Nanoparticle-Containing Structures as a Substrate for Surface-Enhanced Raman Scattering

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Metallic nanostructures were prepared through the alternate immersion of derivatized glass slides in solutions of gold nanoparticles (NPs) and a propanedithiol linker molecule. Nanostructures consisting of 1−17 depositions of gold NPs were synthesized, and these substrates were characterized using UV−vis spectroscopy and atomic force microscopy. Subsequently, the surface-enhanced Raman scattering (SERS) of oxazine 720 was obtained at two excitation wavelengths (632 and 785 nm) from all substrates. Maximum SERS enhancement was observed for 9 and 13 NP depositions for 632 and 785 nm excitations, respectively. The difference in the number of NP depositions required for maximum enhancement is attributed to different wavelengths which can excite distinct aggregate structures within the metallic substrate. Therefore, these NP-containing structures can be “tuned” to yield maximum SERS enhancement for the excitation source being used by varying the number of NP depositions.

1. Introduction

Surface-enhanced Raman scattering (SERS) is one of the most sensitive methods for the detection of adsorbed molecules on nanostructured metal surfaces.1−4 Signal enhancements on the order of 10⁴−10¹⁴ are routinely observed, and in some systems enhancements of up to 10¹⁵ can be obtained.5 Such enhancement factors make this technique readily applicable to the study of submonolayer quantities of analytes.

Typically, the SERS effect occurs when a species is adsorbed onto a rough surface of a free electron metal such as Au, Ag, or Cu.2 Excitation with visible light and near-IR is generally used.3 The origin of SERS stems from two different mechanisms.4 In the first, called the electromagnetic mechanism (EM), the excitation and localization of surface plasmons within the metallic nanostructure enhance the local electromagnetic field.4 In the second, known as the chemical mechanism, a charge-transfer (CT) complex between the adsorbed molecule and the atomic scale roughness at the metal surface (adatoms) results in an enhanced polarizability of the adsorbate. In this case, the difference in energy between the frontier molecular orbitals of the adsorbed molecule and the metal’s Fermi level is within the laser excitation, leading to an additional contribution similar to the resonance Raman effect.5,6 The contribution of the CT resonance can be controlled by tuning the position of the Fermi level, as is commonly observed in electrochemical SERS.

SERS has attracted the attention of analytical chemists since its discovery because of its ability to yield structural information from minute amounts of adsorbed species. However, SERS has not lived up to its potential due to a lack of suitable reproducible substrates for sample analysis. For analytical applications, the requirements of an ideal SERS substrate are numerous, but the most important include low cost, highly enhancing, rugged, and reproducible.7

Colloidal gold and silver, and in particular their aggregates, were first observed to be highly SERS enhancing.8−11 Structures containing aggregates in the 100−1000 nm size regime were particularly useful for trace analytical applications.5,8 The aggregate structures were prepared by the “activation” (or aggregation) of the colloidal sol using a NaCl solution, in the presence of a small amount of analyte. While this method does provide a remarkable enhancement of the Raman signal, it does not provide a reproducible aggregate structure. As a consequence, obtaining quantitatively reproducible SERS signals becomes troublesome.

SERS-active substrates can be prepared by the vapor deposition of metals (Au, Ag, and Cu) onto supporting surfaces such as glass or SiO2/Si.12,13 The temperature of the substrates, the film thickness, and the rate of deposition have all been modulated to provide a substrate with increased enhancement.14−16 Metals have also been evaporated on rough surfaces such as alumina-covered glass, TiO2-coated glass, and filter paper.17

An alternative preparation strategy for SERS substrates is one pioneered by the van Duyne group, which provides a method for...
creating controllable, predictable, and reproducible SERS substrates. In nanosphere lithography (NSL), polystyrene (PS) nanoparticles (typically hundreds of nanometers in diameter) are spin coated onto a glass surface to form a two-dimensional hexagonal array. The PS beads act as a “mask” for the layer of silver that is then vapor deposited over the top of the assembly. Silver deposits at the interstices of the beads, after which the beads are removed by sonication. What remains behind is a periodic particle array (PPA) of silver islands dispersed across the glass surface. This PPA can be tuned by varying the diameter of the PS beads, the angle of vapor deposition, and the amount of silver deposited to give structures of different geometries or separations. This allows for a tunability of the surface roughness to correspond to a particular excitation wavelength, and thus to yield maximum SERS enhancement.

