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I am submitting herewith a dissertation written by Jenny M. Oran entitled “The Rational Design and Lithographic Fabrication of Surface Enhanced Raman Spectroscopy Substrates.” I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Chemistry.

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**THE RATIONAL DESIGN AND LITHOGRAPHIC
FABRICATION OF SURFACE ENHANCED RAMAN
SPECTROSCOPY SUBSTRATES**

A Dissertation
Presented for the
Doctor of Philosophy Degree
The University of Tennessee, Knoxville

Jenny M. Oran
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Dedication

To my wonderful parents,

Gary and Jan Oran.

Without your encouragement, patience, support, and
love, I would not be the person I am today.

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Abstract

The analytical capabilities of surface-enhanced Raman spectroscopy (SERS) reside in the performance characteristics of the SERS-active substrate. Signal enhancement observed in SERS is attributable to the presence of noble metal nanostructures on substrate surfaces. The rational design and control of variables such as shape and size, and distribution, density, and spacing of these nanostructures can lead to substrates that have greater analytical sensitivity and yield more reproducible enhancement. Through systematic control of the morphology of our SERS substrates, we have created ordered periodic arrays as well as random aggregates of nanoscale particles using electron beam lithography (EBL). A unique aspect of these EBL-created substrates is that the morphology is known with great precision. Once fabricated, the arrays and/or aggregates are coated with a SERS-active noble metal through physical vapor deposition (PVD).

Both the uniform and random lithographically produced nanopatterns are studied by surface enhanced Raman spectroscopy to examine the Stokes responses of various analytes, while scanning electron microscopy (SEM) is used to examine pattern surfaces post lithographic development and post noble metal deposition. In the case of the ordered structures, raw and normalized SERS data is seen to correlate with data from simple electrostatic calculations as well as the broad background continuum underlying each spectrum collected. Borrowing from the biological concepts of cloning and combinatorial chemistry, random morphology patterns are designed and spectrally mapped to locate “hot spots” within aggregates. Regardless of the type of substrate, ordered or random, by

using EBL, the substrates can be reproducibly fabricated, yielding consistent analyte environments each time the substrate is created.

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Nomenclature

α	polarizability of molecule/analyte bond
α_0	equilibrium polarizability
c	speed of light ($2.997\,924\,58 \times 10^8$ m/s)
cm^{-1}	wavenumbers
E	electric field of the incident radiation (laser beam)
E_0	maximum amplitude of the incident electromagnetic field
ΔE	difference in energy between vibrational energy levels in Raman diagram
ϵ	dielectric constant
F	field enhancement factor
μ	induced electric dipole
μm	micrometer (micron)
m	dipole moment
nm	nanometer
ν	frequency (vibration or radiation)
ν_{ex}	frequency of incident radiation (excitation frequency)
$\nu_{\text{ex}} - \nu_{\text{v}}$	Stokes frequency
$\nu_{\text{ex}} + \nu_{\text{v}}$	anti-Stokes frequency
r	internuclear separation at any instant
r_{m}	maximum internuclear separation relative to position at equilibrium
AFM	Atomic force microscopy

CAD	Computer aided design
CCD	Charge-coupled device
DDA	Discrete dipole approximation
EBL	Electron beam lithography
HOMO	Highest occupied molecular orbital
LSP	Localized surface plasmons
LUMO	Lowest unoccupied molecular orbital
NSL	Nanosphere lithography
PPA	Periodic particle array
PS	Polystyrene
PVD	Physical vapor deposition
QCM	Quartz crystal microbalance
R6G	Rhodamine 6G
RIE	Reactive ion etching
RSD	Relative standard deviations
SEM	Scanning electron microscopy
SERS	Surface enhanced Raman spectroscopy
SERRS	Surface enhanced resonance Raman spectroscopy
SPR	Surface plasmon resonance
TEM	Transmission electron microscopy
M	Molarity (moles/liter)
ma-2403	Methacrylate-based EBL resist

PDMS	Poly(dimethylsiloxane)
SEF	Surface enhanced fluorescence
NSOM	Near-field scanning optical microscopy

Chapter 1 Introduction

1.1 *The Raman Effect*

Raman spectroscopy is a useful, highly selective technique for the determination of structural information from analyte molecules. It is characterized as yielding narrow, well-resolved vibrational bands that essentially provide a “fingerprint” of the analyte, surface process, and/or interface reaction being examined [1, 2]. C.V. Raman first discovered the Raman method in 1928. He found that the wavelength of the incident beam impinging on particular analyte molecules differs from a small fraction of the radiation scattered by those molecules, and that the chemical structures of the molecules are responsible for the observed shifts in wavelength.

The theory of Raman scattering shows that the observed Raman signal of a molecule results from the same type of quantized vibrational changes that are seen with infrared absorption. The difference between the incident and scattered visible radiation wavelengths falls within the mid-infrared regions of the spectrum. Raman and infrared absorption are complementary techniques, though their spectra are often quite similar. One advantage that the collection of Raman spectra has over infrared absorption is that water does not interfere, therefore Raman signals can be obtained for aqueous solutions. In addition, glass or quartz cells can be used as sample holders as they also do not cause interference. Even with these advantages, Raman was not widely used for molecular structure determination until the 1960's, when lasers became available. Another drawback is that fluorescence often competes with the Raman signal of certain samples

or it can be observed if there are impurities present in the sample. However, this issue has been mostly overcome by the use of near-infrared laser sources. Surface-enhanced Raman techniques can also aid in quenching fluorescence.

Typically, Raman spectra are collected by irradiating a sample with a laser source - often monochromatic, visible or near-infrared radiation - and the scattered radiation from the sample is measured by a spectrometer. Three types of scattered radiation are emitted by the analyte when obtaining a Raman spectrum, Stokes, anti-Stokes, and Rayleigh. The last type, Rayleigh, is much more intense than the other two and is emitted at the same wavelength as the excitation source. There is no energy lost in Rayleigh scattering, and as a result this process is said to be *elastic*. Stokes lines can be found at wavenumber shifts (the difference in wavenumbers, cm^{-1} , between the source and the observed signal) that are smaller than the Rayleigh line (red shifted), while anti-Stokes shifts are found at wavenumbers greater than the Rayleigh line (blue shifted). The energy level diagram in Figure 1-1 shows the mechanism by which Raman spectra are generated. The arrow thickness is roughly proportional to the signal strength observed in the transitions. In general, the Raman effect is seen when incident radiation impinges upon a molecule, interacts with the electron cloud of the bonds of that molecule and excites electrons in the cloud to a virtual state.

The location of the electrons within the vibrational levels of the ground state prior to being elevated to the virtual excited state will determine which shift will be observed. For a Stokes shift, the electrons will start out in the lowest vibrational level of the ground state and relax to an excited level of the ground state. For the observation of an anti-

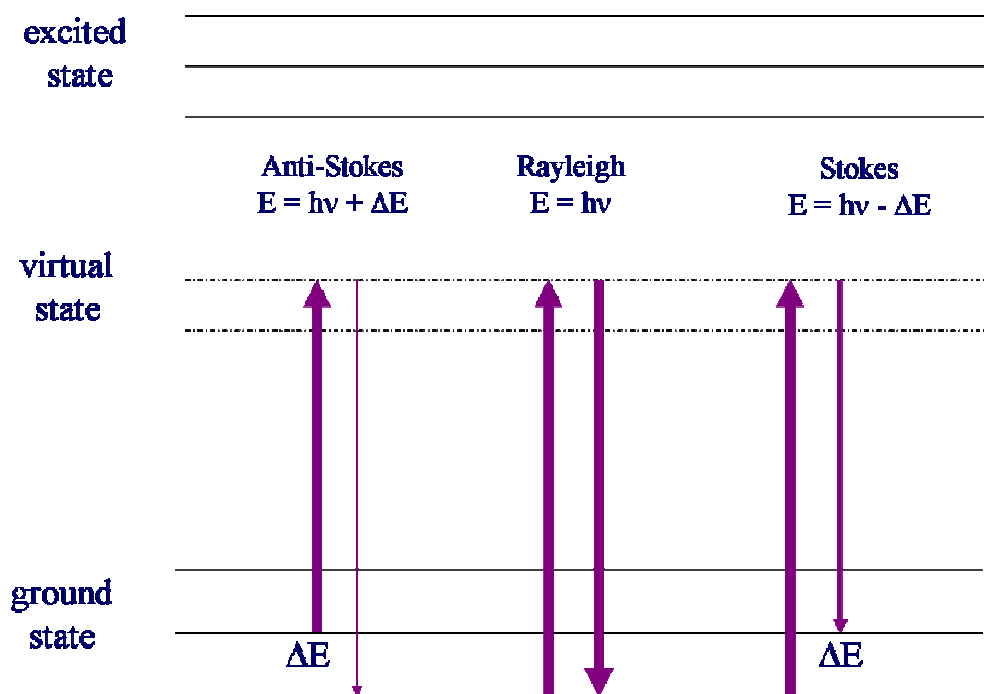


Figure 1-1. Raman energy level diagram.

Stokes shift, the electrons will be in an excited vibrational level of the ground state and relax to a lower vibrational level. At room temperature, the fraction of molecules starting out in this excited vibrational level will be small, so this process is much less likely to occur unless the temperature is increased. It should be noted that the energy changes that cause Stokes and anti-Stokes scattering differ from Rayleigh scattering by frequencies that correspond to $\pm \Delta E$, the energy of the first vibrational level of the ground state [3]. Regardless of which type of shift is observed, there must be a change in the polarizability (deformation) of the electron cloud, in order for the Raman effect to occur [4]. It is important to note that the degree of the Raman shifts are independent of the wavelength of excitation. Therefore, different excitation sources can be used – depending on experimental needs – and the same characteristic spectrum can be obtained for the same analyte each time.

Wave Model of Raman Scattering. To begin, assume that a beam of radiation of frequency, ν_{ex} , is incident upon an analyte solution. The electric field, E , of this radiation can be described by the following equation:

$$E = E_0 \cos(2\pi \nu_{\text{ex}} t) \quad 1-1$$

where E_0 is the amplitude of the wave. The interaction of the electron cloud of the analyte bond and the electric field of the radiation induces a dipole moment, m , which is given by:

$$m = \alpha E = \alpha E_0 \cos(2\pi \nu_{\text{ex}} t) \quad 1-2$$

where α is the polarizability of the bond. It is a proportionality constant, and is a measure of the deformability of the analyte bond in an electric field.

To be Raman active, the polarizability of a bond must vary as a function of the distance between nuclei given by the following:

$$\alpha = \alpha_0 + (r - r_{eq})\left(\frac{\delta\alpha}{\delta r}\right) \quad 1-3$$

where α_0 is the polarizability of the bond at the equilibrium internuclear distance r_{eq} , and r is the internuclear separation at any instant. As the frequency of vibration, ν_v , changes, so does the change in internuclear separation. It proceeds by this equation:

$$r - r_{eq} = r_m \cos(2\pi \nu_v t) \quad 1-4$$

where r_m is the maximum internuclear separation relative to position at equilibrium. The following equation, is what is given when equation 1-4 is substituted into 1-3:

$$\alpha = \alpha_0 + r_m \cos(2\pi \nu_v t)\left(\frac{\delta\alpha}{\delta r}\right) \quad 1-5$$

An equation for the induced dipole moment can be obtained when substituting equation 1-5 into equation 1-2.

$$m = \alpha_0 E_0 \cos(2\pi \nu_{ex} t) + E_0 r_m \cos(2\pi \nu_v t)\left(\frac{\delta\alpha}{\delta r}\right) \cos(2\pi \nu_{ex} t)$$

In trigonometry, we know that:

$$\cos x \cos y = [\cos(x + y) + \cos(x - y)]/2 \quad 1-6$$

Using this, equation 1-5 yields:

$$\begin{aligned} m = & \alpha_0 E_0 \cos(2\pi \nu_{ex} t) + \\ & \frac{E_0}{2} r_m \cos[2\pi(\nu_{ex} - \nu_v)t]\left(\frac{\delta\alpha}{\delta r}\right) + \\ & \frac{E_0}{2} r_m \cos[2\pi(\nu_{ex} + \nu_v)t]\left(\frac{\delta\alpha}{\delta r}\right) \end{aligned} \quad 1-7$$

The first term in the equation 1-7 represents the Rayleigh scattering, occurring at the excitation frequency, ν_{ex} . The second and third terms correspond to the Stokes ($\nu_{\text{ex}} - \nu_{\text{v}}$) and the anti-Stokes ($\nu_{\text{ex}} + \nu_{\text{v}}$) frequencies, respectively. In order for Raman lines to be obtained, the polarizability of the bond must vary as a function of distance, that is $\delta\alpha/\delta r$ must be greater than zero [3]. This selection rule criteria differs from that for IR absorption processes wherein the vibration must change the dipole moment of the molecule.

Instrumentation. Modern Raman instruments consist of three major components: a laser source, a sample illumination system, and a spectrometer. Raman instruments and their components have stricter performance-related requirements than most other molecular spectroscopy instruments due to the fact that the Raman scattering signal is much weaker than the signal resulting from Rayleigh scattering.

Typical sources for Raman spectrometers are usually lasers. This is because their high intensity yields Raman scatter of great enough intensity that it can be measured with a reasonable signal-to-noise ratio. The intensity of the Raman scatter varies as the fourth power of the frequency of the source. Lasers with shorter wavelengths provide much more intense Raman lines, but sometimes cause photodecomposition of samples because of their greater energy. Argon (488.0 or 514.5 nm) and krypton (530.9 or 647.1 nm) ion lasers are sources that emit in the blue and green region of the spectrum that fall into this category. Laser sources having longer wavelengths – in the near-infrared – are advantageous in that they can be operated at higher powers with less photodecomposition of the sample, and they are not energetic enough to populate many fluorescence-

producing excited electronic states of molecules, making interference from fluorescence less of a problem. These longer wavelength sources include diode (782 or 830 nm) and Nd/YAG (1064 nm) lasers. Another source that falls in the middle and is used in the studies presented herein is a He/Ne laser, which emits in the red at 632.8 nm [3].

As far as sampling goes for Raman spectroscopy, sample handling and preparation is fairly simple because water and glass do not interfere with the collection of Raman scatter. Samples can be made as aqueous solutions and/or can be contained in glass/quartz containers. Laser sources are easily focused to small spot sizes, making the investigation of small samples easy.

The third main component needed for collection of Raman spectra is the spectrometer. Figure 1-2 is a diagram of the Raman instrument used in the studies conducted and discussed in later chapters. All spectra were collected using a JY-Horiba LabRam Spectrograph. With this instrument, a microscope objective is used to deliver the 633 nm line of an electrically cooled Helium/Neon laser with a spot size of approximately 25 μm to the sample (if using the 10X microscope objective). The laser beam is reflected off a mirror and into the back of the LabRam through a small hole. It passes through a neutral density filter (used to attenuate laser power depending on sampling needs), is reflected through a pinhole and then through the microscope objective where it is tightly focused onto the sample. All spectra are collected with a 180° scattering geometry and sample acquisition times are typically set to 1 s. The scatter from the sample proceeds back through the microscope objective, where it then passes through a holographic notch filter (used to eliminate the wavelength of radiation coming from the

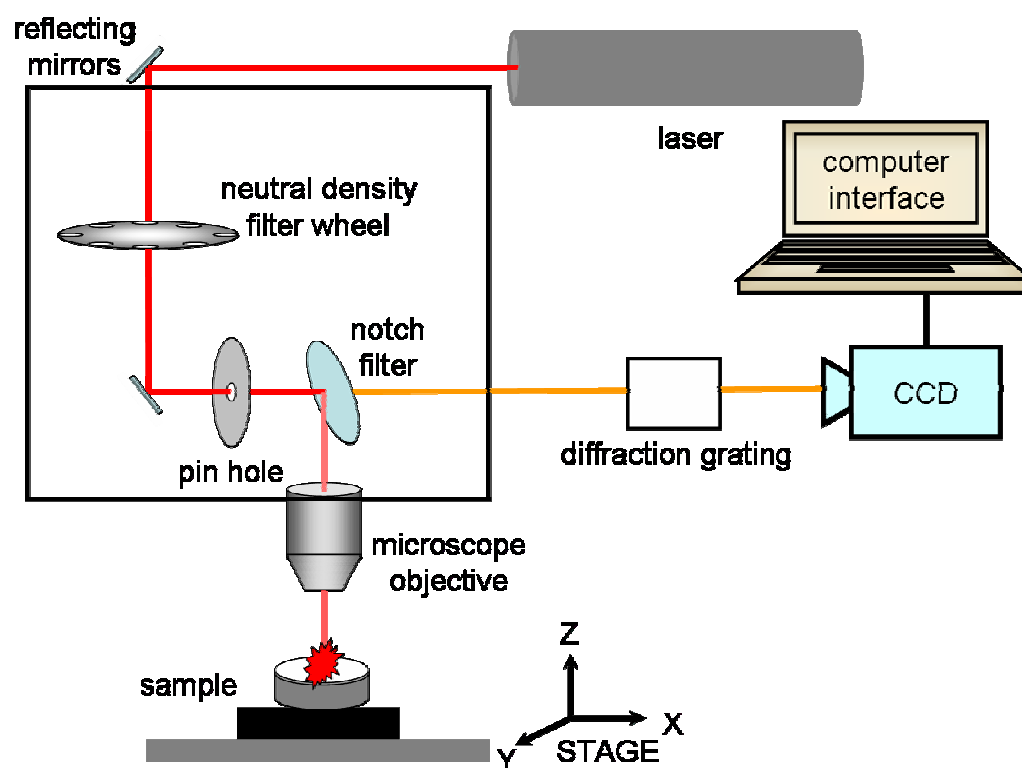


Figure 1-2. Schematic of JY Horiba LabRam spectrometer.

source – the Rayleigh scatter). It then impinges upon the diffraction grating (containing 600 grooves/mm) that disperses the scatter onto the charge-coupled device (CCD) detector.

Resonance Raman Spectroscopy. Another type of Raman spectroscopy to note is resonance Raman. This occurs when Raman line intensities are greatly increased due to an overlap of the exciting wavelength with the electronic absorption peak of the analyte. Symmetric vibrations can be enhanced by as much as 10^2 - 10^6 . As a result, resonance Raman spectra have been collected for analytes having concentrations as low as 10^{-8} M. Since resonance Raman is limited to bands associated with the chromophore being analyzed, spectra usually consist of very few lines. This also means that a resonance Raman spectrum can be acquired selectively due to the fact that it can be targeted for specific absorption bands. To collect resonance Raman spectra with the most success, tunable lasers are often used. Since analyte absorption peaks often occur in the ultraviolet region of the spectrum and tunable lasers are intense sources, sample decomposition often occurs.

