERS; TERS; nanoparticles; electric-field; stark shifts; optical rectification

1. INTRODUCTION

Since the initial correlation between plasmon resonances and enhanced electric fields associated with noble metal nanostructures,¹ significant advances have demonstrated the utility of plasmonics for chemical sensing as well as a number of other applications. The largest enhancements observed in surface enhanced Raman scattering (SERS) are attribute to the excitation of localized surface plasmon resonances (LSPR).² Coupling between adjacent nanostructures and the dielectric environment has been shown to affect the plasmon resonance frequency and the observed SERS signal.³ The development of tip enhanced Raman scattering (TERS), and its super-resolution microscopy capabilities, has further enabled new experiments that controllably assess the relationships between Raman signals and the nanostructures.⁴ In addition to chemical sensing with SERS and TERS, the

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excitation of plasmon resonances has been shown to have potential for applications such as solar cells,^{5, 6} molecular electronics,⁷ and chemical catalysis.⁸⁻¹⁰

The most intense electric fields arising from plasmon excitation are commonly attributed to the gaps between nanostructures.⁴ Indeed, gap-modes have been used for single molecule detection and imaging in SERS and TERS experiments.^{11, 12} Interestingly, experiments and calculations also suggest that the electric fields outside the gaps can be substantial. Calculations by Schatz and coworkers performed more than a decade ago indicated that the electric fields around a nanoparticle dimer are sensitive to the spacing of particles.¹³ Super-resolution imaging results show single molecule SERS detection at locations beyond the gap.¹⁴ Further calculations suggest that dipoles near nanoparticle aggregates can have quite substantial scattering enhancements.¹⁵ It has been further reported that in very narrow gaps, approximately 1 nm or less, quantum effects and tunneling can significantly alter the electric fields in the junction between the particles.^{16, 17}

The methods to experimentally measure the electric fields around nanostructures are evolving. Perhaps the oldest method of assessing the electric field is to determine the enhancement of the Raman signal from a molecule in a plasmonic environment. While it is often challenging to determine the un-enhanced signal of the molecule, or the actual number of molecules being enhanced, the combination of experiments and calculations has provided insight into the electric fields in well-defined geometries.¹² As noted above, the calculations become complicated when the gaps are small, and current computers can only perform full quantum calculations on particles that are smaller than what is used experimentally. Another recent method to visualize plasmon resonances uses transmission electron microscopy to map the modes at discrete electron energies.¹⁸ This approach is a powerful method for samples amenable to TEM.

As experiments have shown that different plasmon resonances can excite molecules in different physical locations,^{19, 20} the ability to correlate electric fields to the observed molecular signals becomes increasingly important. In particular, the signals that arise from coupled nanostructures, such as dimers and more complex geometries, are complicated to interpret. In this report we will examine an example of extreme selectivity observed in TERS experiments with coupled nanostructures and propose a new method to assess electric fields in both TERS and SERS experiments.

2. METHODOLOGY

2.1 SERS substrate fabrication

SERS substrates used in this study were formed by either chemical vapor deposition of silver onto a porous anodized aluminum oxide filter,²¹ or by electrodeposition of silver or gold onto a wire embedded in polystyrene.^{22, 23}

2.2 SERS measurements

A combination of a commercial Renishaw In Via Raman microscope and a home built Raman microscope were used to obtain the SERS spectra reported. The various microscopes were equipped with either a 632.8 nm HeNe laser (Thorlabs, Inc.) or a 660 nm single

longitudinal mode diode laser (Laser Quantum). In general, the focused laser intensity at the sample was less than 1 mW, unless otherwise noted. The home built microscope consists of a Horiba-Jobin Yvon i330 imaging spectrograph and peltier cooled synapse CCD camera. Edge filters (Semrock) were used to reject the Raleigh scattering.

2.3 AFM measurements

Atomic force microscopy (AFM) images were obtained using a MV4000 AFM (Nanonics Imaging, LTD). The MV4000 uses a phase-locked-loop and a tuning fork to control and record the height of the AFM tip over the sample surface. AFM measurements were performed using tapered glass cantilever probes (Nanonics Imaging, LTC) with a nominal tip apex of 20 nm.

2.4 TERS measurements

TERS measurements were performed using the MV4000 AFM and a gold-ball CMP TERS tip (Nanonics Imaging, LTD). The diameter of the gold ball TERS tip was nominally 150-200 nm, as specified by the manufacturer, and was used as received. The TERS tip was placed in the focus of radially polarized laser beam, which was achieved using a liquid-crystal mode converter (ArcOptix, Inc). The Raman excitation excited the TERS tip from above as previously reported,^{24, 25} and the back-scattered photons were collected and measured using the home built Raman spectrometer described above.