The creation of nanostructured arrays can also be accomplished through the use of electron-beam lithography. Gold or silver is vapor deposited onto a SiO\textsubscript{2}/Si wafer, and then an electron beam is used to create arrays with patterns of features ca. 100 nm in size. Focused ion beams (FIBs) have also been used to generate nanohole arrays which are also Raman enhancing.

SERS-active “sandwich” structures have been prepared by Zhang et al. This method involved the assembly of functionalized azobenzenethiols onto surfaces that consisted of gold, silver foil, or etched silver foil. What made this investigation particularly interesting was that a further layer of silver was vapor deposited on top of the azobenzene to give a sandwich-like architecture. Vibrational stretches from the azobenzene were particularly enhanced when protrusions of 100 nm were created on the surface of the substrate. Features larger or smaller than this size showed a decrease in SERS signal. In addition, Zhang also showed that the enhancement factor decreases exponentially with increasing distance of the azobenzene group from the underlying substrate or the overlying silver layer. This is a critical reminder that this enhancement is a surface effect, and decays rapidly away from the surface.

Solution-based assembly strategies for the development of SERS-active substrates have also been a focal point of research in the past several years. In particular, the assembly of gold nanoparticles on a glass surface has been heavily studied. Chumanov et al. initially demonstrated the utility of depositing metallic nanoparticles (NPs) on derivatized glass slides as a substrate for surface-enhanced spectroscopy. Another initial paper in this field was produced by the Natan group and similarly involved the deposition of gold nanoparticles onto a derivatized glass surface. These substrates were characterized for their suitability and were found to be SERS-active. Many papers were produced by this research group characterizing these new substrates and the underlying theory behind them.

Subsequently, Bright et al. published a paper in which a layer of silver was chemically deposited over the top of a two-dimensional colloidal monolayer deposited from the colloidal solution. It was believed that the combination of silver (a strong SERS-active metal) and the underlying morphology of the surface would yield a highly enhanced SERS signal. While a large signal enhancement was observed, the electrochemical deposition process must be precisely tuned to yield optimum results.

The construction of multilayer substrates consisting of alternate layers of colloidal monolayers and a bifunctional linker molecule has also been reported. It was suggested that this structure could be an interesting SERS substrate because it could offer roughness tunability based on the number of colloid layers deposited. It is that paper that provides the motivation for this work.

One final extrapolation of Natan’s work was performed by Mulvaney et al. and involved the construction of three-layer substrates consisting of a combination of nanoparticle assemblies and evaporated thin films. A layer of colloidal gold was deposited onto a glass surface with subsequent chemical deposition of Ag and then vapor deposition of another layer of Ag. The well-defined surface morphology of the Au colloid layer and the optical properties of the Ag layers were expected to yield enhanced SERS signals. Enhancement factors on the order of 10\textsuperscript{8} and picogram detection limits were reported.

A further advancement in the application of immobilized Au nanoparticles onto glass surfaces for SERS is reported in this work. SERS substrates consisting of alternating depositions of gold NPs and a dithiol molecule were synthesized. The substrates were then rigorously characterized using UV–vis spectroscopy and atomic force microscopy (AFM) to obtain morphological and spectroscopic information. The prepared substrates were examined for use in SERS, and the results correlated with the other morphological and spectroscopic information obtained.

2. Experimental Section

2.1. Chemicals. Unless otherwise noted, ACS reagent grade chemicals were used. HAuCl\textsubscript{4}, sodium citrate dihydrate, and propanedithiol (PDT) were obtained from Sigma-Aldrich. 3-(Mercaptopropyl)trimethoxysilane (MPTMS; 97%) was obtained from Caledon Laboratories Ltd. Oxazine 720 was obtained from LambdaPharm. HCl, H\textsubscript{2}SO\textsubscript{4}, and HNO\textsubscript{3} were obtained from chemical grade.

All water used was from a Barnstead NANOpure Diamond water purification system. The ultrapure water had a resistivity greater than 18.2 M\(\Omega\) cm.

2.2. NP Synthesis. The use of clean glassware is critically important for the successful synthesis of gold NPs. All glassware used here went through a rigorous cleaning procedure, which involved soaking in a hot (ca. 100 °C) H\textsubscript{2}SO\textsubscript{4} bath overnight. The glass was subsequently rinsed with deionized water and heated at 110 °C in
an oven to dry. To remove any remaining impurities, the glassware was immersed in a bath of Aqua Regia solution (3:1 HCl/HNO₃) for 20 min and rinsed with deionized H₂O. The glassware was then heated at 110 °C to remove residual H₂O.