The resonance Raman process is different from both normal Raman and fluorescence. When compared to normal Raman, resonance Raman varies energetically. Electrons are promoted into an excited electronic state followed by an immediate relaxation to a vibrational level of the electronic ground state. Also with resonance Raman relaxation to the ground electronic state is not preceded by radiationless relaxation to the lowest vibrational level of the excited electronic state as it is in fluorescence (see Figure 1-3). The time scale for resonance Raman relaxation differs

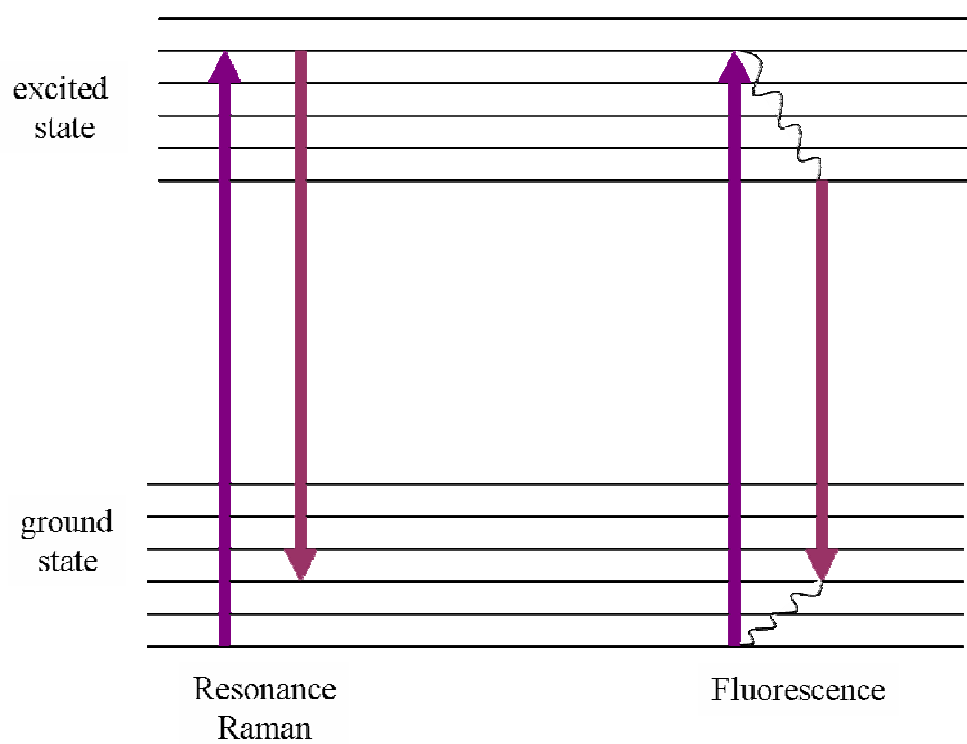


Figure 1-3. Energy level diagram for (left) resonance Raman scattering and (right) fluorescence emission.

from fluorescence emission as well, 10^{-14} vs. 10^{-6} - 10^{-8} s. Fluorescence often interferes with resonance Raman [3].

1.2 *Surface-Enhanced Raman Spectroscopy (SERS): Theory and Mechanisms*

One drawback to conventional Raman spectroscopy is its inherently small cross section. A typical Raman cross section (or the probability of the effect occurring at all) of an analyte molecule is on the order of 10^{-29} cm². This intrinsic weakness results in the inability to achieve low detection limits with conventional Raman [2], so this means that normal Raman studies are limited to examining either neat analytes or analytes having concentrations higher than 0.1M [3]. In order to overcome this limitation, increase sensitivity, and amplify analyte signal, surface-enhanced Raman spectroscopy (SERS) can be used.

In 1974, Fleischmann et al. first observed SERS when the Raman signal of pyridine was enhanced on a roughened silver electrode surface [5]. Their research was focused on creating a chemically specific spectroscopic probe to study electrochemical processes *in situ*. Fleischmann wanted to roughen the electrode to increase its surface area thereby creating more area for molecules to adsorb. Two other groups also noted (independently) increased signal that could not be accounted for by increasing the number of molecules or scatterers present, Jeanmaire and Van Duyne [6] and Albrecht and Creighton [7]. They proposed that enhancement of the scattered intensity was coming from the molecules being in an adsorbed state. Research into the SERS effect increased in the early 1980's. By 1985, facts and features of the experiments and mechanisms governing SERS were

generally agreed upon. That is, there are two accepted (though often debated) mechanisms responsible for the SERS phenomenon.

SERS has been noted for a variety of molecules adsorbed on surfaces of several metals having different morphologies. Typically, silver, gold, and copper are the chosen metals making up SERS substrates, but there have been reports of using alkali metals as well [8]. A theoretical basis for the SERS effect has been the topic of considerable debate [9-12]. In a simplistic sense, the intensity of Raman scattering is proportional to the induced electric dipole, μ , of the observed molecule. In turn, this variable, μ , is proportional to the polarizability of the molecule, α , and the magnitude of the incident electric field, E .

$$\mu \propto \alpha E \qquad 1-8$$

Increasing either the molecular polarizability or the magnitude of the field that is experienced can enhance the observed Raman scattering intensity. Two different enhancement mechanisms have been proposed to account for the SERS effect, the first being electromagnetic models that account for E -related enhancements. These models are independent of the adsorbed analyte and applicable to a broad range of chemicals. Electromagnetic enhancement is the main component responsible for the high sensitivity observed in SERS [13]. Likewise, chemical models account for α -related enhancements [14-19]. The chemical models are less understood and are specific in terms of the adsorbed analyte and the nature of the metallic surface. In general, chemical effects are considered lesser contributions to the SERS effect relative to electromagnetic effects. Discussions of both enhancement mechanisms follow below.

Chemical Enhancement. For systems where both electromagnetic and chemical enhancements exist simultaneously, the effects of both mechanisms are multiplicative. However, studies have been done showing evidence of the chemical enhancement mechanism itself [20-23]. In general, the chemical enhancement contribution to the SERS effect is very short-ranged (0.1 – 0.5 nm), as it is dependent upon the site of adsorption, bond geometry, and the energy levels of the adsorbate molecule. Interpretation of SERS spectra resulting from molecules that are chemically adsorbed to a substrate surface is more challenging than interpreting spectra resulting from electromagnetic enhancement (these spectra are similar to reference Raman spectra). When a molecule bonds to a substrate surface, the electronic structure of the adsorbate is changed and the formation of a complex between the surface and the molecule causes deviation from the reference Raman spectrum [23].

Two models to account for chemical enhancement observed in SERS have been proposed, the charge-transfer model, and the adatom model. In the charge-transfer model, an increase in the molecular polarizability of the molecule is a result of the formation of bonds between the metal of the SERS substrate and the analyte adsorbed to its surface [2]. In this model, chemisorption occurs where an analyte molecule adsorbs to a substrate surface such that the interaction energy is comparable to chemical bond energies and a surface complex forms [23]. Ground-state and radiation-induced charge-transfer processes result from the overlap of metal and adsorbate electronic wavefunctions [2], and have been noted to contribute approximately 10^3 - 10^6 to SERS enhancement [14, 18, 23]. Often, the highest occupied molecular orbital (HOMO) and the lowest unoccupied

molecular orbital (LUMO) of the adsorbate are “symmetrically disposed” in energy with the Fermi level of the metal (see Figure 1-4). These charge-transfer excitations (from metal to adsorbate or vice-versa) can occur at half the energy of the intrinsic intramolecular excitations of the adsorbate [24]. The second model of chemical enhancement, the adatom model, suggests that there is an increase in SERS enhancement when a molecule is adsorbed at an “active site of atomic-scale roughness” on the substrate surface. These sites are no larger than five to six atoms of metal [2]. At these proposed sites of atomic-scale roughness, there is the possibility of additional momentum available for scattering from electron-hole pairs that are present [25].

Electromagnetic Enhancement. Electromagnetic enhancement is more general in nature and is usually seen in SERS when substrates are made of roughened metal surfaces that have features smaller than the wavelength of light being used [26-28]. When electromagnetic radiation impinges on a suitable metal surface, conduction band electrons undergo oscillations of frequency equal to that of the incident light. These oscillating electrons, when at/near the metal surface, are termed surface plasmons [29]. Surface plasmons can be one of two types, propagating – moving across a surface, or localized – on the surface of a particle. In order for localized surface plasmons to be excited by light, substrate surface roughness is a requirement. Under conditions for surface plasmon excitation, the electromagnetic field of the light at the surface of the substrate can be greatly enhanced, leading to greater SERS signal enhancement for analytes located therein, and are dependent upon nanoparticle shape and structure [8, 30, 31]. To obtain large electromagnetic enhancement of the Raman signal of a molecule, it

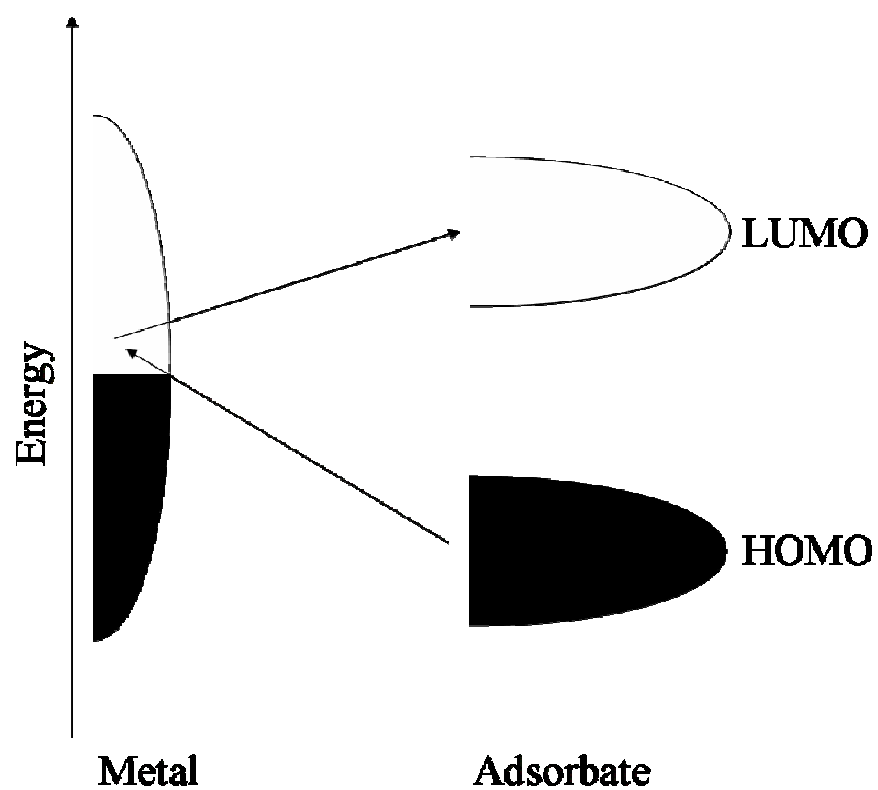


Figure 1-4. Molecular orbital diagram demonstrating possible charge-transfer mechanism for chemical enhancement in SERS.

is necessary to tune the resonance of the surface plasmons to match the wavelength of the incident radiation [32]. Through their interactions with the roughened substrate surface, the amplification of both the incident field of the laser and the scattered Raman field makes up the electromagnetic enhancement mechanism of SERS [8].

A simple example of a metal sphere (whose radius is much smaller than the wavelength of light) in an external electric field can illustrate the electromagnetic mechanism. The electric field is uniform across the particle and the field induced at the surface of the particle is related to the incident/external (laser) field by the following equation:

$$E_{\text{induced}} = \{[\varepsilon_1(\omega) - \varepsilon_2]/[\varepsilon_1(\omega) + 2\varepsilon_2]\}E_{\text{laser}} \quad 1-9$$

where $\varepsilon_1(\omega)$ is the complex, frequency dependent dielectric function of the metal and ε_2 is the relative permittivity of the ambient phase. At a frequency for which $(\varepsilon_1) = -2\varepsilon_2$, the function is fully resonant. The local field experienced by a molecule adsorbed on the surface of the particle is greatly increased by excitation of the surface plasmons. One way to look at this is as if the particle has localized the plane wave of the light as a field centered in the sphere that decays with distance away from the surface in all directions. The particle acts to enhance both the incident field as well as the Raman scatter field, acting as an antenna to amplify the intensity of scattered light. Since this model uses a simple sphere as an example, the numerical factor of 2 in equation 1-9 will change for substrates made up of different structures. The reason for using coinage and alkali metals as substrates is that the resonance condition is satisfied for these metals at the laser

frequencies commonly used in Raman spectroscopy. In addition, the dielectric function for these metals is small at the resonance frequency.

Roughly, the overall enhancement scales as E^4 . Since the Raman scattered light and the incident laser differ in frequency, the enhancement goes as $E_{\text{Raman}}^2 * E_{\text{laser}}^2$. Only for small frequency shifts can both fields be resonant with the surface plasmon. This can be used to explain the change in intensity across the spectrum of vibrational bands. The surface plasmon is excited by either the Raman field or the laser field, but not both. The dipole decay law can be used to explain the range dependence of the electromagnetic part of the SERS effect. Enhancement decreases as $G = [r / (r + d)]^{12}$ for a single molecule located distance d from the surface of a sphere with radius r , or $G = [r / (r + d)]^{10}$, for a monolayer of molecules. If the sphere has a large radius of curvature, the effect appears long-ranged, while with a small radius of curvature, the effect appears to be related to the surface [8]. The research presented in this dissertation is focused on improving substrate morphology in order to exploit the electromagnetic enhancement mechanism of SERS.

1.3 SERS Substrates

Since the discovery of the SERS effect, a wide variety of platforms have been used as substrates. These substrates can be categorized into two groups based on their morphologies: random and uniform. Random morphology substrates are characterized as having non-homogeneous surfaces. These substrates are typically difficult to reproducibly fabricate (except in the special case discussed in chapter 4 herein), meaning that each time they are made, they are just a little different in some way. On the other

hand, uniform morphology substrates are usually prepared in such a way that they can be reproducibly fabricated. These substrates have surfaces made up of ordered metal nanoparticles and provide uniform analyte environments each time they are made/used.

Random Morphology Substrates. The first SERS substrate was a roughened silver electrode discovered by Fleischmann (described previously). The electrode surface is roughened by running the oxidation-reduction cycle in an electrochemical cell containing a solution of silver salts. Advantages of these substrates include the ability to adjust electrode potential allowing for the examination of both charge transfer phenomena between analyte molecules and substrate metal surface, and orientation of molecules at the surface.

Another group of SERS substrates is metal island films fabricated via vacuum deposition. These substrates are simple to make, high in purity, and localized surface plasmon resonance can be altered by varying parameters like film thickness and deposition rate. Typically, thicknesses of 5-20 nm of metal are used for SERS substrates [33]. Both continuous and discontinuous metal films can be prepared. Continuous metal films are made by depositing metal on substrates like glass, quartz, or poly(dimethylsiloxane) [34-38], while discontinuous films are made by depositing on nanoparticle coated surfaces, like silica beads. One advantage to discontinuous films is that particle size can be controlled, thus leading to control over optical properties.

Research done in the Sepaniak group has demonstrated the merits of poly(dimethylsiloxane) (PDMS) nanocomposite as a SERS substrate. In contrast to conventional metal islands deposited on a smooth glass surface, when silver is deposited

onto the PDMS, it embeds itself into the top layer of the polymer giving greater substrate surface area with which analyte can interact. The solid-phase extraction capabilities of the polymer allow analytes to partition into the top layer in order to get closer to the embedded Ag particles yielding greater SERS enhancement than traditional Ag-islands on glass. Since PDMS is an elastomer, it can be stretched and compressed. This allows for the manipulation of the SERS substrate, changing the way the Ag is positioned in the polymer and potentially changing the SERS enhancement observed [39].

Perhaps the most common type of SERS substrate, one that consistently yields large signal enhancement, is simple colloidal gold or silver nanoparticles ranging in size from 10-150 nm [40]. Usually these are formed by the reduction of metal salts, e.g., the reduction of silver nitrate with sodium citrate. In some cases, the reduction is accomplished in more careful fashion to create cubes, rods, or triangular structures [41]. The colloidal systems that are most often used for single molecule and ultra-sensitive detection are usually aggregated clusters that possess some “hot spot(s)” within the aggregate. There is debate as to whether or not the aggregates themselves cause the Raman enhancement or if a “hot” particle happens to get trapped in the aggregate. Nevertheless, research has shown that “hot” aggregates can contain a wide range of particles [42, 43]. Much research has been done using colloidal substrates to support the finding that nanoparticle density plays a role in Raman signal enhancement [44-51]. Using colloidal nanoparticles for SERS has many advantages over substrates like silver islands immobilized on glass. Minimal burning of the sample occurs because the colloid is a fluid solution, allowing for use of higher laser powers at the sample with more

energetic laser lines [13]. Colloidal suspensions permit the acquisition of average spectra because they are governed by Brownian motion [52]. With colloid, analyte must be able to diffuse to the surface in order to adsorb so SERS spectra can be collected. Usually, systems of analyte-colloid are prepared by chemical reduction [13], though they can be prepared using other methods such as ablation, radiolysis, photoreduction [53], and pulsed-laser deposition [54].

While well-designed, random morphology substrates may lead to improved SERS enhancement, it is difficult to reproduce the nanoparticle aggregates that have been found to yield large SERS signal. Figure 1-5 shows examples of some of the random morphology substrates mentioned above. In our work (discussed in greater detail in chapter 4), we combine the randomness of colloidal aggregates with the ability of electron beam lithography (discussed below) to reproducibly fabricate substrates.

1.4 Nanofabrication Techniques

Processes for Preparation of Uniform Morphology SERS Substrates. With random morphology substrates, it is often challenging to obtain reproducible nanoparticle size/surface features. Some of the most interesting SERS substrates are those that are composed of nanostructures confined to a substrate surface. Several groups have developed these more ordered and controlled substrates using a variety of techniques. One method of substrate fabrication uses colloidal nanocrescents that can be magnetically controlled to alter their orientation on a substrate surface to take on SERS hot-spot geometries. The nanocrescents are made of thin layers of metals (Au/Ag/Fe/Au), and

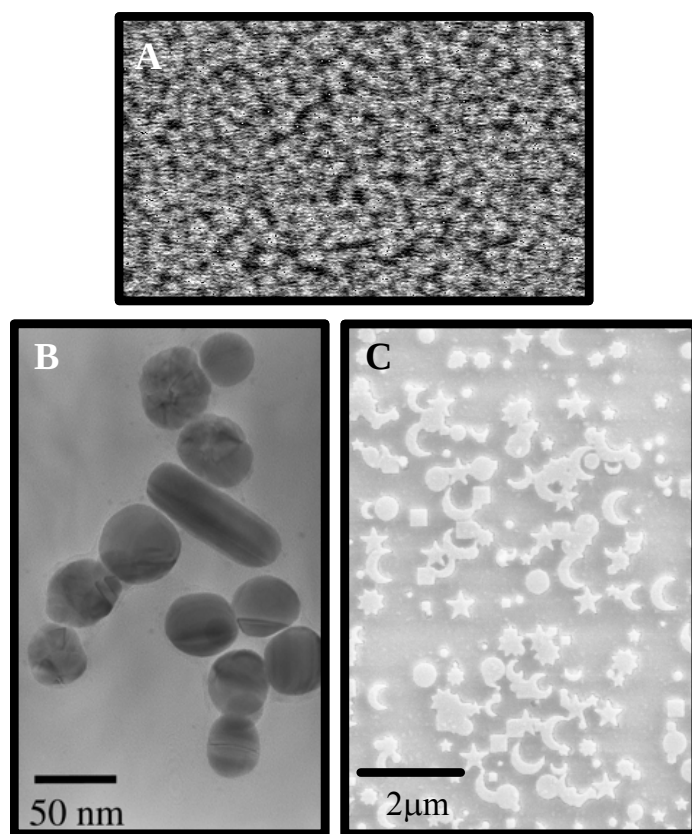


Figure 1-5. Random morphology substrates for SERS: (A) Silver-PDMS nanocomposite, (B) Silver colloid, and (C) Random nanoparticle substrates fabricated via electron beam lithography.

have been used for real-time biomolecular imaging since their gold surface is biocompatible [55]. Other assemblies of colloid materials have been used to produce ordered SERS substrates as well. Hexagonal arrays of spherical cross-section dishes have been formed by electrodeposition of gold through masks made of polystyrene colloid particles assembled on a gold surface. These substrates are made in a similar manner to those made by nanosphere lithography (discussed in the next section), but with the added advantage of being able to control not only spherical dish diameter but also film height. This leads to greater control over optical properties of the substrate [56]. Colloid crystal films have been used as templates to assemble gold nanoparticles in a controlled arrangement to create SERS platforms. They have been used in microfluidic channels to detect levels of sodium cyanide in water, and have performed well over varying ranges of analyte concentration and pH [57]. One other unique SERS substrate is composed of arrays of pyramidal shaped pits. By altering pit dimensions and maintaining pitch, it was shown that surface plasmons could be tuned to yield improved SERS enhancement [58].