2.5 Dark-field Microscopy

The TERS microscope was equipped with reflective dark-field capabilities, which were used to record the wide-field scattering image from our samples. A 100W tungsten lamp was used for illumination through a reflective dark-field microscope objective (Olympus, BD LMPlanFLN, NA=0.5). Images were recorded with a commercial color cMOS microscope camera (Thorlabs, Inc.)

2.6 Data Analysis

Data analysis was performed using an open source peak fitting routine for MatLab.²⁶ A Gaussian lineshape was fit to the peaks in the Raman spectra, and the fit was optimized to obtain the lowest % RMS error.

2.7 Computational Modeling

Finite element method simulations were performed using COMSOL 4.2a. Gold nanoparticles and slabs were embedded in a medium with a dielectric constant $n=1$. The electric field was modeled to propagate along the z -axis at a wavelength 632.8 nm. Here, it is important to note that, the expression describing radial polarization includes an integral that has no closed form solution and, therefore, cannot easily be modeled by COMSOL. Because we are only interested in the scattered electric field in the immediate vicinity of the nanoparticle, we approximate the electric field at the focus by modeling polarization components of equal magnitude along the y and z -axes.

3. RESULTS AND DISCUSSION

3.1 TERS selectivity in coupled nanostructures

In order to obtain significant enhancements in TERS experiments, it is common to form a gap resonance between the TERS tip and a metal surface.²⁷ The metal surface acts to create an image of the approaching tip, resulting in high electric field enhancements between the tip and the surface. This approach has been used for high sensitivity spectroscopy and imaging studies with both AFM and STM based TERS measurements.^{11, 28} However, the required metallic surface limits this approach to molecular and thin film systems. To increase the sensitivity of TERS experiments for investigating single cells and tissues, our laboratory has been investigating the Raman enhancements observed between a nanoparticle-TERS tip and a nanoparticle probe. In the case where the nanoparticle probe is functionalized with a small molecule or peptide specifically recognized by a protein receptor, we have been able to selectively detect the protein receptor.

In our initial proof of concept experiment, a 50 nm biotinylated gold nanoparticle was dispersed and detected on a streptavidin coated glass slide.²⁹ The strong binding interaction between biotin and streptavidin bound the nanoparticles to the glass surface to enable subsequent TERS imaging. Figure 1 shows the spectrum observed when the nanoparticle tip interacts with the nanoparticle on the surface.

The bands in the spectrum are observed to increase and decrease uniformly as the tip comes into contact and passes over the nanoparticle probe on the surface. In previous work, it was shown that maxima in the Raman scattering were observed on either side of the nanoparticle, resulting from the combined interactions between the nanoparticles and a linearly polarized laser.³⁰ The single maximum observed in this experiment is attributed to radial polarization used.

To assess the utility of this approach in intact cells, antibody functionalized nanoparticles were attached to SW480 cancer cells in culture.³¹ The cells were fixed and then studied by dark-field and TERS microscopy. Increased TERS scattering was observed to correspond to the positions of nanoparticles observed in the dark-field microscopy. COMSOL modeling of the nanoparticles under a pseudo-radial polarization supported our earlier claim that polarization effects account for the single maximum in the TERS imaging maps. In particular, the COMSOL models shows that the coupling is asymmetric and only enhances on one side of the nanoparticle probe. Unfortunately, the Raman signals observed all corresponded to the antibody on the nanoparticle. The observed signal enhancements are consistent with molecules in a gap, and questioned the origin of the streptavidin signals observed above.

To understand how the observed enhanced Raman spectrum correlated to the physical location of the molecules, we performed a series of experiments comparing the Raman signals from streptavidin and biotin functionalized nanoparticles probed by both SERS of aggregated nanoparticles and our TERS methodology.³² These results indicated that the majority of bands observed could be attributed to the streptavidin protein. Furthermore, differences were observed in the TERS spectra where streptavidin was intentionally put in

the gap (streptavidin functionalized nanoparticles) and when the streptavidin was bound to the surface. The spectral differences observed suggest that differences in the electric field environment may enhance different parts of the protein when on the glass surface or in the gap junction. Peaks were missing from the protein on glass samples that were observed in the other experiments. Importantly, the SERS spectra from aggregated nanoparticles contained all the peaks observed in the TERS experiments.