The standard procedure for the synthesis of colloidal gold is a method published by Frens. In this work, a slight modification to the Frens procedure published by Grabar et al. was used. A 500 mL portion of a 1 mM solution of HAuCl₄ was brought to a rolling boil with stirring, and then 50 mL of 38.8 mM sodium citrate was added. The solution color instantly changed from yellow to colorless. Heating and stirring were continued for 10 min, which resulted in the solution changing to a wine-red color. After this time period, heating was discontinued and stirring was allowed to continue for another 15 min to complete the reaction.

To improve the longevity of the prepared colloidal gold solutions, they were stored in an amber bottle at 4 °C. Colloid solutions were observed to be stable for up to 1 year without appreciable aggregation or bacterial growth.

2.3. NP Depositions on Glass. Glass microscope slides (Fisher, 3 in. x 1 in.) were cut into approximately 1 in. x 1 in. squares using a glass cutter. The slides were then sonicated in 95% ethanol followed by deionized water for 10 min to dislodge any visible particulate matter.

The slides were then submerged in a 70 °C piranha solution for 10 min to remove any organic material. Piranha solution consists of a 3:1 (v/v) mixture of concentrated H₂SO₄ and 30% H₂O₂. The slides were then rinsed copiously with H₂O followed by MeOH.

Once cleaned, the glass slides were placed in a 5% (v/v) solution of MPTMS in MeOH. The slides were allowed to derivate for a minimum of 12 h. Afterward, they were removed from the silane solution and rinsed copiously with MeOH, followed by H₂O, and then dried under a gentle stream of nitrogen.

The derivatized slides were placed into a solution of Au NPs and allowed to sit for 24 h to complete nanoparticle self-assembly and form a single nanoparticle-layer substrate. After the prescribed time period, the substrates were removed from the NP solution and rinsed with H₂O. The substrates were stored in H₂O when not being immediately used.

To add further nanoparticle layers, the substrates were immersed in a 5 mM methanolic solution of PDT for 15 min, removed, and rinsed with copious amounts of methanol followed by H₂O. The substrates were then immersed in a Au NP solution for 1 h. The substrates were then removed and rinsed with copious amounts of H₂O followed by methanol. This process was repeated until the desired number of NP depositions was obtained.

2.4. UV-Vis Characterization. UV-Vis characterization of the Au NP solutions was performed using a Cary 50 UV-Vis instrument controlled by the Cary WinUV software. The scan range was 400–900 nm with a scan rate of 600 nm/min and 2 nm resolution. Disposable cuvette cells were used for all measurements. A deionized water spectrum, obtained under the same experimental conditions as other measurements, was used as a background.

The Au nanostructures were characterized using a Cary 1E UV-Vis spectrophotometer in absorbance mode. Instrument control was performed using the Cary WinUV software. Spectra obtained using dry Au NP-coated glass exposed to air are referred to as ex situ, and experiments involving the substrates in the presence of a solvent are dubed in situ. For ex situ measurements, a filter holder (Melles Griot) was attached to a machined metal plate. This allowed for the substrate to be placed in the sample beam of the instrument. Ex situ scans were acquired from 400 to 900 nm at 333 nm/min with a resolution of 2 nm. All in situ measurements were performed in water. A background scan consisting of a silane-derivated slide was obtained using the same instrumental settings.

2.5. AFM Characterization. AFM scans were obtained using a ThermoMicroscopes Digital AFM instrument operated under ambient conditions. Noncontact mode was used for all scans.

Noncontact AFM tips were obtained from ThermoMicroscopes (model 1650-00). AFM tips are of the I-type geometry and are made of silicon. The nominal dimensions of the tips were 120 µm length, 26–28 µm width, and a thickness of 3.6–4.4 µm. The tips had a force constant of 28–56 N/m and a nominal resonant frequency between 307 and 384 kHz.

Scans were obtained at a 1000 nm by 1000 nm scan range, with a total of 200 lines scanned. The scan speed was 1000 nm/s. Detector settings (proportional, integral, and derivative) and the tip set point were optimized for each scan.

2.6. SERS Measurements. A 5 mM methanolic solution of oxazine was prepared, and this solution was placed onto the SERS substrate to cover the entire surface. The ethanol was allowed to evaporate over a period of 10 min and resulted in the deposition of oxazine onto the nanoparticle surface. Residual or excess oxazine was removed by rinsing the substrate with copious amounts of water. The substrates were then dried under a gentle stream of nitrogen and then placed in the Raman microscope system for measurement.