Nanosphere Lithography (NSL). Another technique widely used in creating ordered SERS substrates is nanosphere lithography [59, 60]. This technique is a general fabrication process for the production of surfaces of periodic particle arrays (PPA) having nanoscale features. Substrates made by NSL have the appearance of truncated tetrahedrons. The first step in NSL involves the preparation of a mask - single or double layer depending on concentration of polystyrene (PS) nanospheres - by spin coating PS nanospheres onto the substrate of interest (glass slides, silicon wafers, etc.). A surfactant is often added to the PS nanosphere solution to assist in the wetting of the substrate

surface. After spin coating, thin films of metal (Ag, Au, Cr, CaF_2 , or cobalt phthalocyanine) are vapor deposited over the PS nanospheres onto the substrates. The nanospheres are then removed by sonication in methylene chloride (or in the case of the CoPc removed with tape). Deposited metal that has gone through the spaces between the PS nanoparticles remain on the substrate surface forming the periodic particle array pattern. The spacing between the particles of the PPA is defined by the mask geometry and the size of the PS nanospheres [59].

With NSL, surface plasmon resonances (SPR) of nanoparticle systems can be systematically examined by controlling nanoparticle shape, size, and spacing. It is a low cost, simple method that uses readily available materials for the fabrication of homogeneous (in size and shape) nanoparticle arrays that can be tuned to the laser wavelength being used for SERS experiments. This technique is often used to compare theory to experiment for nanoparticle optical property studies [61, 62].

Electron Beam Lithography (EBL). Simply stated, electron beam lithography is the process of exposing electrically sensitive surfaces to an electron beam in order to fabricate sub-micron and nanometer scale features. Its development followed soon after the scanning electron microscope (SEM) in 1955 [63]. With the improvement of electron optics around 1976, resolution below 100 nm was achievable with EBL, and it continues to be exploited to fabricate sub-100 nm structures to this day [64-70]. When compared to traditional photolithography, EBL is able to reach much higher resolution due to the fact that the electron beam can be tightly focused down to a spot size of 1 nm [71]. Until recently, EBL has been primarily used for fabrication of nanoelectronic devices.

In order for EBL nanofabrication to be successful with high precision and resolution, conditions must be set so that electron scattering causes minimal resist exposure. This adverse effect can happen one of two ways, (1) with very high energy or (2) with very low energy electrons. In case (1), the beam broadening in the resist due to elastic scattering is small, and the beam diffuses deeply into the resist, while in case (2), the energy of the electrons is low enough that it is not able to scatter over large distances in the resist. A simplified diagram of an EBL system is given in Figure 1-6. It contains three crucial components, the electron gun, the vacuum system, and the control system. The electron gun, supplies, accelerates, and focuses a beam of electrons onto a substrate. First, electrons are made by cathodes or electron emitters. They are then accelerated by electrostatic fields, yielding higher kinetic energy, and shaped into a beam. Next, a series of electric and magnetic lenses focus and deflect the beam to a point on the substrate. Manipulation of the electron beam can only happen when it is under high vacuum. The final part of the EBL instrument, the control system, coordinates the movement of the electron beam over the substrate (or the movement of the substrate under the beam, depending on what type of scanning is used) with the blinking on and off of the beam, so that only the AutoCAD designed pattern is transferred to the substrate.

EBL is based on the principle that certain substances (i.e. electron beam resists) change their properties when exposed to electrons. Digital images (in our case, arrays of various shapes, created using AutoCAD software) can be directly patterned onto a substrate of interest through computer control of the position of the electron beam [72]. The electron source has several advantages over conventional optical and ultraviolet

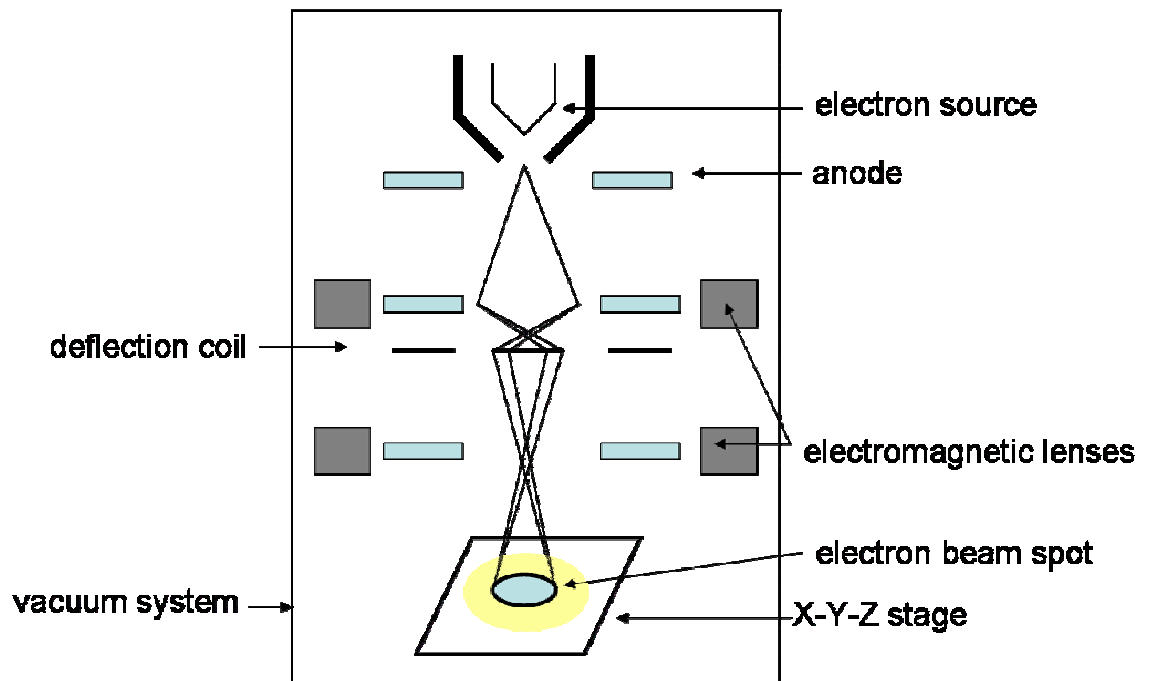


Figure 1-6. Simplified diagram of an electron beam lithography system.

photolithography processes. It is characterized as having a high diffraction-limited resolution that allows for the creation of nanometer-sized features. EBL can be used to *directly* write onto a resist-coated substrate, eliminating the need for photomasks that are expensive and time-consuming to manufacture.

The direct writing method is the most common EBL approach [73]. Direct patterning of substrates proceeds through a series of steps – depicted in Figure 1-7. The steps shown in Figure 1-7, are those that are taken in the research described herein. First, a silicon wafer is coated with the electron beam sensitive resist via spin coating and then baked to make a uniform layer of resist. Two different types of resists are used in EBL – positive (more soluble in developer post exposure due to the breaking of bonds during irradiation) and negative (less soluble in developer post exposure due to cross-linking of polymer chains – used in our studies) [71]. The coated wafer is then exposed to the electron beam according to a pre-designed pattern. With this method, resist is exposed one pixel at a time using a finely focused Gaussian round beam that moves with the wafer. Since exposure occurs one pixel at a time, this approach is limited with respect to the time it takes to expose a wafer if the pattern being transferred is complicated [73]. Once exposed, the pattern is developed on the wafer using a developing solvent specific to the resist used. Details regarding the pattern design and the developing processes used in the presented work can be found in chapters 2, 3, and 4.

Direct Fabrication Procedure

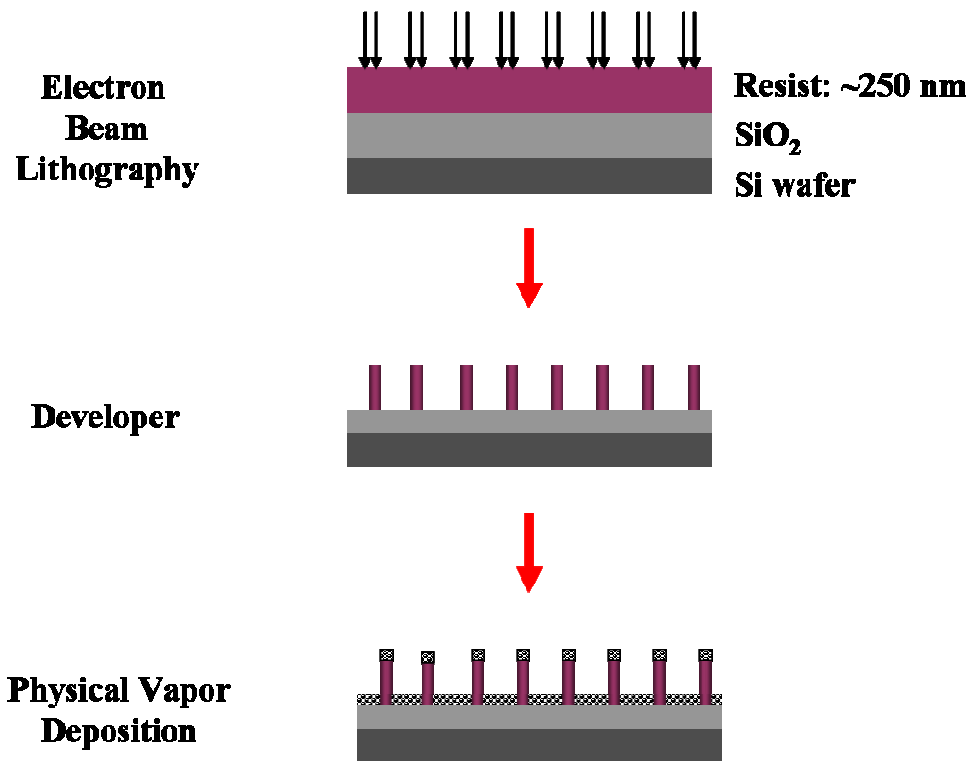


Figure 1-7. Steps taken in the direct EBL fabrication of SERS substrates.

Several research groups use EBL for the fabrication of SERS substrates. When combined with vapor deposition of SERS-active metal, and a lift-off technique (dissolving of unwanted resist post vapor deposition of metal), EBL is often used to create regular arrays of metallic nanoparticles for SERS [1, 74-76]. Our research group has demonstrated a *direct* EBL technique for the fabrication of nanostructured SERS substrates. With EBL, substrates can be made having more uniform/homogeneous morphologies for SERS by controlling various aspects of nanoparticles composing the SERS-active platforms. In our first published work using EBL for the fabrication of SERS substrates, several nanoparticle symmetries, geometries, and particle shapes, sizes and spatial arrangements have been explored. This work showed that particles shaped as elliptical discs arranged around a C2 axis of symmetry gave the best SERS signal when compared to other geometric shapes and symmetries [77]. Chapter 2 will go into greater detail about this work, followed by discussion of the research that was done afterward to further develop uniform morphology SERS substrates (Chapter 3). Chapter 4 will discuss work “in-progress” to *reproducibly* fabricate random morphology SERS substrates via EBL.

Facilities Used for Nanofabrication. For the experiments described in the chapters that follow, two different EBL tools were used – one located at UT in Dr. David Joy’s lab and one at the Center for Nanophase Materials Sciences (CNMS) at Oak Ridge National Laboratory (ORNL). Both instruments are state of the art, sophisticated electron beam lithography systems used for high-resolution nanopatterning (see Figure 1-8). The tools are manufactured by JEOL, and require extensive training to be able to



Figure 1-8. Picture of the JEOL JBX 9300FS Electron Beam Lithography system similar to the EBL tool located at CNMS.

independently use them. Our access to these EBL tools was often limited as they belong to other research groups whose needs sometimes preceded ours, and they are located in other facilities. The EBL at CNMS is located inside a clean room facility. To gain access to this particular instrument, we wrote a proposal describing work discussed in chapters 3 and 4, and it was accepted. Clean room training was required and completed, as was all ORNL site training.

1.5 Statement of the Problem

Some of the major drawbacks to SERS as an analytical technique involve inherent limitations to the substrates themselves. These include inhomogeneity in enhancement sites across substrate surfaces, limited effective surface area that is debilitating to calibration as it restricts the linear dynamic range, quick oxidation of substrates when exposed to air, water, or other oxidizing agents, and degradation in substrate sensitivity if photolytic or thermal effects occur. As a result, significant qualitative and quantitative reproducibility problems arise.

In order to overcome these limitations and improve the figures of merit of the SERS method, our research has focused on the fabrication of both uniform and random morphology SERS substrates using direct EBL and physical vapor deposition. There are several advantages to creating SERS substrates using these techniques. Theoretical calculations can be applied to predict enhancement factors and subsequently, predict and design ordered nanostructured patterns that will yield greater signal. The use of computer aided design (CAD) software allows the design of arrays of nanostructures that have

rapidly manipulated features, including size, shape, geometric pattern, and gap to produce novel substrates. Highly complex and unusual arrays can be generated even though they cannot be quickly modeled. Due to the homogeneity of the substrate surface features, high densities of identical analyte environments can be fabricated that are reproducible across their surface as well as between substrates. Using advanced tools, EBL and PVD, we have aimed at improving upon the limitations of conventional SERS substrates by fabricating both ordered and random arrays of nanostructures as platforms for SERS.

Chapter 2 Preliminary Studies for the Fabrication of SERS Substrates via Electron Beam Lithography

2.1 Preliminary Work

Prior to working with our EBL resist, we found that it was best to determine the optimum exposure dose for fabricating patterns. Each time a new bottle of resist was obtained, the following study was performed. Simple 50 x 50 μm arrays of 500 nm squares with 100 nm spacing were patterned across silicon wafers using different base doses for each array. The arrays were subsequently developed and examined by SEM. The optimum base dose was determined by examining SEM images and looking at the development of each array. Figure 2-1 shows arrays of squares at varying base doses (A. 150 $\mu\text{C}/\text{cm}^2$, B. 160 $\mu\text{C}/\text{cm}^2$, and C. 170 $\mu\text{C}/\text{cm}^2$) for the resist used for studies discussed in Chapter 3. It is seen here that the optimum base dose for this particular resist was 170 $\mu\text{C}/\text{cm}^2$.

Two other parameters that must be determined when new resist was obtained were the best method for soft-baking the spin-coated silicon wafers and the time needed for pattern development post EBL exposure. In our initial studies (discussed in this chapter) and in studies in Chapter 4 (completed at Oak Ridge National Laboratory), wafers were soft-baked on a hot plate, while in other studies (Chapter 3) an oven was used. Reasons for this are unclear, though it seemed to depend on the bottle of resist being used. The resist would not develop off the wafers if the incorrect method for soft-

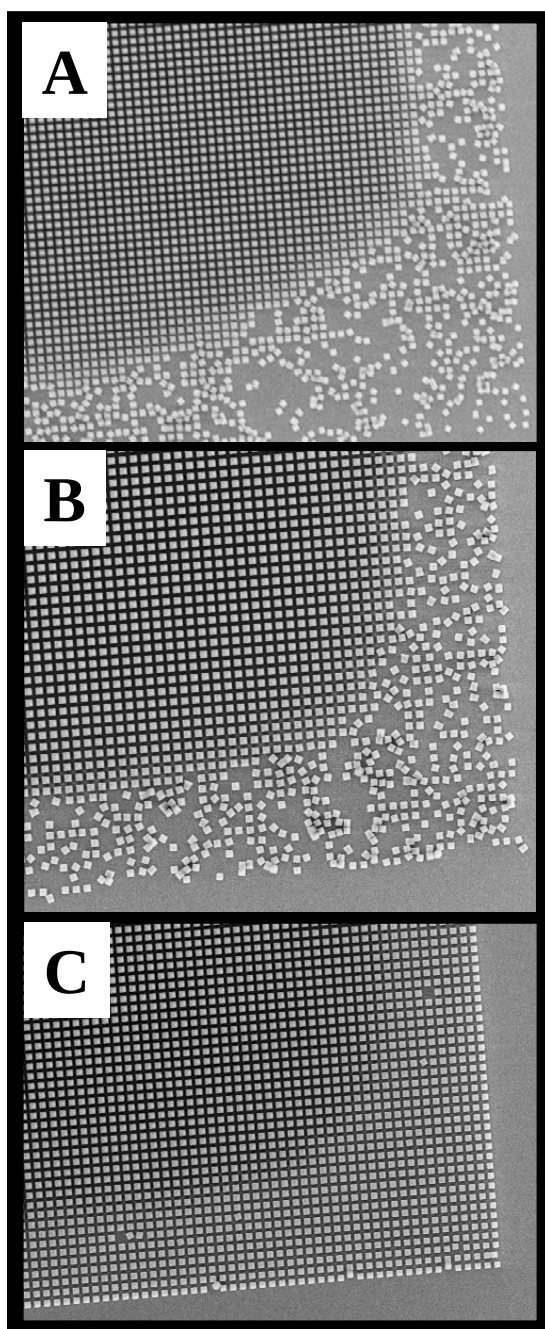


Figure 2-1. Examples of different base doses used in base dose study completed with ma-N 2403 EBL resist (A. $150 \mu\text{C}/\text{cm}^2$, B. $160 \mu\text{C}/\text{cm}^2$, C. $170 \mu\text{C}/\text{cm}^2$).

baking was employed. With developing, the correct time was determined by trial and error for each new bottle of resist. In order to ensure that at least a few sets of patterns would be obtained from EBL experiments, wafers were often cut into quarters so developing could be attempted on each quarter instead of the entire wafer at once for the first EBL run with each new bottle of resist. Once the correct time was determined, entire wafers could be developed at one time.

There are many contributors to the work described below. As for my involvement, I assisted in every aspect of learning the techniques for fabricating patterns (AutoCAD pattern creation, wafer preparation, EBL operation, wafer development) and collecting data. The following, a slightly modified version of a publication, is published as:

M. A. De Jesus, K. S. Giesfeldt, J. M. Oran, N. A. Abu-Hatab, N. V. Lavrik, and M. J. Sepaniak. *Appl. Spectrosc.* **59**, 1501, (2005).

2.2 *Introduction to First EBL Studies*

It has been determined that electromagnetic radiation incident on certain metals with nanoscale features can initiate localized surface plasmons (LSPs) and induce strong electromagnetic fields, which can result in very significant enhancements of Raman scattering [78,79]. Unfortunately, issues of substrate selectivity and limitations in analytical figures of merit, such as reproducibility and dynamic range, have inhibited the general acceptance of surface enhanced Raman spectrometry (SERS) for routine analytical applications. Nevertheless, SERS has been shown to be very useful for

chemical and bio-analytical applications due to narrow spectral bands, which result in unique spectral fingerprints and structural information [80-82]. Moreover, under certain specialized conditions leading to enhancements in signals of roughly 10^{14} , spectra have been acquired for single molecules [83, 84].

Traditional SERS substrates, such as colloidal solutions [85, 86], colloidal particles encapsulated in sol-gels [87], metal-island films on glass [88, 89], and metallized polymers [34, 90, 91], tend to have random morphologies and fractal-like qualities. The complex and rather inhomogeneous nature of these surfaces has obscured the physical understanding of the SERS effect and hindered efforts to correlate theory with experiment. More recently, there has been a drive for uniform morphologies in order to reproducibly produce *tunable* localized surface plasmon resonances. Some researchers have attempted to confront this problem by synthetically controlling the size and shape of colloidal nanoparticles [92-95]. However, while most of these methods have greatly improved the distribution of size and shape of the nanoparticles themselves, there is less control over the spacing between the particles. Other groups have explored the production of regularly structured substrates via vapor deposition of metal over polymeric nanospheres and silica posts [96, 97].

Lithographic techniques such as nanosphere lithography and electron-beam lithography (EBL) have emerged as promising alternatives for the fabrication of new substrates with uniform nanoscale features [97-101]. These techniques also offer the possibility to create new substrates with controlled inter-particle coupling, which can be an important contributor to SERS enhancement [74, 102]. EBL, in particular, promises

the relative straightforwardness of creating well-defined nanoparticle geometry and unique spatial arrangements that the other techniques cannot similarly achieve. These components can be manipulated to produce uniquely tuned LSPs, probe particle size, shape, and orientation with respect to laser polarization, and produce homogeneous analyte environments that, in a practical sense, are useful for reproducible trace analysis.