Our results with biotin suggested that small molecule ligands have more utility than large proteins due to the distance dependence reported for SERS experiments.^{33, 34} We tested this by functionalizing nanoparticles with a short cyclic peptide sequence: arginine-glycine-aspartic acid-phenylalanine-cysteine (c-RGDfC).³⁵ This sequence is reported to be specific for the $\alpha_v\beta_3$ integrin in human cells.³⁶ Based on our results with biotin and streptavidin, we also procured the purified $\alpha_v\beta_3$ integrin for comparative analysis by SERS with aggregated nanoparticles.

Figure 2 illustrates the remarkable result obtained using the c-RGDfC functionalized nanoparticles.³⁵ TERS detection of the c-RGDfC nanoparticles on a cell membrane, yield the same spectrum as is obtained from SERS experiments with just the functionalized nanoparticles and purified protein receptor. Control experiments done with bare nanoparticles and a non-binding peptide sequence did not produce the same spectrum. SERS spectra from aggregated nanoparticles on the cell surface also failed to produce the same spectrum observed in Figure 2.

To test the similarity between the TERS and SERS results, we developed a partial least squares discriminant analysis (PLS-DA) method to compare the spectral similarity.³⁷ We tested this algorithm with both the c-RGDfC- $\alpha_v\beta_3$ integrin results and with biotin-streptavidin samples. Interestingly, the observed TERS spectra show statistically similar spectra to the SERS spectra obtained from a purified protein binding to the functionalized nanoparticles, suggesting the amino acid residues being enhanced are similar in both cases. We further tested this by using a streptavidin mutant that removed a tryptophan we believed to reside near the nanoparticle probe. The mutated protein showed no statistical similarity in the PLS-DA algorithm to the SERS result of the streptavidin, suggesting this amino acid contributes substantially to the observed TERS and SERS spectra of the native protein.

The selectivity observed raises interesting questions. Where exactly is the receptor on the cell surface? How many receptors are being probed? Why don't we see the SERS spectra from other molecules on the cell surface? The differences in results from SERS imaging of nanoparticles aggregated on the cell surface versus the TERS detection of nanoparticles bound to the cell surface suggests that the plasmonic environment is both different and critical. We are in the process of investigating the origin and utility of these effects.

3.2 Vibrational Stark shifts as electric field reporters in coupled nanostructures

One of the challenges in identifying the origin of the enhanced Raman signals observed from coupled nanostructures is identifying the electric fields around the structures. Intensity based measurements are problematic because of the bias toward hotspots and challenges determining how many molecules are present. In the case of nanoparticles, TEM

measurements are possible. In cases with well-defined geometries, the fields can be modeled; however, recent debate about the importance of quantum effects in narrow gaps raises questions about the accuracy of these calculations and the ability to scale them to larger systems. Recent results from our lab suggest that frequency shifts from nitrile molecules adsorbed to plasmonic structures can report on the local electric fields.^{22, 23}

The initial result in our lab showed a correlation between the frequency of an adsorbed cyanide molecule on an electrodeposited gold surface and the SERS intensity of a co-adsorbed thiophenol molecule.²² The C-N stretch mode near 2200 cm^{-1} was observed to shift to higher energies in regions that exhibited larger SERS signals. We hypothesized that the shift correlated to the strength of the plasmonically induced electric field. To test this hypothesis we formed a gap-junction over the electrodeposited gold surface using a nanoparticle TERS tip. When the tip was brought near the surface in the laser focus, a reversible 130 cm^{-1} blue shift was observed for part of the CN stretching band.

Figure 3 compares the shift observed in the TERS measurement to a COMSOL simulation of sphere over a gold film. While not an exact match, there are features that can be evaluated to qualitatively assess the electric fields in this geometry. It is worth noting that parameters, such as roughness³⁸ and the thickness of the gold film³⁹, are known to perturb the result. Nonetheless, the model provides a picture for interpreting the TERS result.

In Figure 3 it is evident that the shifted signal arises from a smaller, higher intensity region between the TERS tip and the gold surface. The COMSOL calculation suggests that the enhancement within the gap is on the order of 10^6 , which seems reasonable. To further evaluate the TERS result, we need to explore the origin of shift.

It has been reported that metallic nanostructures undergo optical rectification when the plasmon resonance frequency is excited.⁴⁰ Optical rectification establishes a DC potential on the surface, which we believe results in a vibrational Stark shift in the nitrile frequency. In addition to our work with CN, a plasmonically induced Stark Shift was independently reported for CO between two spherical nanoparticles.⁴¹

To further explore these effects, we further investigated vibrational Stark shifts by intentionally adsorbing mercaptoalkylnitriles onto SERS active materials.²³ The strong affinity for sulfur to gold and silver provides a straightforward method to attach nitrile probes to the surface. In this study, we examined differences between CN from the electrodeposition process, n-mercaptobutylnitrile with a flexible C-C linker, and p-mercaptobenzonitrile with a rigid phenyl ring between the surface and the CN moiety. In all three cases, the observed Stark shift showed the expected linear increase with increased incident laser power. Interestingly, p-mercaptobenzonitrile showed a narrower CN frequency band, suggesting a more uniform adsorption geometry. The shifts were examined on heterogeneous electrodeposited silver surfaces and also on flat gold films with gold nanoparticles dispersed on top. The observed behavior further supports the shifts arise from optical rectification.