All Raman measurements were performed on a Renishaw inVia Raman microscope system. The Raman signal output was sent to a PC computer running the WIRE software (version 2.0, Renishaw Instruments).

The excitation source was a 632 or 785 nm laser light and was operated at 10% of the total laser power (approximately 5 mW). A 50× objective was used for all measurements. Spectra were acquired from 100 to 2000 cm⁻¹ at 4 cm⁻¹ resolution. For 785 nm excitation, five accumulations of 5 s exposure were performed. For 632 nm excitation, five accumulations of 1 s exposure were used.

3. Results and Discussion

3.1. Substrate Characterization. Regular glass slides do not facilitate adsorption of Au NPs onto their surface. Therefore, surface derivatization was necessary and was accomplished through the reaction of MPTMS with the pendant hydroxyl groups of the glass slide. The net result is a pendant thiol group emanating from the surface of the modified glass slide. Using the well-known gold thiol chemistry, NP deposition will occur when a derivatized glass slide is immersed in a gold NP solution.

What makes gold NPs particularly attractive is their incredibly high molar absorptivity coefficient. Commonly reported values are on the order of 10⁴ M⁻¹ cm⁻¹, which is much greater than those of many common organic dyes. This intense absorption is due to the excitation of localized surface plasmons (SPs). The inset of Figure 1 shows the visible absorption spectrum of an aqueous solution of gold NPs prepared in this work. Of note is the pronounced absorption near 520 nm due to the SP absorption.

The UV–vis spectrum of a monolayer of Au NPs deposited onto a derivatized glass slide is shown in Figure 1. A pronounced absorption at 520 nm is also noted, which is due to the SP excitation of the deposited NPs. Indeed, even a monolayer of Au NPs is markedly colored when observed by the naked eye. Also note that the plasmon maximum for the UV–vis spectrum of an NP substrate, obtained in situ, looked markedly similar to that for Au NPs in solution (Figure 1), which is indicative of a small level of aggregation.

The NP substrates were then treated with a solution of PDT. This bifunctional molecule acted as a linker to facilitate adsorption of additional gold NPs.

several glass slides modified with a varying number of NP depositions obtained using the ex situ condition. The UV-\textvisiblespectra in Figure 2 show a continuous red shift as additional Au NPs are deposited. A broad envelope at longer wavelengths also develops with an increase in the number of deposited Au NPs. A similar red shift in the main SP absorption plus the development of a broad envelope at long wavelengths as the number of Au NP depositions increased was also observed when similar experiments were performed using the “in situ” arrangement (data not shown).

The surface plasmons of gold colloids are extremely sensitive to their local environment.\(^{36}\) When aggregation of the colloids occurs, that is to say, when the colloids irreversibly approach too closely, the dipole approximation breaks down.\(^{38}\) Instead, one must treat each of the colloids as a multipole, because adjacent colloids thus have an effect on each other.\(^{38}\) This effect results in the formation of an absorption feature that is red shifted and is characteristic for aggregated colloids. This phenomenon suggests that, as more gold NP layers are deposited onto the substrate, the colloids become increasingly aggregated to form large structures within the substrate.\(^{38}\) The broad red-shifted peak shown in Figure 2 indicates the aggregation of the metallic NPs upon drying. It is well-known that the dielectric environment has a direct effect on the morphology of NP aggregates.\(^{39}\) Similar changes in the optical characteristics of immobilized NPs upon drying have also been observed.\(^{32}\)

Figure 3 shows the dependence of both the integrated peak area of the UV-\textvisiblespectra (Figure 3a) and the wavelength of maximum absorption ($\lambda_{\text{max}}$) (Figure 3b) on the number of NP depositions. Only the values for the ex situ experiments are shown in Figure 3. As additional layers of gold NPs are adsorbed, an overall increase in the UV-\textvisiblesorption is observed, as shown in Figure 2. Previous studies of similar systems show a linear increase of the absorption with increasing number of layers, as predicted by Beer’s law.\(^{32}\) In those cases, however, the absorption


at one wavelength, measured for all of the substrates, was used. However, in the spectra presented in Figure 2 there is a continuous shift in the wavelength of maximum absorption, and thus, this would not be a preferred manner to quantify the absorbance of the substrate. Instead, the individual spectra from Figure 2 were integrated between 475 and 900 nm, yielding a value proportional to the integrated absorption coefficient. This quantity thus represents the overall absorption due to colloids in different environments (aggregated or not) that exist within the substrate. The change in the integrated absorption with increasing number of NP depositions is shown in Figure 3a. Two linear regions are observed. The first linear region extends up to 10 NP depositions and presents a steeper slope. The increase in the absorption with the number of depositions is gentler after 10 NP depositions.