EBL coupled with plasma etching has been shown to produce highly ordered surfaces that are very SERS active [96]. Others have explored EBL and reactive-ion etching (RIE) which is often coupled with lift-off techniques to produce two-dimensional arrays and gratings with highly reproducible particle size, shape, arrangement, and proximity [98, 103, 104]. This has allowed the development of substrates with narrow LSP resonance bands that can be targeted for specific excitation sources. In some cases, structures have been created by EBL with interparticle gaps of less than 50 nm [1, 99]. However, the uniformity of the size and shape of the individual particles is frequently limited by imperfections in the thin e-beam resist films and by damage induced by the developing processes. Unfortunately, the high cost of EBL and RIE inhibits the mass-production of SERS substrates with these techniques.

In this work, we present the use of a *direct* EBL technique, which circumvents the use of lift-off and/or RIE, for the fabrication of densely packed polymeric arrays of pillars (of polymer e-beam resist) that are metallized via physical vapor deposition to create isolated metal nanoparticles. The flexibility of this EBL technique permits the rapid generation of patterns with unique sized and shaped nanoparticles in controlled spatial arrangements. Thus, we can survey a fairly large number of conceptual designs

for SERS, such as cubic, hexagonal, and elliptical nanoparticles. It is important to note that it is the presence of nearly vertical walls on the EBL patterned pillars that lead to formation of nanoscale discontinuities (gaps) in the evaporated metal films. By incorporating an iterative process between the EBL and SERS, we demonstrate that it is possible to rapidly optimize nanostructures for a given laser frequency. Although concomitant theoretical studies are always useful, the ease and speed with which such surveys can be conducted obviates performing such studies prior to experimental fabrication. The impact of the nanoparticles shape, size, and spacing, and geometrical arrangement on the Raman signals of 1,10-phenanthroline and Rhodamine 6G is studied and reported herein. The importance of these variables to improve the performance characteristics of SERS substrates is presented.

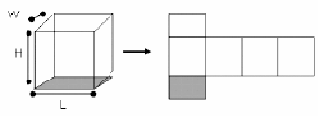
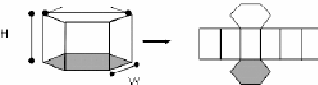
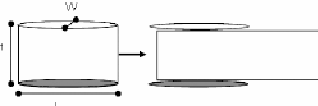
2.3 *Experimental*

SERS Instrumentation. All SERS spectra were acquired using a modified version of a LabRam Spectrograph from JY-Horiba. The instrument set-up has been described in detail previously [34, 38, 105]. In general terms, the instrument uses an Olympus BX-40 microscope with a 10X (0.25 NA, ∞) objective that delivers up to 8.9 mW of the 632.8 nm line from an electrically cooled He-Ne laser. The laser spot size in these studies was approximately 20-25 μm . All spectra were acquired in a 180° scattering geometry with a 2936 cm^{-1} spectral window. In this work, all sample acquisition times were set to 1 second.

Preparation of SERS-active substrates. A series of two-dimensional models of square, hexagonal and elliptical nano-arrays were created in AutoCAD 2005. Pattern size varies between 50-300 nm in diameter with an inter-particle spacing of 50-200 nm (see Table 2-1 for details). Each drawing was subsequently converted to GDS-II format by using the LinkCAD conversion program. The files were transferred and programmed into the EBL computer. A 2” Si wafer (Wafer World, FL) was cleaned with a piranha solution for 5-10 min and rinsed with deionized water (18M Ω , Barnstead E-Pure), and ethanol (HPLC, Fisher). The wafers were then heated at $\sim 300^{\circ}\text{C}$ for 15 min. to free the substrate of any physically adsorbed humidity. Once the Si surface was dried, a 250 nm film of ma-N 2403, a methacrylate-based negative e-beam resist, was uniformly applied to the surface by spin coating at 3000 rpm for 30 s. The coated wafer was then baked at 90°C on a hotplate for 60 seconds and placed into the EBL system. Film thickness of the ma-N 2403 resist was estimated from a chart provided by the manufacturer and is based on spin rate.

A Jeol JBX-6000 FS/E electron beam lithography system with a 50 keV thermal field-emission gun was used for the nanofabrication of the SERS active substrates. The resist film was exposed to $120\text{ }\mu\text{C}/\text{cm}^2$, yielding an array of nanofeatured patterns. Each array is approximately $50 \times 50\text{ }\mu\text{m}$ in size with $100\text{ }\mu\text{m}$ spacing in x and y directions between each uniquely patterned array. Once exposed, the patterns were developed in alkaline ma-D 332 developer for 20 s and rinsed in deionized water (18 M Ω , Barnstead E-Pure) for 3 min.

Table 2-1. Data on nanocomposite Patterns.

Pattern	Ag-Coating (nm)				Patterns	Ag Surface	# Loci	Shape	Surface Area Diagram
	L	W	H	Gap	per grating	Area (mm ²)	per grating		
SP-A	100	100	25	50	111222	2224	444889		
SP-B	200	200	25	200	15625	938	62500		
SP-C	200	200	25	150	20449	1227	81796		
SP-D	300	300	25	150	12358	1483	49432		
HP-A	190	100	25	50	63798	2362	382788		
EP-P	150	50	25	50	104114	4380	208229		
EP-C2	150	50	25	50	125000	5258	250000		
EP-C3	150	50	25	50	45514	1915	91027		
EP-C4	150	50	25	50	93840	3948	187680		
EP-C5	150	50	25	50	89404	3761	178809		
EP-C6	150	50	25	50	180428	7590	360857		

The SERS-active substrates were prepared with a Physical Vapor Deposition (PVD) chamber from Cooke Vacuum Products, Inc. Samples were mounted 25 cm above and normal to the effusive source. Average mass thickness and deposition rates were measured for each film with a Maxtec quartz-crystal microbalance (QCM) that is mounted adjacent to the arrays. The ma-N 2403 arrays were coated with 25 nm of 99.999% Ag (Alfa Aesar, MA), or in one case 99.999% Au (Gatewest, Canada), under high vacuum conditions (1×10^{-6} torr). Once prepared, the SERS active substrates were stored in a vacuum dessicator held at 10^{-4} torr in the dark prior to use (see Table I for substrate specifications).

All SEM micrographs were collected with a Hitachi S4300-E SEM with a field-emission gun operating at 4kV. An electron beam diameter of ~ 4.5 nm was used to obtain the high-resolution images in secondary electron detection mode. This reduced the sample damage and the charge build up while producing high-resolution images of coated and uncoated polymer surfaces.

Sample Preparation. The test analyte solutions used in these studies were 1×10^{-6} M Rhodamine 6G (98+%, Allied Chemicals) and 1×10^{-4} M 1,10-phenanthroline (98+%, Aldrich), prepared in deionized water (18 M Ω , Barnstead E-Pure).

Data acquisition and analysis. The nano-array chips were placed at the bottom of plastic Petri dishes, filled with a 2 mL aliquot of each sample solution. The maximum SERS signal was obtained by fine-focusing the microscope objective and the spectroscopic data was collected by moving the stage at 10 μm intervals (1 spectral acquisition per step) over a 1600 μm^2 area (N=16). All Ag substrates were irradiated with

2.2 mW of the 632.8 line of a He-Ne laser for 1 second, while the Au substrates were exposed to 4.1 mW for 1 second. Average spectra were collected on 3-5 arrays of the same pattern. The average of these spectra for each pattern was used for the interpretation of the analytical results.

The available Ag-surface area (Ag-SA) was estimated by calculating the analyte accessible surface area of an individual patterned metal nanoparticle times the total number of particles in the arrays. The Ag deposition conditions were adjusted in such a way that the Ag layers at the bottom of the arrays were beyond the percolation threshold, thus, rendering the non-pillar portion of the array area largely inactive for SERS. The number of enhancement points, or loci, was estimated by multiplying the total number of patterns within the array times the number of corners/points for a given geometric shape.

2.4 *Results and Discussion*

In principle, the systematic adjustment of experimental variables such as particle size, spacing, shape and geometric distribution can be used to produce intense excitation of SERS responses over reasonably large volumetric areas of substrate. Most traditional nanolithographic work is based on the combination of EBL and reactive ion etching (RIE) to create arrays of isolated Ag (or Au) nanoparticles on silicon. Our group has demonstrated the potential benefits of using metal-polymer nanocomposites as random morphology substrates for SERS applications [34, 38, 39, 105]. These benefits can be further exploited by systematic nanostructuring of a polymeric surface via EBL, creating a uniform and more homogenous substrate over extended areas of nanostructured

polymers (e-beam resist in this case). EBL can facilitate the rational design of SERS substrates by providing an efficient and rather reproducible method to create nanostructured surfaces with a variety of shape, sizes and orientations.

Studies with Square Pillars. In order to study the impact that the particle size and spacing have on SERS signal of a nanostructured substrate, a series of square pillars (SP) were prepared. Each pattern was arranged as a square array of approximately 2500 μm^2 . The nanoparticle size and spacing within the array was varied from 100-300 nm and 50-200 nm, respectively (see Table 2-1 and Figures 2-2 to 2-5). The success of the nanolithographic procedure was corroborated via SEM. Figure 2-2 focuses on the square pillar arrangements. The collected micrographs show a series of nanopillar polymer arrays with approximate heights of 250 nm. A minor increase in surface roughness as well as the rounding of the nanostructured edges was observed after the deposition of 25 nm of Ag onto the ma-N 2403 nanostructures (Figure 2-2). It should be pointed out that the 25 nm thickness is an average effective thickness. In fact, due to the large vertical protrusion of the polymer nanopillars and a strong Ag-Ag cohesion, the metal may preferentially deposit on the pillars and, therefore, exhibit thicknesses that are somewhat variable (pattern to pattern) and greater than 25 nm. Inspection of our extensive SEM images of various patterns and metal thicknesses confirm this tendency.

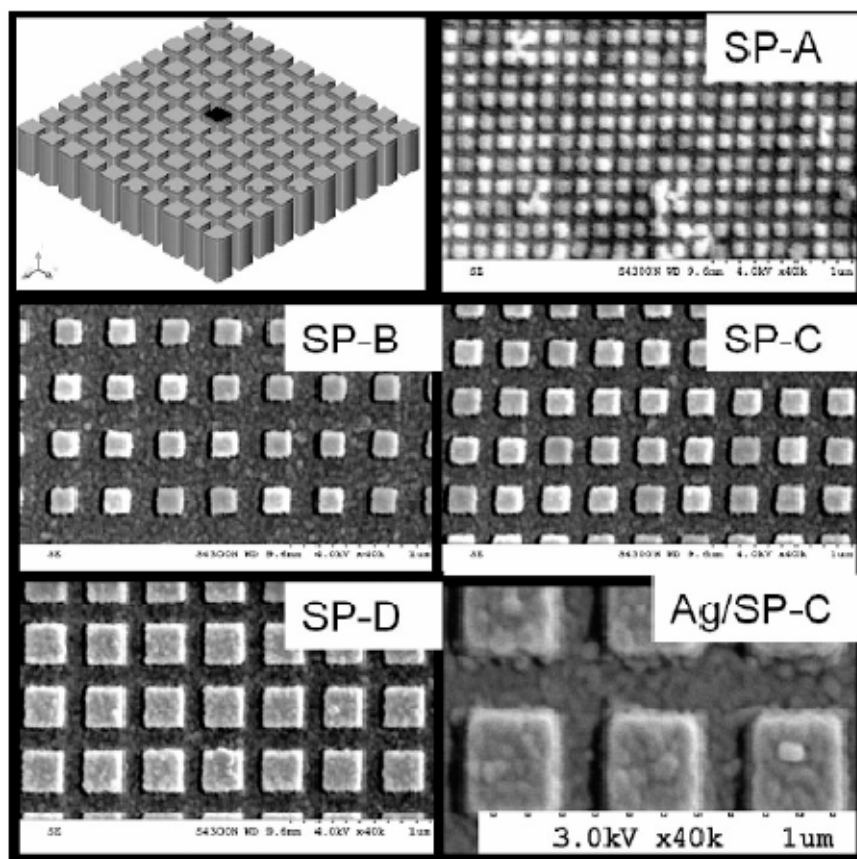


Figure 2-2. Scanning electron micrographs of a series of ma-N 2403 250 nm high square pillars on a Si wafer. The upper left figure is a 3D depiction of an array coated with 25 nm of Ag. The dimensions of each pattern side and their respective gaps (in nm), are: 100, 50 (SP-A); 200, 200 (SP-B); 200, 150 (SP-C); 300, 150 (SP-D). Ag/SP-C is an SEM showing the appearance of the substrate upon the deposition of 25 nm of Ag at 1.0 Å/s (the image was zoomed in for clarity). All micrographs were collected at a 40K magnification.

A comparison of the SERS spectra of an aqueous solution of R6G (1×10^{-6} M) on nanostructured (SP-B) and native Ag/ma-N 2403 surfaces exhibited a nearly two orders of magnitude increase in the R6G bands when adsorbed on the nanostructured areas (Figure 2-3). For the spectra shown, background subtraction was not performed to negate the very small signal from the ma-N 2403 that is in contact with the metal. Of course this thickness for the smooth surface is greater than the optimum for silver islands on smooth glass (~ 8 nm) [106], and silver deposited in our prior work on PDMS polymer (~ 20 nm) [34]. Nevertheless, the significance of nanostructuring is seen in this comparison.

The effects of square nanopillar dimensions and particle density were studied using the magnitude of the 1,10-phenanthroline SERS band centered at 706 cm^{-1} (see Figure 2-4). The gaps between nanoparticles were varied in these structures (see Figure 2-2), but probably not over a range wherein interparticle surface plasmon effects are in play. In addition to the raw signals (706 cm^{-1} band area), the signals were normalized in two manners. To accommodate the notion that the most active regions of the nanoparticles are on (or extremely near) the surface, the middle histogram bar in Figure 2-4 was normalized to the exposed Ag surface area within the array. Computational simulations using the discrete dipole approximation (DDA) suggest that strongest signal enhancement arises from molecules proximal to the nanoparticles vertices (loci) [107-109]. Thus, the far right histogram bars in the figure were normalized to the number of exposed corners in the arrays. The notion here is that all surface area is not created equal in a SERS sense and extra significance is given to the loci. Since relative signals are most significant in this treatment, each type of bar is scaled to a maximum of 100 %.

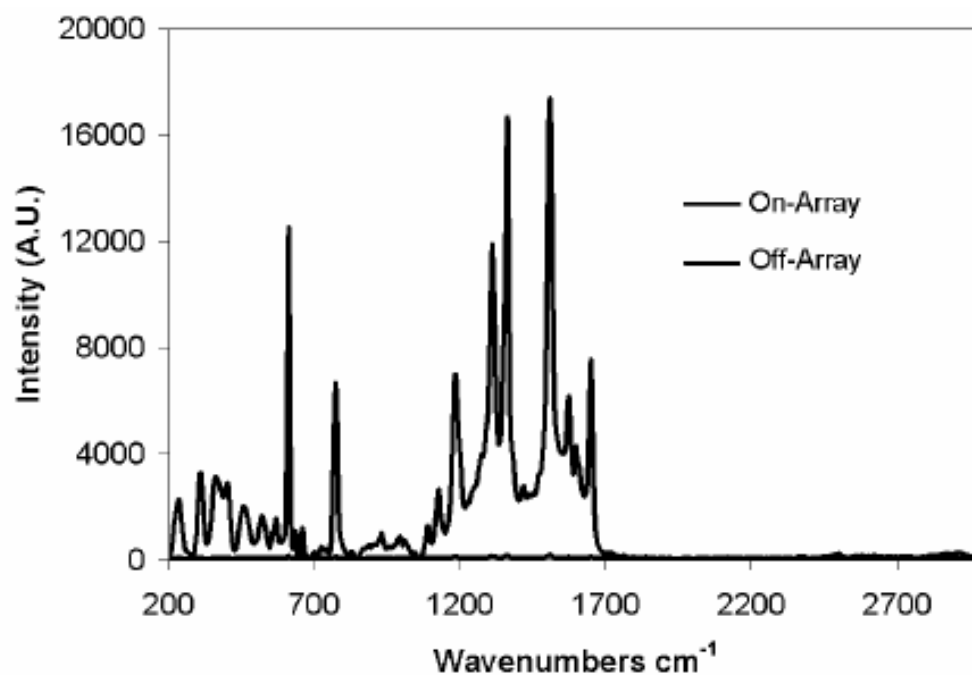


Figure 2-3. Generation of strong R6G (1×10^{-6} M) SERS signals upon the nanostructuring of the ma-N 2403 surface (gray), relative to the ma-N 2403 off the array surface (black). All spectra were irradiated for 1 s with $2.1 \pm (0.1)$ mW of 632.81 nm radiation of a He-Ne laser onto a 25 nm Ag film.

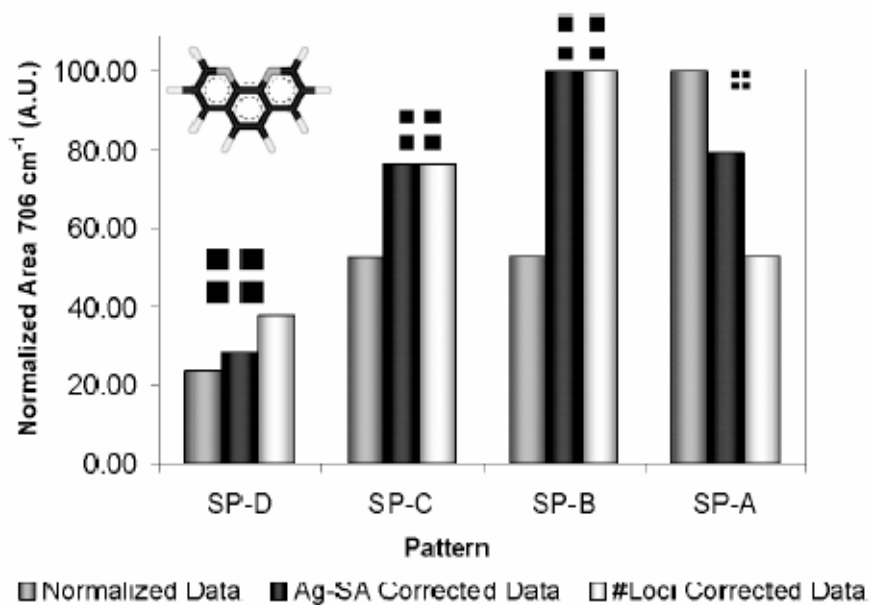


Figure 2-4. Effects of nanoparticle size and gap on the 706 cm^{-1} band signal of 1,10-phenanthroline ($1 \times 10^{-4}\text{ M}$): Normalized experimental data (gray), corrected relative to the available Ag surface area (black), corrected relative to the available number of loci (light-gray). See text for details.

Several interesting observations can be made when the data is inspected. The lowest to highest raw signals are observed when the density of nanoparticles is increased (SP-D smallest signal with SP-A greatest signal). Based on this trend, one might have expected SP-C to yield a slightly greater raw signal than SP-B since the gaps were smaller and density greater for these SP-C, 200 nm nanoparticle arrays. Instead the signals are essentially equal. Most likely, this reflects the variability in attempting to precisely create the structures by EBL, PVD metal deposition, and in conducting the SERS measurements. This is verified statistically by percent RSD calculations where the average difference in SERS signal between patterns is 20%. Surface area does not appear to be the controlling factor in the observed signals; while the SP-A has the greatest area and raw signal the second greatest area belongs to the SP-D array which gives the smallest raw and area normalized signals.

When considering the loci corrected signals, the 200 nm arrays outperform (appear hotter) than both the larger 300 nm and smaller 100 nm arrays. It is curious to note that while the nanoparticle geometries are quite different in comparison to our EBL pillar structures, in Nie's early colloidal particle work [110], it was determined that the optimum Ag colloid size for 488 nm, 568 nm, and 647 nm excitation were 70 nm, 140 nm, and 200 nm colloidal particles, respectively. This is not inconsistent with our observations for excitation with the output of the 633 nm He-Ne laser used in this work. Since both the level of EM excitation and the effective volume available for excitation are important, both must be considered when designing high performing substrates. Although we were not equipped to use Ar^+ laser excitation at 488 nm or 514 nm with our

spectrometer, it would have been interesting to determine if the SP-A would capitalize on both the hotness and the volume (or nanoparticle density) factors with the shorter excitation wavelength. The advantage of being able to rationally and rapidly survey uniform substrate structures with this EBL approach to fabrication is seen in this study. The performance of the nanostructured substrates is also affected by the differences in dielectric constants of the metal nanoparticles and their surrounding medium, as demonstrated in Figure 2-5. The results show over a 40% drop off in signal intensity for a 1×10^{-6} M solution of R6G when the experiment is performed in air (by simply air drying the liquid phase sample) rather than water. This drop in signal could also be due to other factors such as sample degradation, differences in analyte concentration between the two phases, or the dielectric related to the analyte coating the surface when dry. Similarly, changing the metallic surface from silver to gold induces a 50 fold decrease in signal, even after doubling the power of the incident beam. This dramatic signal drop is due in part to the differences in the dielectric constant of Ag when compared to that of Au. Ag exhibits a more efficient excitation of its LSPs in the visible region than Au. The poor SERS response of the Au substrates in the visible region can be slightly compensated by extending the acquisition time or possibly by tailoring the nanoparticle shape and size.