Optical rectification is a known second order nonlinear process, where a time invariant field arises from the second order susceptibility, $\chi^{(2)}$.⁴² This DC potential then perturbs the nitrile

bond giving rise to the observed Stark shift (μ). From the observed Stark shift it is then possible to determine the local optical field (E_{AC}) through the following relation:

$$E_{AC} = \sqrt{\frac{\Delta\mu}{\chi^{(2)}}} \quad (1)$$

From the optical field, it is then possible to determine approximate enhancement factors via the $|E|^4$ approximation. The key advantage to this method is the frequency shift is independent of intensity or the number of adsorbed molecules. From the example in Figure 3, this shift appears sensitive to differences in electric field environments on the length scale of nanometers. It is worth noting that optical rectification is also sensitive to tunneling and nanostructure that may differ from SERS enhancements, yet our results thus far indicate this is an promising method to determine electric fields. Perhaps the most challenging aspect of this method is the lack of data regarding the value of $\chi^{(2)}$.²³ Improved values for the second order susceptibility could provide a quantitative measure of the electric field.

Figure 4 shows the SEM image of and electrodeposited silver electrode and the map of the CN frequency at each pixel in a SERS map. The CN frequency provides a qualitative measurement of the electric fields on the surface. The variation in the CN frequency is consistent with the heterogeneous SERS enhancements observed from these surfaces. Further work will investigate how the observed frequencies scale across different SERS substrates and other plasmonic materials.

4. CONCLUSIONS

We have reported on the selective Raman enhancement of molecules bound to coupled nanostructure systems. Specifically, we show that a nanoparticle with a ligand specific to a protein receptor can selectively enhance the Raman spectrum of the protein even in a complex chemical environment like a cell membrane. This selectivity appears to depend on the controlled interaction between the nanoparticle probe and the nanoparticle TERS tip. The origin of the selectivity is not entirely clear, but changes in the electric field around the particles seem to be important. To experimentally assess these electric fields, we present a new method that uses changes in the vibrational frequency of an adsorbed nitrile functional group to assess the local electric field. The nitrile frequency is reported to shift in response to an optically rectified electric field, which is directly related to the local field enhancements. This methodology suggests a new way to correlate the electric fields that give rise to Raman enhancements in SERS and TERS experiments.

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Figure 1.

The Raman spectrum that arises from a nanoparticle TERS tip interacting with a biotinylated nanoparticle on a streptavidin protein surface, as illustrated, is shown. Raman bands attributed to biotin and streptavidin are marked “B” and “S”, respectively. The amino acids and protein structures that are associated with the S bands are noted. The inset shows the TERS image from which the spectrum was obtained. The pixel size in the inset image is 20 nm, and the scale bar (white) is 100 nm.

Figure 2.

The interaction between a c-RGDfC functionalized 80 nm nanoparticle and a nanoparticle TERS tip on the intact membrane of human colon cancer cell is illustrated. The TERS map, plotted from the intensity of the 1004 cm^{-1} band, shows a signal characteristic of a nanoparticle on the cell surface, the topography of which is shown below. Inspection of the most intense pixel in the TERS map, shows a spectrum with significant similarity to the SERS spectrum obtained from purified $\alpha_v\beta_3$ integrin mixed with the functionalized nanoparticles.

Figure 3.

A) The Raman spectrum observed when a nanoparticle TERS tip is in contact with an electrodeposited Au surface with adsorbed cyanide is shown (blue). The spectrum when the tip is retracted is shown (red). A control experiment using the same TERS tip on a flat template stripped gold surface is shown for comparison (black). The zoom region shows the changes in the CN stretch frequency for the three experiments. B) The COMSOL result for a 100 nm gold sphere 2 nm over a 50 nm gold slab, illuminated at 632.8 nm with polarization components simulating the conditions of a radial polarization. The scattered electric field (E), and the effective $|E|^4$ enhancement is plotted along the line between the sphere and the slab.

Figure 4. The SEM of an electrodeposited silver SERS surface (left) and the map of CN frequency variation across a similar surface shows heterogeneity, reflective of the SERS activity on the surface.