As shown in Figure 3b, there is initially a dramatic increase in the wavelength of maximum absorption with the number of NP depositions, shifting from 595 nm for a monolayer of Au NPs to 655 nm after seven NP depositions. After seven Au NP depositions, the magnitude of the wavelength shift with Au NP depositions is substantially lower, due to the colloids reaching the minimum approachable distance between each other. This behavior is similar to that obtained by Li et al.

While some morphological information can be inferred from the UV-vis spectra, namely, the degree of aggregation of NPs, a rigorous morphological characterization must be performed using an imaging technique such as AFM. AFM allows for the measurement of features at the nanometer level, such as individual NPs or NP aggregates. A selection of AFM images for the substrates prepared for this work is shown in Figure 4.

With one NP layer deposited on the derivatized glass slide, individual colloids can be observed protruding from the slide (Figure 4a). Examining the representative line scan obtained from the topographic image, one can see that the protrusions emanating from the surface are approximately 10 nm high. This value is on the order of the diameter of gold NPs used here. While height measurements obtained from AFM can be used as is, lateral dimensions are affected by an artifact called tip-sample convolution. Measurements made from the line scans were corrected for tip-sample convolution using the method outlined by Doron et al.

With increasing NP depositions, an overall increase in the size and span of the structures is observed (Figure 4b–d). This suggests that the NPs are transitioning from individual entities on the surface into larger aggregate structures with additional NP depositions. This is in agreement with the UV-vis data (Figures 2 and 3), which showed an SP maximum at longer wavelengths with increasing depositions. The two linear regions shown in Figure 3a should also be related to the surface morphology, since a simple linear Beer law plot should not be expected due to other extinction channels from the nanostructures, such as scattering and reflection.

The average feature size of the NP aggregates was measured from the representative line scans, and the change with increasing NP depositions is shown in Figure 5.

The ability to fine-tune the overall substrate morphology on the basis of the number of NP depositions makes this technique particularly interesting for use in SERS. The underlying roughness can be tuned until the substrate yields the maximum enhancement factor through the SERS mechanism. This aspect will be further explored in the next section.

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3.2. SERS Data. Oxazine 720 was chosen as the Raman analyte for this work because of its well-characterized chemical and spectroscopic properties. All SERS data were obtained using only the dried substrates (ex situ). The substrates needed to be dried for AFM measurements. The morphological changes induced upon drying were irreversible; therefore, placing the slides back into aqueous solution for SERS measurements would induce more uncontrolled morphological changes and had a direct impact on the reproducibility of the SERS intensities. Moreover, the procedure for the reproducible deposition of the dye, described in the Experimental Section, is also more suitable for ex situ SERS.

The SERS spectrum of oxazine 720 was measured on the various NP substrates, and the results are shown in Figure 6a for 632 nm excitation and in Figure 6b for 785 nm excitation. A variety of Raman transitions were observed in Figure 6, and these transitions can be correlated with the Raman spectrum of a sample of solid oxazine dye. A small Raman signal is initially observed when oxazine is deposited onto one NP layer, suggesting that the substrate is only weakly enhancing. With increasing NP depositions the oxazine Raman signal undergoes a dramatic increase in intensity. From examining the spectra in Figure 6a, note that all peaks in the Raman spectra were observed to change concomitantly with increasing NP depositions. To examine the dependence of the Raman signal on the number of NP depositions, the intensity of the 591 cm$^{-1}$ band was plotted as a function of the number of NP depositions, and the results are presented in Figure 7.

For 632 nm excitation, the oxazine signal undergoes a nearly constant increase with increasing NP depositions before the maximum signal enhancement is achieved for nine NP depositions, as shown in Figure 7a. The average feature of the substrate with nine NP depositions obtained by AFM and shown in Figure 5 was around 40 nm. The signal at 11 NP depositions is dramatically reduced, and the signal for 13–17 NP depositions slowly decreases as the average feature size increases.