Nanoparticle Shape Studies. Nanoparticle shape is considered an important parameter in determining the strength of the generated LSPs and in tuning the resonance maximum to a given wavelength [111-113]. In view of this observation, a series of pillars of elliptical, and hexagonal geometries were fabricated in addition to the previously

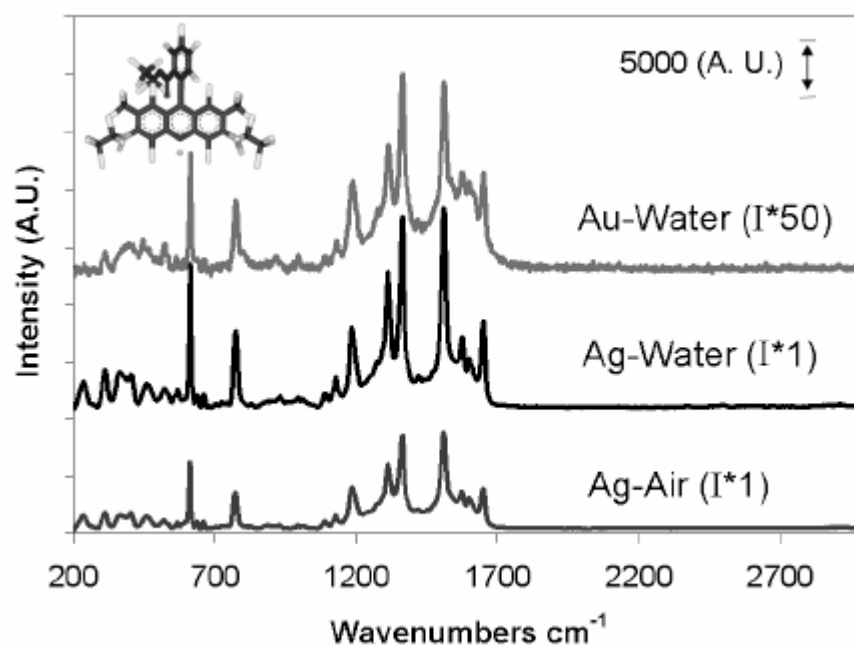


Figure 2-5. Spectra of R6G (1×10^{-6} M) in different dielectric environments for SP-A morphology: gold nanoparticles and water solution (top); silver nanoparticles and water solution (middle), silver nanoparticles dry (bottom). All silver substrates were irradiated for 1 s with $2.1 \pm (0.1)$ mW of 632.81 nm radiation from a He-Ne laser while the gold substrate was irradiated with $4.1 \pm (0.1)$ mW for 1s.

studied square patterns to compare their ability to generate large SERS signals using 633 nm for excitation (Figures 2-6 and 2-7). The nanoparticle spacing was set to 50 nm to provide a uniform comparison with the SP-A pattern. The magnitude of the 783 cm^{-1} SERS band of R6G ($1 \times 10^{-6}\text{ M}$) was used in this comparison since it is located in a region that is transparent to the ma-N 2403 background, and as prior experience indicated that photolytic changes in the band do not seem to occur. The results show an increase of over 20% in the apparent R6G SERS when the nanoparticle shape was changed from a square pillar (SP-A) to a hexagonal (HP-A) or elliptical (EP-C2) one (Figure 2-7). Correcting the observed signals over the available Ag surface area indicates that the improved SERS response on EP-C2 occurs due to an increase in the packing density of the array. In comparing the area corrected HP-A to SP-A, the former provides slightly greater signal, which may be a result of a greater number of loci per nanoparticle. Other geometries, such as rectangular and triangular pillars were also studied by our group, but their SERS performance was considerably inferior to those reported herein.

Geometric Arrangement (Symmetry) Studies. The elliptical patterns were chosen for a study of the effects of geometric arrangement on the magnitude of SERS signal since they were relatively easy to pattern in a variety of highly symmetric orientations. A new series of patterns were nanolithographically fabricated with C_2 , C_3 , C_4 , C_5 and C_6 symmetry axes. The abovementioned nanostructures were constructed with 50 nm spacing and a 1:3 (50:150 nm) lateral aspect ratio (Figure 2-8). Two factors are expected to influence the generation of LSPs and the magnitude of SERS signal in this study. First, the orientation of the excitation polarization vector relative to the

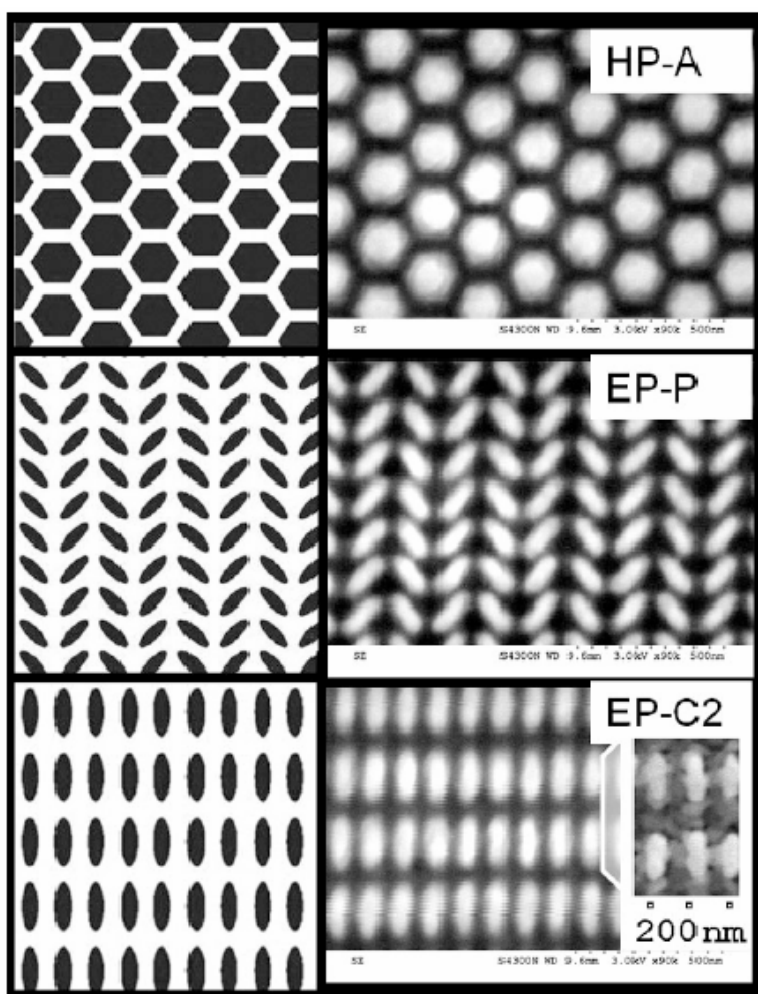


Figure 2-6. Scanning electron micrographs of a series of hexagonal and elliptical ma-N 2403 pillars on a Si wafer (right). The insets (left) are the AutoCAD drawings used by the EBL to generate these patterns. Each side of the hexagons is 100 nm long with a spacing of 50 nm between patterns. Each elliptical pattern has an aspect ratio of 1:3 with a spacing and short axis dimension of 50 nm. All micrographs were collected at 90K. The bottom right inset shows a 40K micrograph of the EP-C2 surface after the deposition of 25 nm of Ag at 1Å/s.

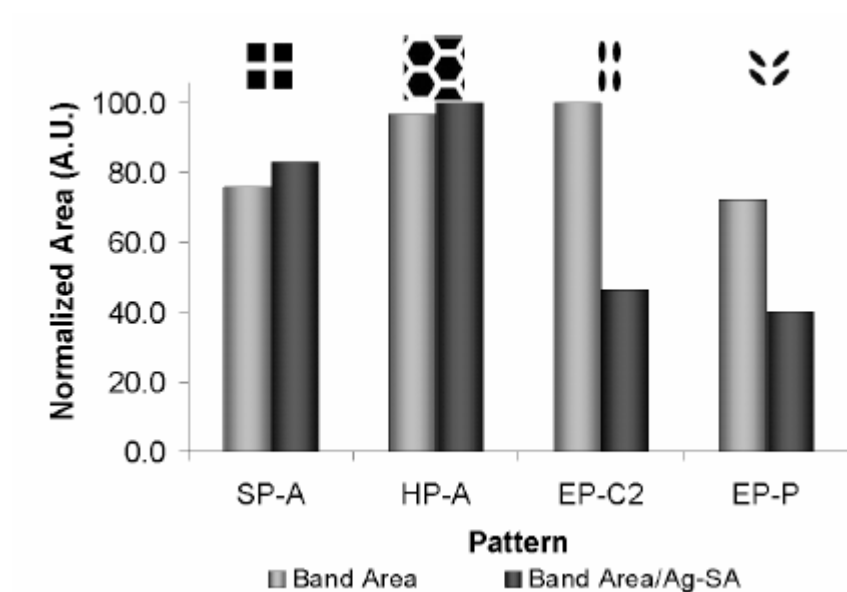


Figure 2-7. Changes in the 783 cm^{-1} band SERS signal of R6G ($1 \times 10^{-6}\text{M}$), as a function of the geometric pattern: normalized band area (gray); normalized band area over Ag surface area (black).

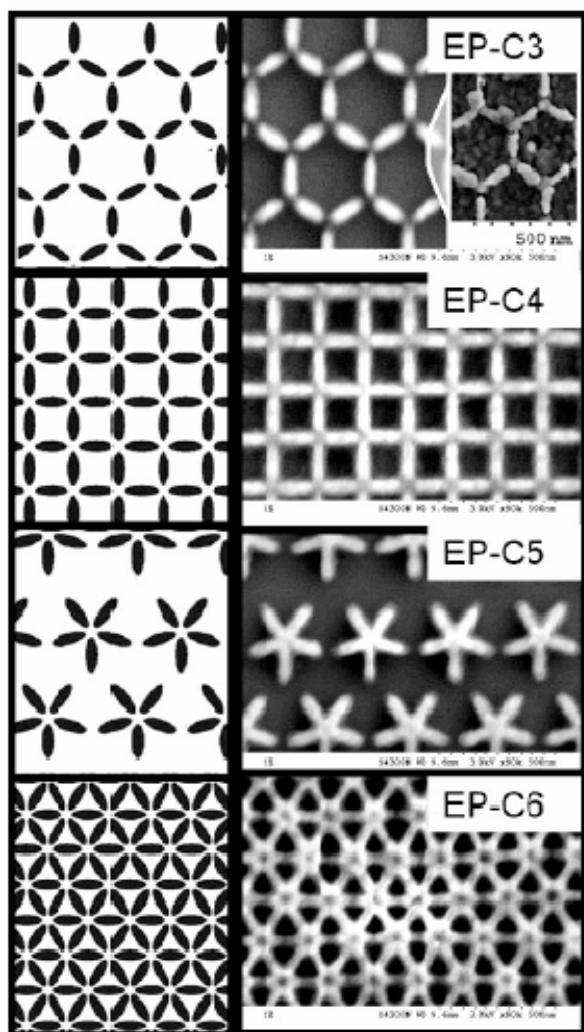


Figure 2-8. Scanning electron micrographs of a series of elliptical ma-N 2403 patterns with different point group symmetries (right). The insets (left) are the AutoCAD drawings used by the EBL to generate these patterns. Each pattern has an aspect ratio of 1:3 with a spacing and short axis diameter of 50 nm. All micrographs were collected at 90K magnification. The top right inset shows a 40K micrograph of the EP-C3 surface after the deposition of 25 nm of Ag at 1Å/s.

nanoparticle axis is known to influence the coupling of the excitation radiation into LSPs, with a parallel orientation (long axis of the particle along the vector) being the preferred orientation [31, 114, 115]. Second, interparticle coupling of LSPs is possible if interparticle spacing is very small. The limits on the gaps between the polymer pillars created via EBL are determined by the chemical nature of the e-beam resist, with the ma-N 2403 capable of 50 nm (or slightly less) features. Theory predicts this gap is too large to observe interparticle effects [76, 108, 116, 117]. However, by positioning multiple loci (ends of ellipses) in close proximity, by having extended structures with potential long range effects, and given that metal vapor deposition tends to overcoat the pillars and close the gaps to some degree, it was anticipated that some interparticle effects might actually occur in this study.

The raw and loci normalized responses (again the magnitude of R6G 783 cm^{-1} band) for this family of elliptical patterns were determined (Figure 2-9). The polarization vector effect should be best for the EP-C2 case and indeed both the raw and normalized responses are very good for this pattern. As expected, the response is slightly better than for the slanted EP-P pattern. It is curious that the loci normalized response for the EP-C3 pattern is the highest despite an expected less favorable polarization effect. This may indicate the favorable influence of interparticle effects. However, as the density of loci around a point increase with C3 through C6 symmetries the signals decrease substantially. You will note the micrographs in Figure 2-8 are demonstrating some fuzziness due to charging effects and this is greatest when many close loci are involved.

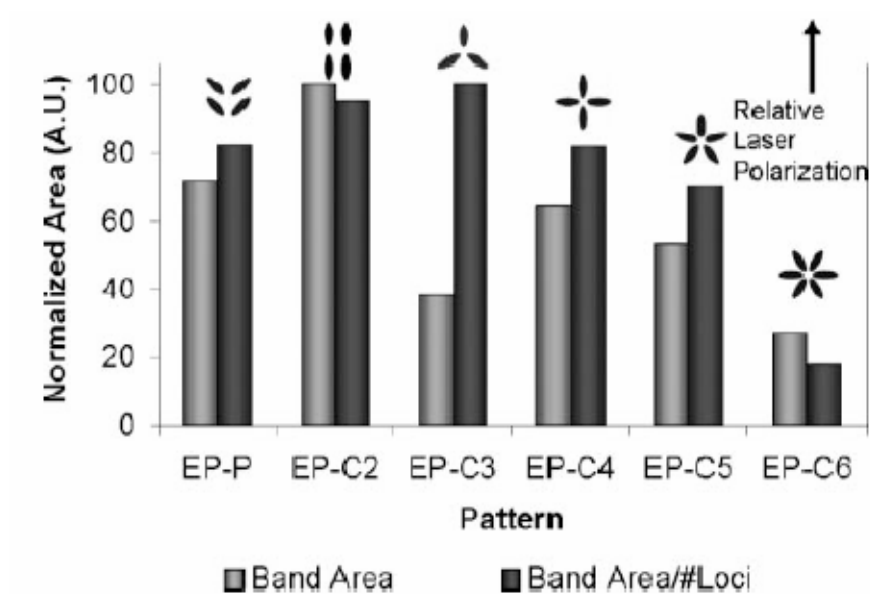


Figure 2-9. Changes in the 783 cm^{-1} band SERS signal of R6G ($1 \times 10^{-6}\text{M}$), as a function of the pattern orientation & symmetry: normalized band area (gray); normalized band area over the total number of loci (black). The direction of laser polarization relative to pattern orientation is shown.

Thus, the exact natures of the structures we are actually creating via EBL with vapor deposition of metal are not known, although we do expect they are periodic arrays. It seems from Figure 2-8 that the C3 pattern is actually three isolated ellipses pointing toward each other, but the C5 pattern may be more of a five sided star than isolated ellipses as created with the AutoCAD program. More refining of our nanofabrication procedures is clearly needed to better elucidate the effects of symmetry in this type of study.

A simple and clever way to evaluate the performance of the nanostructured surfaces at a given wavelength is by normalizing the inverse Rayleigh response of the substrate and comparing it with the SERS signal of the analyte. While several effects determine the relative magnitude of the laser backscatter, the effect of coupling to LSPs is to diminish the magnitude of the backscatter. Thus, if that coupling, due to a combination of particle hotness or density, is the dominant effect, then the inverse relationship should hold true. For the elliptical patterns, this approach shows a directly proportional relationship between the inverse Rayleigh signal and the raw 783 cm^{-1} SERS signal of a $1 \times 10^{-6}\text{ M}$ solution of R6G (Figure 2-10).

In summary, the nanolithography fabrication of metal-polymer nanostructures is a promising approach towards the rational design of SERS substrates. The systematic adjustment of the nanostructure characteristics such as size, packing density and spatial orientation is a valuable alternative for the creation of homogeneous and highly sensitive substrates tuned to a particular operating frequency. In principle, this approach provides

an effective means to maximize the sensitivity of the substrates so they can be used for the quantitative detection of ultra trace amounts of an analyte rather than its mere

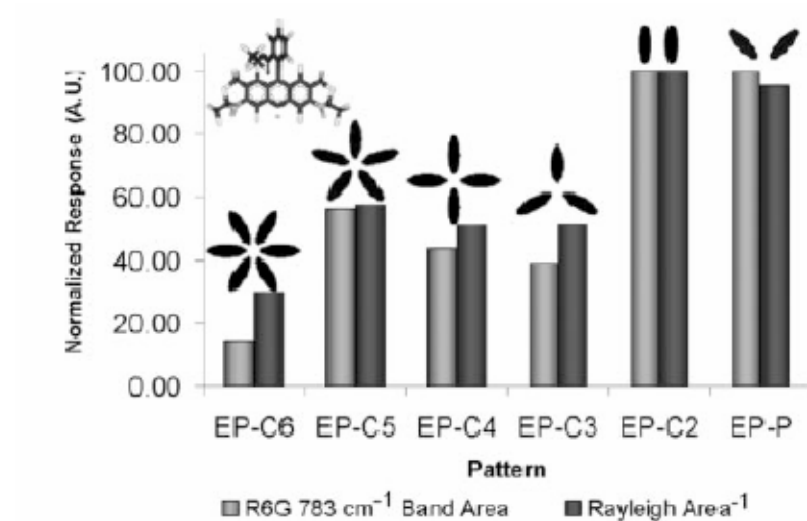


Figure 2-10. Changes in the Rayleigh (black) response (magnitude of laser backscatter) with respect to R6G SERS signal at 783 cm^{-1} (gray).

identification. The use of metal-polymer nanostructures for SERS detection provides some unique features such as: simplicity, reduced-cost, and enhanced sensitivity over extended areas. Consequently, nanostructure substrates are a promising alternative for the implementation of SERS as a routine analytical technique. A more detailed evaluation of arrays of elliptical nanoparticles is presented in the next chapter.

Chapter 3 Nanofabricated Periodic Arrays of Silver Elliptical Discs as SERS Substrates

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3.1 Introduction

Surface-enhanced Raman spectroscopy (SERS) is a useful analytical technique for determining the structural properties of molecules on or near metallic surfaces [9]. A theoretical basis for the SERS effect has been the topic of considerable debate [9-12]. In a simplistic sense, the intensity of Raman scattering is proportional to the induced electric dipole, μ , of the observed molecule. In turn, this variable, μ , is proportional to the polarizability of the molecule, α , and the magnitude of the incident electric field, E . The following was presented previously as equation 1-8:

$$\mu \propto \alpha E$$

Increasing either the molecular polarizability or the magnitude of the field that is experienced can enhance the observed Raman scattering intensity. Electromagnetic models have been proposed to account for E -related enhancements. These models are independent of the adsorbed analyte and applicable to a broad range of chemicals.

Likewise, chemical models have been proposed for α -related enhancements [14-19]. The chemical models are less understood and are specific in terms of the adsorbed analyte and the nature of the metallic surface. In general, chemical effects are considered lesser contributions to the SERS effect relative to electromagnetic effects.

Electromagnetic enhancement is more general in nature and is usually seen in SERS when substrates are made of roughened metal surfaces, typically gold, silver, or copper, that have features smaller than the wavelength of light being used [26-28]. When electromagnetic radiation impinges on a suitable metal surface, conduction band electrons undergo oscillations of frequency equal to that of the incident light. These oscillating electrons, when at/near the metal surface, are termed surface plasmons [29]. They produce a secondary electric field, which adds to the incident field. Surface plasmons have the ability to enhance the local electromagnetic field in the area near the nanoparticles composing the substrate, leading to greater SERS signal enhancement for analytes located therein, and are dependent upon nanoparticle shape and structure [30, 31]. To obtain large electromagnetic enhancement of the Raman signal of a molecule, it is necessary to tune the resonance of the surface plasmons to match the wavelength of the incident radiation [32]. In addition, the molecule itself can further polarize the metal at the scattering frequency and thereby amplify the Raman signal. As a result, research groups have focused on exploiting the electromagnetic enhancement mechanism of SERS by engineering substrates with both random and controlled morphologies that can be used to tune the observed surface plasmon resonance. Greater detail was given in chapter 1 concerning the enhancement mechanisms governing SERS.

While well-designed, random morphology substrates may lead to improved SERS enhancement, it is difficult to obtain reproducible nanoparticle size/surface features when fabricating these types of substrates. To overcome this difficulty, several groups have developed more ordered and controlled substrates using a variety of techniques. One method of substrate fabrication uses biocompatible nanocrescents that can be magnetically controlled to alter their orientation on the substrate surface to take on SERS hot-spot geometries [55]. Various assemblies of colloid materials have been used to produce ordered SERS substrates as well. Hexagonal arrays of spherical cross-section dishes have been formed by electrodeposition of gold through masks made of polystyrene colloid particles assembled on a gold surface [56]. Colloid crystal films have been used as templates to assemble gold nanoparticles in a controlled arrangement to create SERS platforms [57]. Unique SERS substrates have been formed in arrays of pyramidal shaped pits that have shown tunability of surface plasmons by altering pit dimensions while maintaining pitch [58]. Another technique widely used in creating ordered SERS substrates is nanosphere lithography [59, 60]. These techniques are discussed in more detail in chapter 1.

One other method of note is electron beam lithography (EBL). In his work, Kahl, et. al, combines EBL with a lift-off technique in order to create regular arrays of metallic nanoparticles for SERS [1]. Previous work in our group, has demonstrated a *direct* EBL technique for the fabrication of nanostructured SERS substrates. With EBL, substrates can be made having more uniform/homogeneous morphologies for SERS by controlling various aspects of nanoparticles composing the SERS-active platforms. In our previous

work (chapter 2), several nanoparticle symmetries, geometries, and particle shapes, sizes and spatial arrangements were explored. This work showed that particles shaped as elliptical discs arranged around a C2 axis of symmetry gave the best SERS signal when compared to other geometric shapes and symmetries [77].

From these results, we decided to focus on the improvement of our EBL-SERS substrates by using arrays of elliptical nano-discs fabricated using our unique, *direct* EBL process. We chose first to optimize the deposition conditions used for making SERS-active substrates by studying various sizes, gaps, and aspect ratios of ellipses arranged in rows of dimers. Once conditions were optimized, several studies were completed. Experiments were done to examine the effect of surface roughness and surface area of the nanoparticles in relation to SERS enhancement. Reproducibility and homogeneity of the EBL-substrate surfaces were examined via SERS signal of Rhodamine 6G (R6G). The relationship between the broad background continuum over the Stokes-shifted SERS range and the magnitude of the SERS signal for each array was also studied and correlated. Finally, a simple computational model was used to gain insight into the role of electromagnetic enhancement effects in these substrates' SERS performance [118].

3.2 *Experimental*

Surface-Enhanced Raman Spectroscopy Instrumentation. All SERS spectra were collected using a JY-Horiba LabRam Spectrograph. Details of the instrument setup have been described previously [34, 105]. In general, a 10X (0.25 NA, ∞) microscope objective was used to deliver 2.1 mW of the 633 nm line of an electrically cooled HeNe

laser with a spot size of approximately 25 μm . However, for the substrate homogeneity study and enhancement factor determination, a 50X (0.45 NA, ∞) microscope objective was used. The laser spot size was $<10\text{ }\mu\text{m}$ in that case. All spectra were collected with a 180° scattering geometry and sample acquisition times were set to 1 s. The laser polarization vector was along the long dimension of the elliptical discs.

Attempts to acquire extinction spectra for these substrates were pursued by simply replacing the laser source with the spectrometer's white light source that is normally used for visualization and aligning. The reference intensity at each wavelength was determined by the reflectivity just off the EBL-created patterns. Normally SERS spectra are manually corrected for the broad background scatter using the LabSpec 4.12 software of our Raman system. The total spectral area that is subtracted is available (see below) and is considered to reflect the magnitude of the substrate dependent extinction of the laser irradiation and SERS activity.

Preparation of Surface-Enhanced Raman Spectroscopy Active Substrates. A series of arrays of nano-size ellipses were created in AutoCAD 2005. All arrays were 40 X 40 μm in dimension and contained ellipses arranged in sets of dimers ranging in size from 300:50 nm – 300:300 nm (long axis:short axis) with inter-particle spacing of 100 nm and internal dimer gaps of 50 nm (see Table 3-1 for details). Each AutoCAD drawing was converted to GDS-II format by using the LinkCAD conversion program. All files were then transferred to the EBL system computer and converted to the format readable by the instrument. A 2-in. Si wafer (Wafer World, FL) was used as obtained and a 250

Table 3-1. Data for all fabricated nano-arrays. All vertical gaps between dimer sets are 100 nm. The shaded areas point out where the patterns differ from one another.

Pattern Name	Aspect Ratio	Dimensions (nm)				Number of Particles	Ag Surface Area (nm ² /Particle)	Ag Surface Area (μm ² / Pattern Set)
		L	W	H	Internal Dimer Gap			
6:1_50	6:1	300	50	20	50	28302	10.95x10 ⁴	3.10x10 ³
6:2_50	6:2	300	100	20	50	16960	19.30x10 ⁴	3.27x10 ³
6:3_50	6:3	300	150	20	50	15370	27.72x10 ⁴	4.26x10 ³
6:4_50	6:4	300	200	20	50	14098	36.19x10 ⁴	5.10x10 ³
6:5_50	6:5	300	250	20	50	10600	44.70x10 ⁴	4.74x10 ³
6:6_50	6:6	300	300	20	50	10600	53.25x10 ⁴	5.65x10 ³

nm film of ma-N 2403, a methacrylate-based negative photoresist, was applied via spin coating at 3000 rpm for 30 s. Once coated, the wafer was baked at 90 °C for 60 s and placed under vacuum in the EBL system. Resist film thickness was estimated from a chart provided by the manufacturer and is based on spin rate.

A Jeol JBX-6000 FS/E electron beam lithography system with a 50 keV thermal field-emission gun was used for the fabrication of the nano-arrays. The resist film was exposed to a beam of 180 $\mu\text{C}/\text{cm}^2$ to produce the patterns. Each 40 X 40 μm array was spaced 100 μm in the y direction and 500 μm in the x direction to yield 3 – 5 rows of evenly spaced unique patterns. Once the exposure was complete, the patterns were developed in alkaline ma-D 332 developer for approximately 10 s, rinsed with deionized water (18 M Ω Barnstead E-Pure), and dried.

Substrates were made SERS-active by deposition of 99.999 % Ag (Alfa Aesar, MA) using a physical vapor deposition (PVD) chamber from Cooke Vacuum Products, Inc. Samples were mounted 25 cm above and normal to the effusive source. Average mass thickness and deposition rates were monitored for each film using a quartz-crystal microbalance (QCM) mounted adjacent to the arrays. The ma-N 2403 arrays were coated with varying amounts of Ag at varying deposition rates depending on the study being done. The Ag islands on glass substrate used for performance comparison was formed by depositing an 8 nm average thickness of the metal at 0.2 $\text{\AA}/\text{s}$ on a clean microscope slide. Scanning electron microscopy (SEM) images were collected with a LEO 1525 microscope with a field-emission gun operating at 3.00 kV. Images were obtained in secondary electron detection mode. Sample damage and charge build-up were reduced

under these conditions to yield high-resolution images of Ag-coated and uncoated ma-N 2403 surfaces. Figure 3-1 shows SEM images of several patterns fabricated via EBL and coated with 20 nm Ag at 5.5 Å/sec. Their dimensions and additional information are listed in Table 3-1 with the shaded areas of the table highlighting the main differences in the various arrays. In essence, Figure 3-1 shows a periodic array of isolated Ag discs created on top of 250 nm high pillars, of various programmed lateral dimensions, of the e-beam resist polymer.

Atomic force microscopy (AFM) was used to examine roughness of substrate surfaces post Ag deposition at various rates. Images were collected in contact mode at a scan rate of 0.56 mm/s using a Asylum Research (Santa Barbara, CA) MFP3D microscope.

Analyte Preparation and Data Acquisition. The analyte used in most studies was 1×10^{-6} M Rhodamine 6G (98+%, Allied Chemicals) prepared in deionized water (18MΩ Barnstead E-Pure). This analyte under the conditions used (see below) is expected to effectively cover (form a monolayer on) the surface of the nano-patterned substrates. For enhancement factor determination, the analyte used was benzenethiol (99+%, Acros Chemicals).

Details of how data was collected and processed have been described previously [77]. Very briefly, the wafer containing the rows of patterns was placed at the bottom of a plastic Petri dish that was filled with approximately 2 mL of analyte solution. SERS signal was optimized by fine-focusing the microscope objective, and the spectroscopic data was collected by rastering the laser beam across each pattern at 10 μm intervals

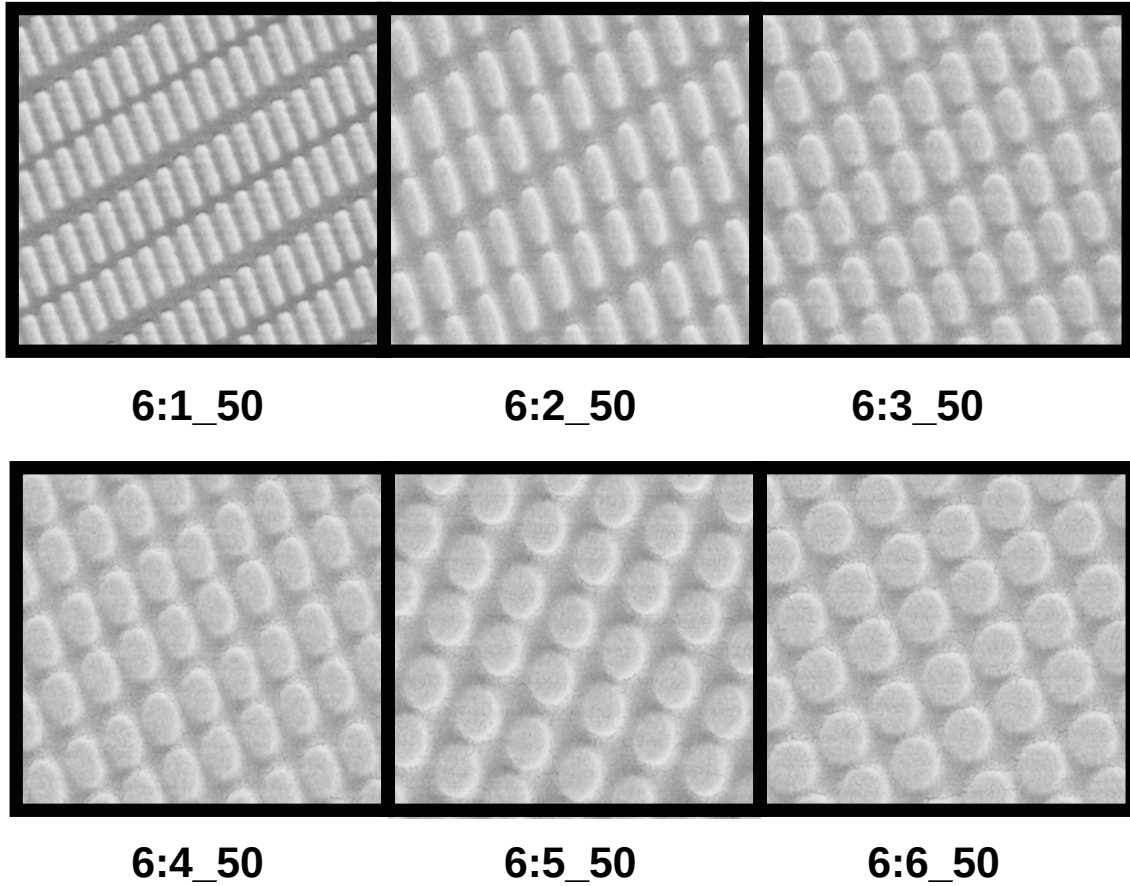


Figure 3-1. Scanning electron micrographs of a series of ma-N 2403 250 nm high elliptical pillars on a Si wafer. The dimensions of the nano-discs in each pattern are listed in Table 3-1. All micrographs were collected at a 45K magnification.

(1 spectral acquisition per step) over a $1600\text{ }\mu\text{m}^2$ area ($N = 16$). Spectra were collected on 3 – 5 arrays of the same pattern and averaged. The average of these spectra was used in interpreting results for each pattern.

For enhancement factor data collection, the above method of spectra collection was used for collecting data from both the Ag islands on glass and the EBL-ellipse substrates. Since two different substrates were examined using the same analyte, both were placed in the same plastic Petri dish and analyte added. Data was collected from one substrate after the other by moving the stage of our LabRam spectrometer. For collection of Raman spectra, the 50X microscope objective was tightly focused on a glass capillary tube containing neat analyte sample. Spectra were collected for 5 s each and averaged.

Theoretical Calculations. Electromagnetic enhancement effects for the Ag nano-discs were evaluated by treating the discs as ellipsoidal particles and computing the electric field near the surface of an individual, isolated disc when it is exposed to a static external field oriented parallel to the disc's long axis. The disc's dielectric constant was taken to be the frequency-dependent dielectric constant of Ag at the incident laser wavelength ($\epsilon = -15.87 + 1.07 i$) and the discs were embedded in a medium with the dielectric constant of water, $\epsilon = 1.78$. The magnitude of the near-surface field was evaluated by numerical differentiation (using a step size of 0.053 nm) of a mathematical expression for the electric potential outside a dielectric ellipsoid exposed to a static field [118]. These field computations were performed on an ellipsoidal shell 1 nm above a disc's upper surface. A field enhancement factor F was computed as the ratio between the magnitudes of the near-surface field and the static external field.

3.3 *Results and Discussion*

Determination of Optimum Physical Vapor Deposition Conditions. The first step in the optimization of our EBL-SERS substrates was to determine the optimum PVD conditions (i.e. rate of deposition, thickness of Ag deposited) that give the greatest SERS enhancement. Optimum deposition rate was found by depositing 20 nm of Ag at different rates. This thickness was chosen based on previous studies [77]. Silver was deposited on three separate rows of patterns at the following rates: 1.0 Å/sec, 3.5 Å/sec, and 5.5 Å/sec. The results of this study show that the fastest rate of deposition, 5.5 Å/sec, gave the greatest SERS enhancement (see Figure 3-2 and Surface Roughness/Area discussion below).

Subsequently, we determined the best thickness of Ag to deposit on the arrays. Three different thicknesses of silver, 20 nm, 30 nm, and 40 nm, were deposited on three separate rows of patterns at a fixed deposition rate of 5.5 Å/sec. Figure 3-3 depicts the SERS signal of 1×10^{-6} M Rhodamine 6G on patterns 6:2_50 (top) and 6:5_50 (bottom) coated with the three different thicknesses of Ag. Here the greatest enhancement is seen when 20 nm of Ag is deposited, despite the fact that the thinnest disc has the smallest surface area. This may indicate that the 20 nm high discs generate more intense surface plasmon resonances under the conditions employed.

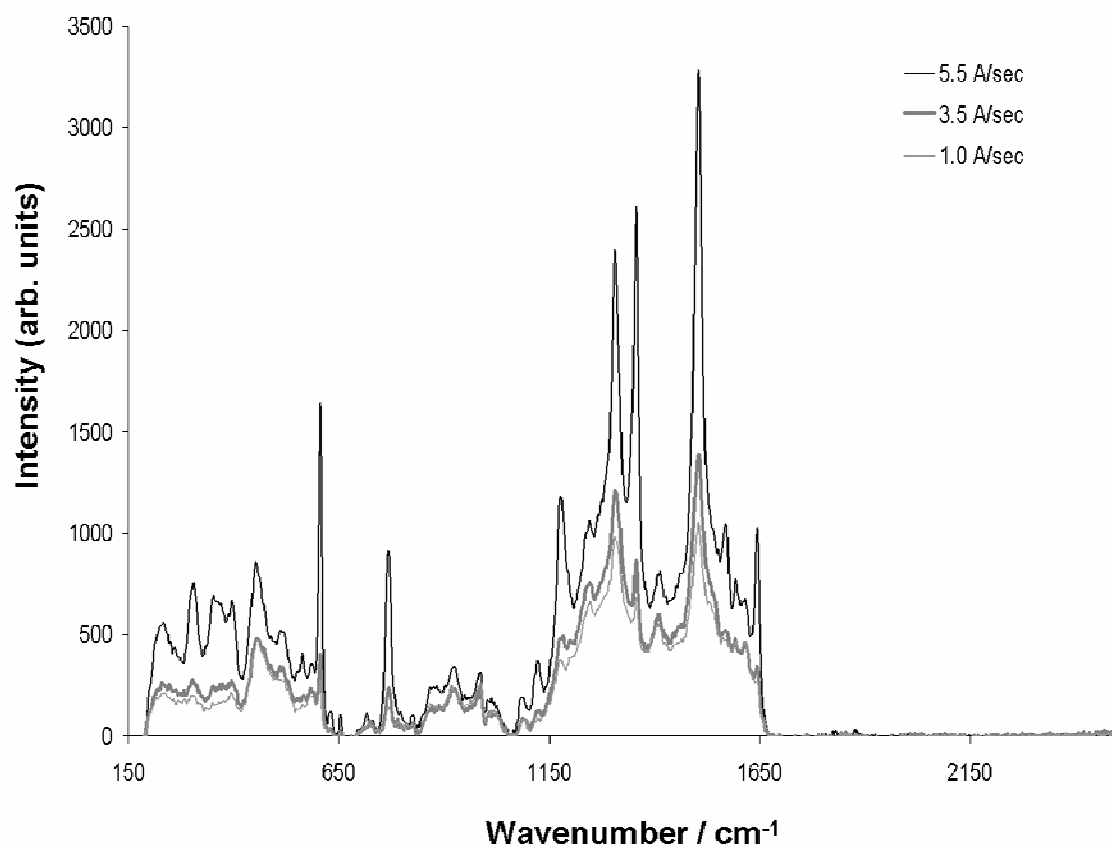


Figure 3-2. SERS spectra of R6G (1×10^{-6} M) collected on three arrays of pattern 6:2_50, each with 20 nm Ag deposited at three different deposition rates. Each pattern was mapped out in a 2x3 grid (total of 6 spectra for each pattern) with $2.1 \pm (0.1)$ W of 633 nm radiation from a He-Ne laser. Signal intensities are in arbitrary units (arb. units).