The results for 785 nm excitation are presented in Figure 7b. One- or three-NP-layer substrates are only weakly enhancing, and this is reflected in the overall poor signal to noise (S/N) ratio of their SERS spectra in Figure 6b. With increasing NP depositions between 5 and 11 layers, an overall and continuous increase in the SERS enhancement is observed, with the peak intensities of the Raman band dramatically increasing. A maximum in the SERS signal is observed for 13 NP depositions, which corresponds to an average feature size of around 55 nm. The SERS signal is increased 100-fold over the one-NP-layer substrate, while the surface area, as measured by AFM, only increased 20%. After 13 Au NP depositions, the SERS signal is dramatically suppressed.

The error bars indicated in Figure 7 are from replicate measurements leading to an RSD on the order of 10–40%, which is comparable to those of previous reports from other SERS substrates.

The substrate’s ability to enhance the Raman process is related to the excitation of surface plasmons within the structure. The frequency (and hence wavelength) required for SP excitation is highly dependent upon the shape and size of the aggregate structures. However, when the SPs are excited, a pronounced absorption in the UV—vis spectrum is observed at that particular wavelength. For example, Figure 3b shows $\lambda_{\text{max}}$ for the UV—vis spectra of the NP substrates prepared here. Several authors argued that $\lambda_{\text{max}}$ would correspond to the excitation wavelength

![Figure 6](image)

**Figure 6.** Raman spectra of oxazine deposited on the nanostructures consisting of 1–17 NP depositions: (a) 632 nm and (b) 785 nm excitation. Spectra shown are for an odd number of NP depositions.

![Figure 7](image)

**Figure 7.** Increase in the 591 cm$^{-1}$ Raman stretch of oxazine with increasing NP depositions at 632 and 785 nm excitation.

that would be capable of exciting the largest proportion of aggregate structures. In turn, this would provide the largest SERS enhancement.

Analyzing the data obtained from 632 nm excitation, shown in Figure 7a, a maximum enhancement was observed for nine NP depositions. From Figure 3b the wavelength of the absorption maximum for a glass substrate after nine NP depositions is ca. 650 nm. This $\lambda_{\text{max}}$ is close to the excitation energy of the laser. On the other hand, for 785 nm excitation, the enhancement maximum is observed for 13 NP layers (Figure 7b), and as shown in Figure 3b, $\lambda_{\text{max}}$ for this substrate is around 665 nm.

A significant decrease in the enhancement is observed for both excitations after the maximum, as illustrated in Figure 7. Similar behavior has been observed by Olson et al. for alkylsilane-coated gold nanoparticles deposited onto hydrophobic surfaces.49

The mismatch between the laser excitation energy and the SP resonance of the nanostructures can be explained on the basis of the fact that only a small region in the aggregate structure is extremely enhancing and contributes significantly to the SERS effect. These localized regions of large electromagnetic field are known as the “hot spots” of the nanostructure. In fact, Etchegoin et al. have shown that the resonances of the hot spots do not coincide with the plasmon resonance distribution measured by UV—vis spectroscopy.50,51 Therefore, the value of $\lambda_{\text{max}}$ cannot be used as a definitive guide for maximum enhancement. A systematic investigation, such as the one presented here, is then important to find the right match between the magnitude of the enhancement and the nanostructure.

**4. Conclusions**

Au nanostructures, consisting of alternate depositions of gold NPs and a dithiol linker molecule, were synthesized in this work. The nanostructures were characterized using UV—vis spectroscopy, owing to the intense optical absorption inherent to the gold NPs. A shift of the absorption features to longer wavelengths was noted with increasing NP depositions and is attributed to increasing aggregation of the gold NPs. In addition, the nanostructures were examined using AFM imaging. An overall increase in aggregate structure size was observed with increasing NP depositions.

The ability of these nanostructures to act as a substrate in SERS was then determined by adding the Raman-active analyte oxazine 720 onto the surface of the structures. SERS spectra were obtained using 632 and 785 nm excitation. Maximum SERS enhancement was observed for 9 and 13 NP depositions for 632 and 785 nm excitations, respectively.

The increased enhancement is attributed to SP excitation within the aggregate structures. Different excitation wavelengths are capable of exciting different aggregate structures, which are attributed to the different results observed at 632 and 785 nm. Therefore, it has been shown that this substrate can be tuned to yield maximum enhancement for a particular excitation wavelength, on the basis of the number of NP depositions that occur.

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