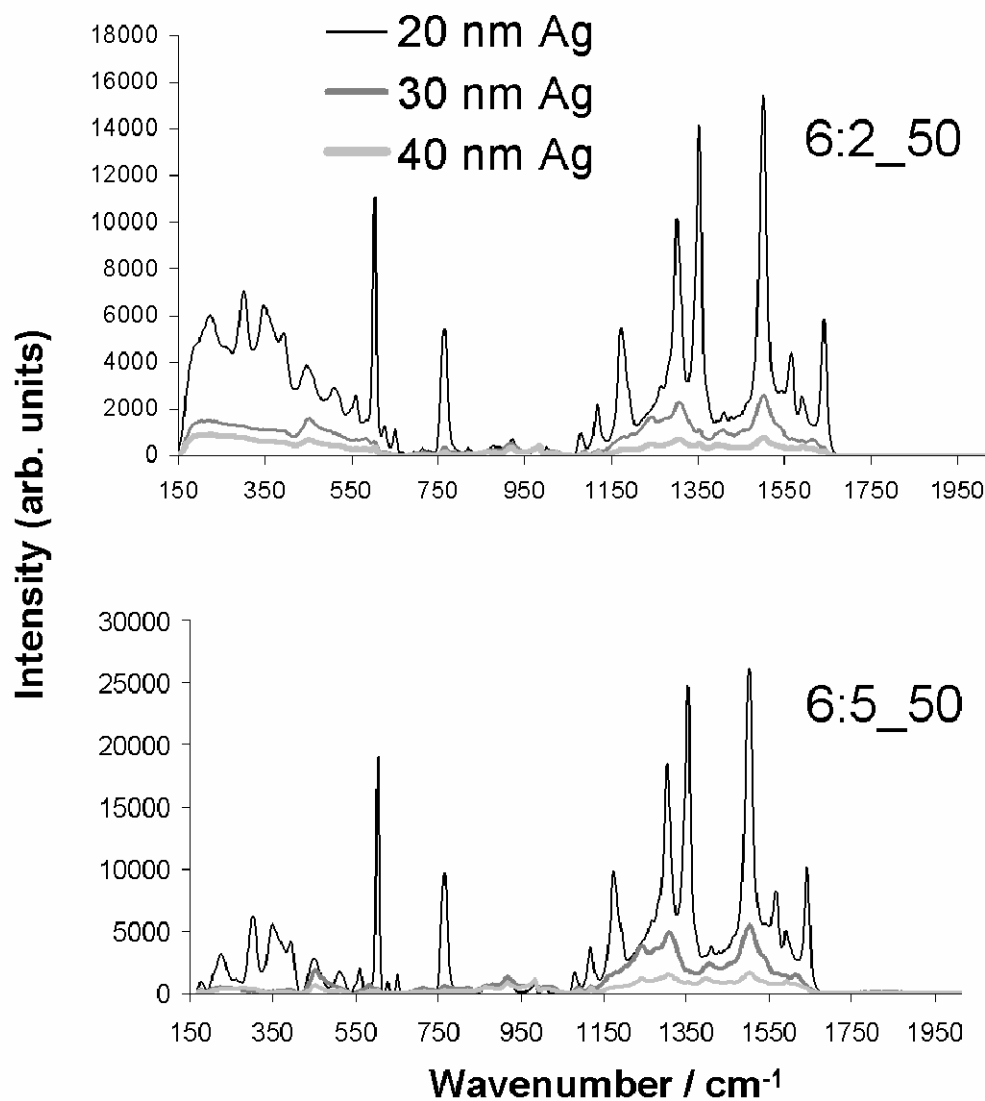


Figure 3-3. SERS spectra of R6G ($1 \times 10^{-6} \text{M}$) collected on patterns 6:2_50 and 6:5_50 with three different thickness of Ag deposited at $5.5 \text{ \AA/s}$. Each pattern was mapped out in a 4×3 grid (total of 12 spectra for each pattern) under the conditions listed in Figure 2 caption.

Reproducibility and Homogeneity of Arrays. In addition to creating periodic arrays as SERS substrates with predetermined morphology, it is expected that the EBL approach will produce substrates in a more reproducible manner than with random fabrication approaches. Thus, while conducting the studies discussed in this article, substrate reproducibility was examined. This study looked at the reproducibility between different sets of the same array pattern. SERS spectra were collected on three rows of patterns – all coated at the same time using optimum deposition parameters. Every row contained one pattern of each array listed in Table 3-1. The average SERS band area of the 767 cm^{-1} band of $1 \times 10^{-6}\text{ M}$ Rhodamine 6G for the three patterns (one from each row) was calculated and the reproducibility between the different patterns of the same array of nano-discs was determined. Table 3-2 shows a range in reproducibility from 12 % to 28 % as particle size increases and aspect ratio decreases.

With this study, the homogeneity within the arrays was also examined. Previous work done in our group has shown the merits of using Ag coated PDMS as a SERS substrate [34]. One drawback to this type of random morphology substrate is the inhomogeneity of the substrate surface. Figure 3-4 is a rastering experiment that compares signals for a uniform morphology EBL-SERS substrate to a random morphology Ag-PDMS substrate. It can be seen that the surface of the EBL-SERS substrate shows much less SERS inhomogeneity than the Ag-PDMS substrate. Note the random “hot spot” (point of greatest SERS enhancement) circled on the Ag-PDMS substrate. This hot spot shows ten times the amount of signal when compared to the spot of least enhancement on the Ag-PDMS nanocomposite. When examining the EBL-SERS

Table 3-2. Reproducibility between three different patterns of the same array.

Pattern	% RSD
6:1_50	12.4 %
6:2_50	21.8 %
6:3_50	20.8 %
6:4_50	23.1 %
6:5_50	24.8 %
6:6_50	28.4 %

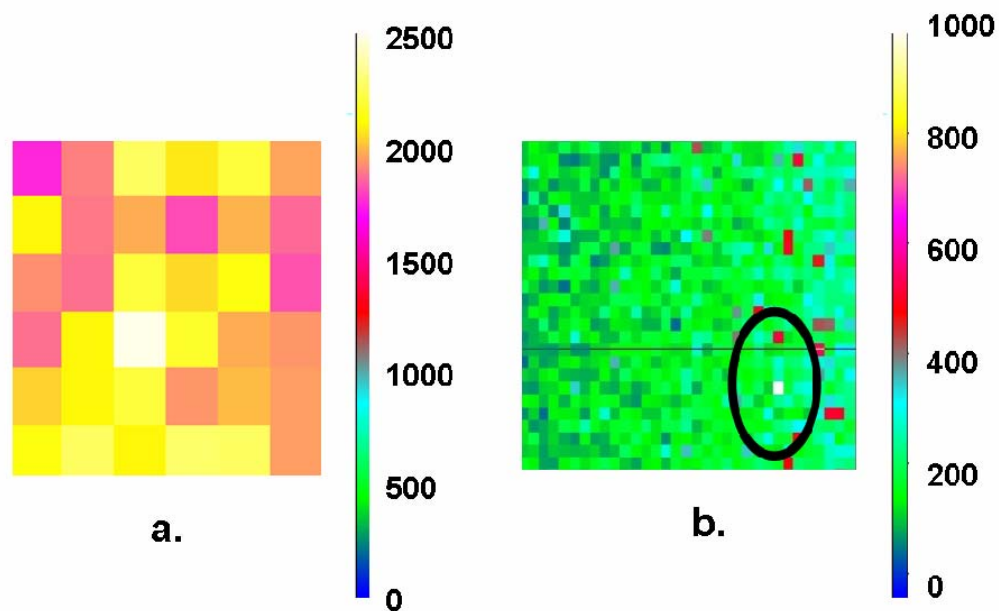


Figure 3-4. Comparison of homogeneity of EBL fabricated substrates a. 6:6_50 and b. Ag-PDMS substrate. The imaging involved displacements of 5 μm for (a) and 25μm for (b) (the dimensions of the spectral maps shown are: (a) 30 x 30 μm, and (b) 1000 x 625 μm). Note the random “hot spot” on the Ag-PDMS substrate (circled).

substrate, the signal at the hottest spot is roughly 33 % greater than that at the spot of lowest signal, considerably more homogeneous than the random morphology Ag-PDMS substrate.

Initial Expectations and Studies. We initially chose to make arrays of dimers of ellipses as this was the particle orientation showing the greatest promise from previous work [77], and because we wanted to monitor the effects of changing the internal dimer gap. Prior theoretical work indicated that as this gap is decreased, enhancement increases [119]. We intended to create the dimers of ellipses to be as close together as possible, but the resolution of our EBL tool only allowed for a gap of approximately 20 nm without some of the ellipse dimers touching each other following metal deposition. Since this was the case, internal dimer gap was changed from 20 nm to 100 nm (in increments of 20 nm) and SERS signal was examined. Surprisingly, we found that as the gap got bigger, the signal increased by approximately 50 %. By manually inspecting SEM images of our gap study patterns, we found that the ones containing the closest gaps had the most variability in gap distance (ranging from ~16 nm to ~22 nm). As the gaps were made bigger, the variability diminished (as observed from the SEM micrographs). Due to the limited resolution of our EBL system and our experimental procedure, it appears that the particles could not be uniformly brought in to close enough proximity to exhibit interparticle coupling, thus this effect could not be successfully studied.

The effect of changing the aspect ratio of the ellipses on SERS signal was also evaluated. We expected that the long, prolate ellipses would give greater enhancement than the round, oblate ones, due to the “lightning rod effect,” where the local electric field

around a sharp nanoparticle feature can be increased by a factor of 10^2 to 10^3 [119-121]. The tips of prolate particles have been predicted to show the largest shape dependent enhancements [122]. It was our expectation that this effect would dominate the total enhancement of the elliptical discs. When we collected SERS data, a different trend was seen, that points toward the significance of the overall area of each disc. Figure 3-5 shows what was observed. As aspect ratio changed from prolate to oblate, i.e. from pattern 6:1_50 to 6:6_50, the signal increased by a factor of 7.5 when normalized to number of discs optically probed.

Despite the ambiguities associated with enhancement factor (EF) calculations [123], we made an effort to estimate the factor for our substrate. The EF for the 6:6_50 pattern was calculated since it was found to be the best SERS performing substrate in these studies. The procedure followed below was found in the literature [124]. First, the spot size of the laser through the 50X microscope objective ($\sim 3 \mu\text{m}$), and the size and spacing of the nanoparticles composing the substrate determined the number of nanoparticles in the probed region. Next, the total surface area of the nanoparticles in the area of interest was determined. This was then multiplied by the reported packing density of benzenethiol (6.8×10^{14} molecules/ cm^2) [125, 126] to yield maximum surface number density (N_{surf}) of adsorbed molecules that led to the observed SERS signal. Neat benzenethiol in a glass capillary tube was used as the Raman standard. The volume of the probed area was found to be 10.6 pL. Using the density of the analyte (1.073 g/mL), the number density (N_{vol}) of molecules leading to observed Raman signal was determined to be 6.3×10^{13} molecules.

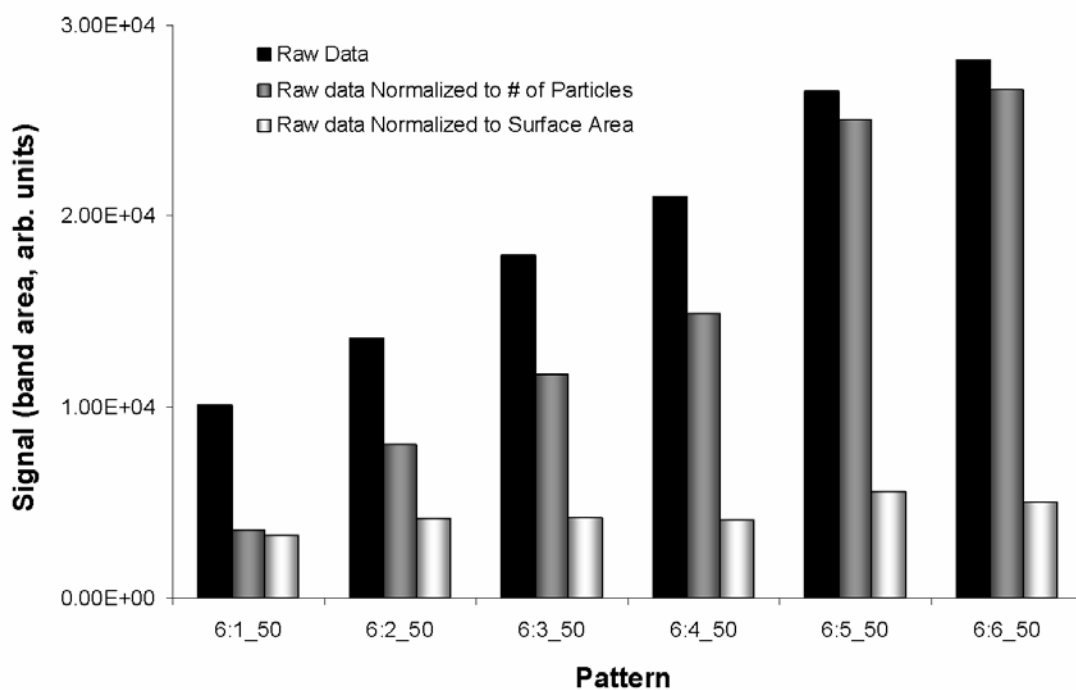


Figure 3-5. Each bar represents the band area (arb. units) of the 767 cm⁻¹ band of 1x10⁻⁶ M R6G as raw signal, normalized to the number of ellipses interrogated, and normalized to the total surface area interrogated. Going from the highest aspect ratio, 6:1_50, to the circular pattern, 6:6_50, the individual particle and the area corrected signals range over a factor of 7.5 and 1.5, respectively.

The SERS EF for the 6:6_50 pattern was determined using the following equation:

$$EF(1575\text{ cm}^{-1}) = \left(\frac{N_{\text{vol}} I_{\text{surf}}}{N_{\text{surf}} I_{\text{vol}}} \right) \quad 1-10$$

where I_{vol} and I_{surf} are the signal areas under the 1575 cm^{-1} peaks for neat benzenethiol (Raman standard), and $1 \times 10^{-6}\text{ M}$ benzenethiol (SERS), respectively. When calculated, the EF was found to be 5×10^6 . Results were compared to a more conventional SERS substrate, silver islands on glass. Within the sample container the spectrometer optic was translated and focused directly onto a piece of Ag island on glass substrate. Since the SERS signal found for benzenethiol using the 6:6_50 pattern was ~ 1.5 times greater than that of the signal obtained from the traditional Ag island film on glass (for which we expect that the surface area probed is somewhat greater), we assume that the enhancement factor for the ellipse pattern is greater than that of the traditional random morphology substrate.

We have attempted to relate optical extinction to SERS performance in prior work with random morphology substrates [34, 127]. In addition, other researchers have found informative correlations between the position of optical extinction maxima relative to laser excitation wavelengths with activity of their substrates [124, 128]. However, with their substrates, optical extinction spectra could be collected in transmission mode or on substrates that have low reflectivity / reflectivity that does not vary greatly over the substrates studied. Unfortunately, when we used the capabilities of our Raman spectrometer (see Experimental section) to acquire extinction spectra in a backscatter

mode the results were less than satisfying. In general, small trends consistent with Figure 3-5 were sometimes observed but never over the entire range of substrate nano-disc patterns. Our sense is that the large and highly variable reflectivity of our very dense nano-disc arrays obscures the more subtle resonances that should correlate with SERS activity trends.

As an alternative optical experiment, we attempted to use the observed broad continuum background as an indication of the magnitude of the extinction of the laser irradiation by the substrate and possible coupling to surface plasmon resonances. Early in the history of SERS it was recognized that this background is associated with SERS [129, 130]. More recently, a correlation between this optical continuum and single molecule SERS effects have been noted [131, 132]. Continuum background spectra have also been observed in other cases. For example, they have been noted underneath the SERS signal of analyte between the nanogaps of gold electrodes. Just as in the research described herein, this background was only present when SERS signal was present and vice-versa. It was also not seen outside of the electrode gaps [133], just as it is not seen off-pattern (i.e. where SERS is not observed) in our work. While perhaps not as desirable as directly acquiring actual extinction spectra, the experiment to quantify the magnitude of the continuum background is quite straightforward. The inset in Figure 6 demonstrates that the raw SERS spectra we obtain reside on a broad background that is subtracted in normal presentation (e.g., Figures 3-2 and 3-3). Our LabSpec software provides the total area under the background correction line. When this is plotted over the range 640 – 710 nm ($180 - 1740 \text{ cm}^{-1}$) in Figure 3-6, the correlation with the raw SERS activities across

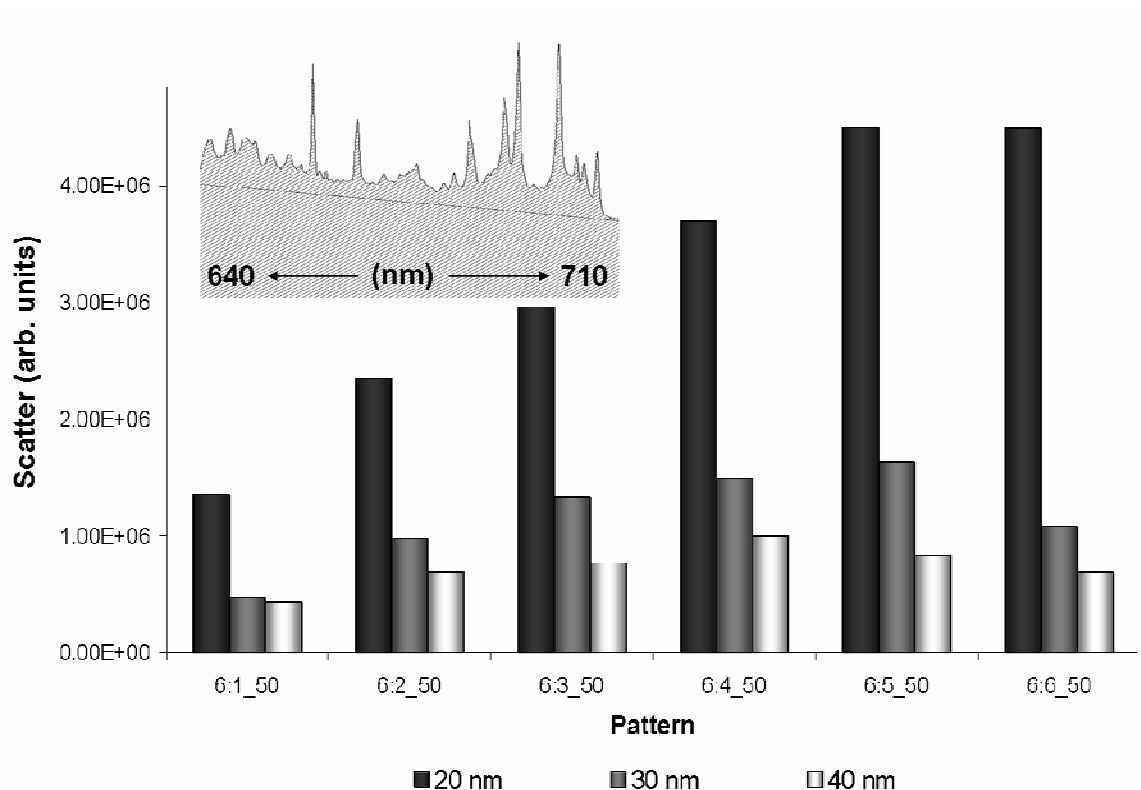


Figure 3-6. The inset is a typical R6G spectrum that shows the broad continuum background that is subtracted to baseline correct in the normal presentation of spectra (Figures 3-2 and 3-3). The area under the dashed line is then plotted in the bar graph for each pattern and at each nano-disc thickness as a pseudo-measure of the extinction properties of the substrates. This bar chart is for the exact patterns used for Figures 3-2 and 3-5 and correlates well with the magnitudes of raw SERS signals in those figures.

the patterns 6:1_50 through 6:6_50 as shown in Figure 3-5 is quite good. Evidence that the broad background is not leakage of laser excitation, backscattered radiation, or the fluorescence of some substance on the substrate is seen in the consistently lower background for the thicker coated substrates (20 nm vs. 30 and 40 nm); again correlated very well with the lower SERS activity of the substrates composed of thicker nano-discs (compare to Figure 3-3). The thicker nano-discs would be expected to exhibit at least as much laser backscatter or fluorescence as the 20 nm thick ones if, indeed, those were the sources of the broad background.

Influence of Surface Area and Surface Roughness. Surface area and roughness of individual nanoparticles appear to play important roles in the SERS enhancement observed in our experiments. A trend can be seen when going from pattern 6:1_50 to 6:6_50; as surface area of the individual particle increases, SERS signal also increases. In fact, Figure 3-5 shows that if the responses are normalized to surface area of discs optically probed, the signals increase only modestly by a factor of 1.5 when going from 6:1_50 to 6:6_50.

These trends in SERS signals are observed when comparing SERS signals from non-nanostructured photoresist surfaces coated with Ag to signals from our Ag coated nanostructured substrates. Both sets of substrates were coated at two different deposition rates, 1.0 and 5.5 Å/sec, to determine why the higher rate gave better enhancement (see Figure 3-2). With the non-nanostructured photoresist surfaces (i.e. off pattern), no signal is apparent regardless of deposition rate. However, when the photoresist is nanostructured and coated with Ag at the higher rate of deposition, the signal increases with nanoparticle

surface area (Figure 3-5). Under SEM examination of the various arrays, it appears that, as the deposition rate increases from 1.0 to 5.5 Å/sec, the silver particles (clusters) that are being deposited become smaller. We believe this leads to a more authentic coverage of the tops of the e-beamed photoresist pillars, and to a greater surface roughness effect for each pillar. Further evidence of this is seen in collected AFM images. Photoresist surfaces coated with 20 nm Ag at 1.0 Å/sec and 5.5 Å/sec were inspected with AFM and the images are shown in Figure 3-7a and 3-7b. These images illustrate the increase in surface roughness obtained for the higher deposition rate when compared to the lower rate.

It is thought that the greater surface roughness created with the higher rate of deposition adds to the geometric surface area of the individual nanoparticles giving more analyte accessible area to each particle making up the various nanostructured substrates. The greater roughness may also coincide with localized hotspots (greater lightning rod effects) on the nanoparticle surfaces themselves that contribute to the increase in observed SERS signal [134]. Since the larger particles have greater geometric surface area to begin with, they exhibit the best SERS enhancement when greater roughness/additional surface area as well as localized hot spots are generated at the higher rate of deposition.

Theoretical Calculations and Correlation with SERS Data. Figure 3-8a and 3-8b show the field enhancement factor F above the surface of two nano-discs, one with long axis:short axis dimensions of 300:150 nm (pattern 6:3_50, Figure 3-8a) and one with dimensions of 300:300 nm (pattern 6:6_50, Figure 3-8b), modeling the nanoparticles

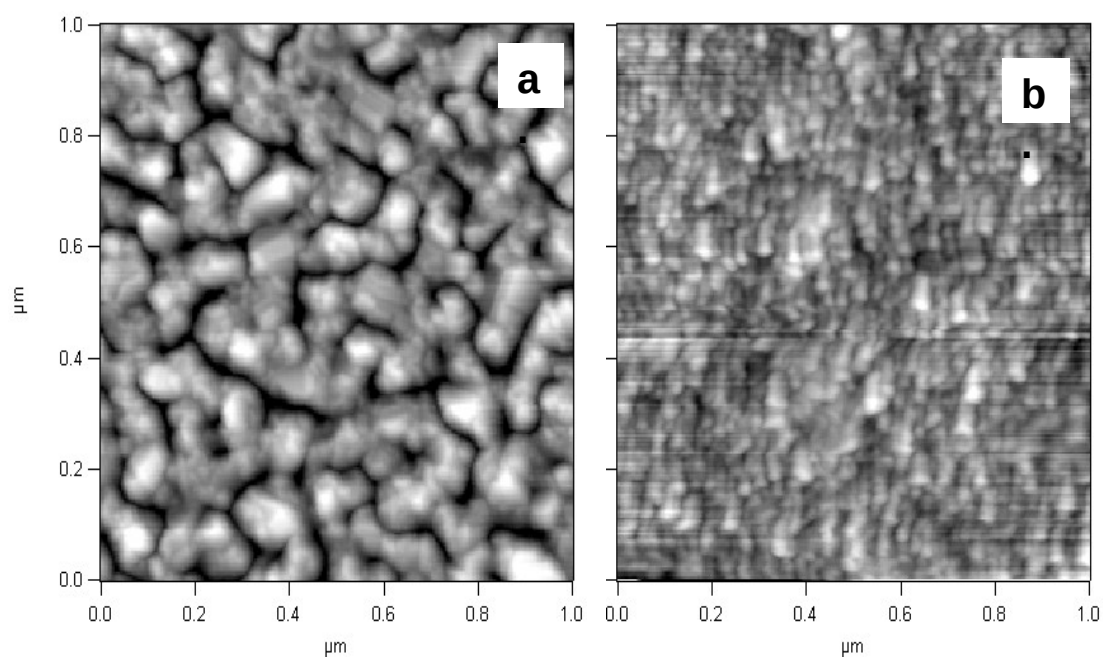


Figure 3-7. AFM images of 20 nm Ag deposited onto ma-N 2403 e-beam resist at two different rates (a. 1.0 $\text{\AA}/\text{sec}$, RMS roughness of 5.7 nm, and b. 5.5 $\text{\AA}/\text{sec}$, RMS roughness of 7.1 nm).

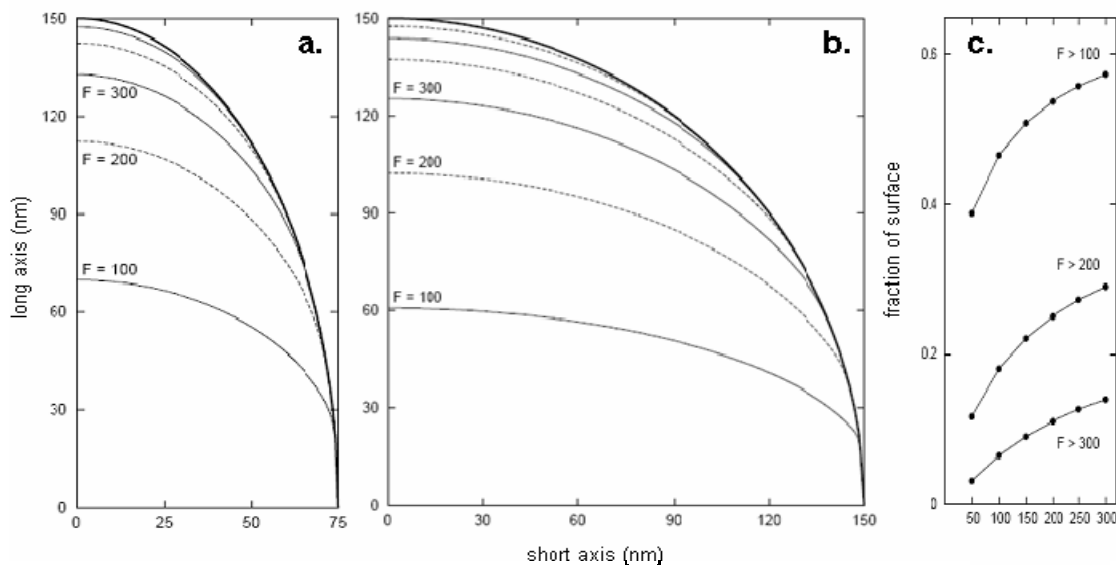


Figure 3-8. Contour plots of the computed field enhancement factor F above the surface of two discs with long axis:short axis dimensions of (a.) 300:150 nm (b.) 300:300 nm. Contour lines begin at $F = 100$ and alternate between solid and dashed; the interval between successive contour levels is 100. The thick solid lines indicate the discs' outer edges. Only one-quarter of the area above each disc is shown; the remaining three quadrants are identical to those shown here by symmetry. (c.) Fraction of a disc's upper surface for which F exceeds a cutoff value, for three cutoff values (100, 200, and 300). The lines drawn are merely intended to guide the eye and have no computational significance.

having 6:3 and 6:6 aspect ratios, respectively. This figure demonstrates that the upper surfaces of both discs have large regions where F is high, suggesting that electromagnetic enhancement effects play an important role in the SERS activity of the nanostructured substrates experimentally studied herein.

Closer inspection of the figure also reveals that the fraction of the upper surface with F values above 100 is similar for both discs; the same is true for the smaller regions defined by the more stringent cutoffs of $F = 200$ or $F = 300$. In fact, except for a small band around the edge of the disc, the contour patterns for the larger disc are well approximated by those for the smaller disc, simply stretched by a factor of two along the horizontal direction.

Figure 3-8c quantifies this observation further; it shows the fraction of a disc's upper surface for which F exceeds a cutoff value (100, 200, or 300) as a function of the length of the disc's short axis. This fraction is only modestly dependent on the overall size of the disc, especially for discs with short axes of 150 nm or longer. This in turn suggests that the overall SERS electromagnetic enhancement for a particular nano-disc should be roughly proportional to its surface area; larger discs will have a larger number of analyte molecules in high- F locations, but the relative size and shape of a given high- F region does not depend strongly on the disc's dimensions. (Similar trends are observed for shells suspended only 0.5 nm above the discs' upper surfaces, or for discs embedded in an external medium whose dielectric constant is that of the e-beam resist polymer: $\epsilon = 2.66$.)

This computational conclusion is in good agreement with the experimental observations. Specifically, the trend lines in Figure 3-8c should mimic the trends observed in the area corrected signals seen in Figure 3-5. For the $F > 200$ cutoff there is an increase in the “fraction of surface” of approximately 2 as the short axis of the disc is increased from 50-300 nm while the area corrected trend shown in Figure 3-5 is a reasonably close factor of 1.5. We note that our model employs the electrostatic approximation for the discs’ responses to a homogeneous static external field; this ignores electrodynamic effects and effects associated with the Stokes shift of the laser wavelength. Our model also considers only isolated discs and ignores lateral interactions among the discs, including possible short and long range effects. Work is in progress to include these effects in a more realistic model that employs the discrete dipole approximation [135].

The good agreement between the experimental trends shown in Figure 3-5 and the computational results shown in Figure 3-8 indicates that our experimental observations are consistent with a model in which the analyte molecules probed in these experiments reside on the upper surface of the Ag nanodisks. The Raman activity of these surface-bound analyte molecules is enhanced by the large near-surface electric fields supported by the nanodisks (see Figures 3-8a and 3-8b), which we have computed from an exact solution to Maxwell’s equations in the electrostatic approximation. Although many previous studies have emphasized the role of the “lightning rod effect” in producing large SERS enhancement factors for long, narrow particles [119-121], this effect plays a relatively minor role in our observation. This may be because the localized “hot spots”

created by the lightning rod effect are at the disc edges and few analyte molecules reside in these hot spots. The work presented here thus shows that a combined experimental and theoretical effort can provide new insight into the (sometimes counterintuitive) SERS enhancement factors obtained for real nanostructured substrates.

In summary, electron beam lithography continues to be a promising approach to the rational design of SERS substrates. Substrate morphology can be altered quickly through computer aided design software, while theoretical calculations and simulations of particle hotness can be used for planning future studies with these substrates and suggesting other structures, orientations, aspect ratio dimensions, etc. to try experimentally. These metal-polymer nanostructures offer homogeneity across the substrate surface as well as reproducible analyte environments. As a result, these nanostructured uniform morphology substrates continue to improve the chances of SERS becoming a routine method of analytical detection.

Chapter 4 A Biologically Inspired Nanofabrication Approach to Creating High-performing, Reproducible Surface Enhanced Raman Spectroscopy Substrates

4.1 Introduction

Surface enhanced Raman scattering is a valuable analytical phenomenon that is the result of the increase in Raman signal from molecules that have been attached to/are in the vicinity of nanometer-sized metallic particles. There are two accepted mechanisms, chemical and electromagnetic, that are generally recognized as being responsible for the observation of the SERS effect [9, 136]. Chemical enhancement mechanisms are dependent upon both analyte molecules adsorbed to the SERS substrate surface and the nature of the metal surface itself. With chemical enhancement, analyte molecules interact with metallic substrate particles at “active sites”, in some cases by creating a charge transfer intermediate state to increase Raman signal [137].

Electromagnetic enhancement is more general in nature and is typically not dependent upon the analyte used. It can be seen in SERS when substrates are made of roughened metal surfaces, typically gold, silver, or copper, that have features smaller than the wavelength of light being used [26-28]. When electromagnetic radiation impinges on the metal composing the SERS substrate, conduction band electrons undergo oscillations of frequency equal to that of the incident light. These oscillating electrons are called surface plasmons when they are at/near the metal surface [29]. They produce a secondary electric field, which adds to the incident field. Surface plasmons have the ability to

enhance the local electromagnetic field in the area near the nanoparticles composing the substrate, leading to greater SERS signal enhancement for analytes located therein. The effect is highly dependent upon nanoparticle shape and structure [30, 31, 85]. As a result, recent research has focused on taking advantage of the electromagnetic enhancement mechanism of SERS by engineering substrates with both random and controlled morphologies that can be used to tune the observed surface plasmon resonance. Greater detail on the electromagnetic mechanism of SERS can be found in chapter 1.

Perhaps the most common type of SERS substrate, one that consistently yields large signal enhancement, is simple colloidal gold or silver nanoparticles having the size range of 10-150 nm [40]. Usually these are formed by the reduction of metal salts, e.g., the reduction of silver nitrate with sodium citrate. By varying preparation parameters like temperature, reducing/stabilizing agents used, or metal ion concentration, the distribution of colloid size and shape can be partially controlled [138]. In some cases, the reduction is accomplished in more careful fashion to create cubes, rods, or triangular structures [41]. Colloid particles can be aggregated using sodium chloride to reduce the background seen in SERS while increasing SERS signal [49, 50, 139-142]. The colloidal systems that are most often used for single molecule and ultra-sensitive detection are usually aggregated clusters that possess some “hot spot(s)” within the aggregate. There is debate as to whether or not the aggregates themselves cause the Raman enhancement or if a “hot” particle happens to get trapped in the aggregate. Nevertheless, research has shown that “hot” aggregates can contain as few as two to six [42] tightly packed particles and be as large as ten to twenty/thirty particles [43]. Much research has been done using colloidal

substrates to support the finding that nanoparticle aggregation/density plays a role in Raman signal enhancement [44-51]. It has been shown that aggregates as small as dimers of nanoparticles can be “hot” [46]. However, in another study by Khan, et al., where the effects of aggregate size/nanoparticle density on surface enhanced resonance Raman scattering (SERRS) signal was examined, Ag colloid solution was evenly distributed across the surface of a TEM grid, and SERRS data was collected and correlated with TEM images. The results showed that as nanoparticle density increased, SERRS activity increased. Regions of the grids that contained large aggregates showed the most intense SERRS signal, while regions with few particles were the least intense. This same study also discussed the fact that active and inactive aggregates share similar features – interparticle spacing and size, etc. Figure 4-1 is from that study [43]. Though the nanoparticle clusters look similar, some of them yield good enhancement while other do not. The problem here arises in that well-designed, random morphology substrates may lead to improved SERS enhancement, but there is an inability to reproduce these chemically generated nanoparticle aggregates that have been found to yield that large SERS signal.

Previous work in our group, has demonstrated a *direct* electron beam lithography (EBL) technique for the fabrication of nanostructured SERS substrates [77, 143]. With EBL, the morphology of the SERS-active substrate can be controlled since the nanoparticles composing the substrate are chosen and laid out using computer aided design software. Recently, we found that substrates made of ordered arrays of ellipses having aspect ratios of 300:300 nm and 300:250 nm gave better SERS enhancement than

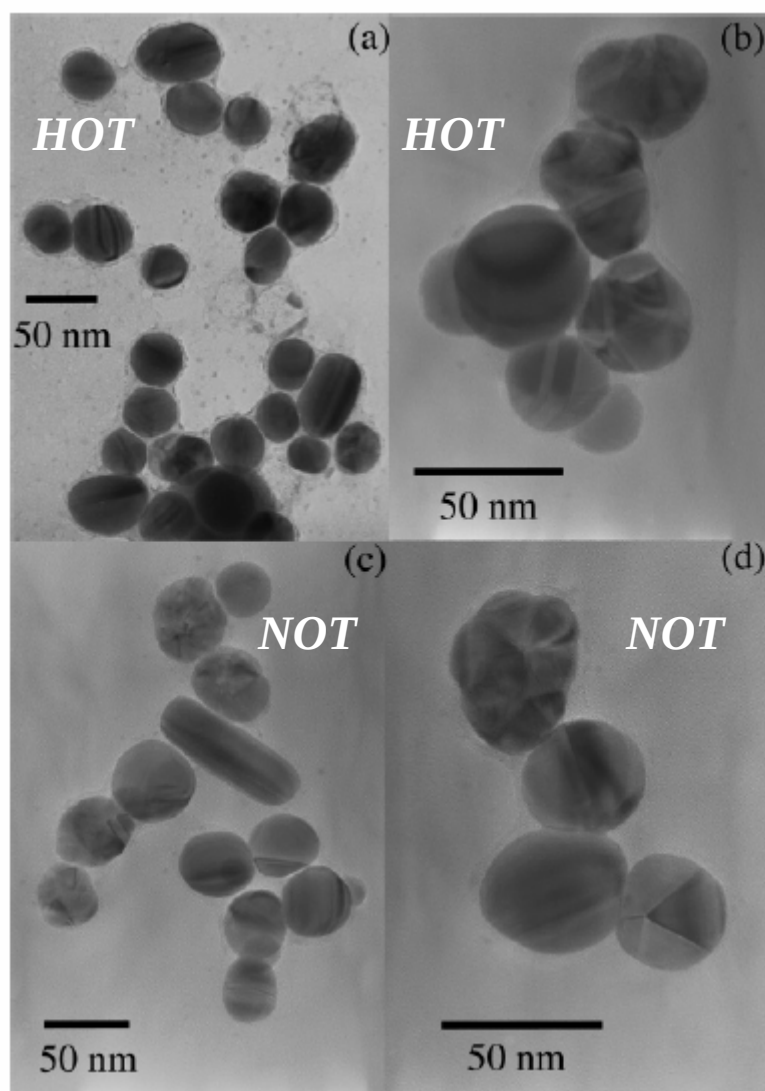


Figure 4-1. TEM images of SERS-active (a & b) and SERS-inactive (c & d) nanoparticle clusters.

smaller, prolate ellipses (300:50 nm to 300:200 nm) [143]. We have also done research with randomly shaped aggregated colloid and colloid shaped as cubes that have yielded good SERS enhancement as well [127].

Resisting the compulsion to exploit the precision capabilities of EBL to create ordered, uniform substrates, the purpose of our current work is to determine whether EBL can be used to study and clone random, “hot” clusters. Along those lines, we wanted to combine the two types of substrates to fabricate SERS substrates of greater enhancement that can be reproducibly fabricated. In this work, we borrow from the biological concepts of combinatorial drug discovery chemistry and cell-cloning to demonstrate a novel approach to the fabrication of SERS substrates (see below). We combine the randomness of colloidal aggregate substrates with the ability of the EBL system to reproduce substrate morphology by designing arrays containing pseudo-aggregates made up of different shapes ranging in size from approximately 100 – 700 nm. This chapter reports preliminary results of an on-going project to fabricate random, aggregate or fractal-like SERS substrates via EBL in a reproducible manner.

4.2 *Experimental*

Instrumentation. All spectra were collected using a JY-Horiba LabRam Spectrograph. Details of the instrument setup have been described previously [34, 105]. In general, a 50X (0.45 NA, ∞) long working distance microscope objective was used to deliver 2.1 mW of the 633 nm line of an electrically cooled HeNe laser with a spot size of approximately 2-5 μm . All spectra were collected with a 180° scattering geometry and

sample acquisition times were set to 1 s. SERS spectra are manually corrected for the broad background scatter and the fluorescence signal obtained using the LabSpec 4.12 software of our Raman system. In some instances, the surface enhanced fluorescence (SEF), is used as a quantitative measure of substrate performance.

Preparation of SERS-Active Substrates. A 50 X 50 μm pattern containing five different shapes of varying sizes (Figure 4-2A), randomly positioned, was created in AutoCAD 2005 (called “original aggregate array”). The shapes were copied in AutoCAD and blindly pasted into the array containing 5 X 5 μm squares arranged in a grid. Once the desired density of shapes was achieved, we wanted to fine-tune our ability to later find desired areas of the random patterns, so shapes that were positioned on the actual grid squares were deleted. This allowed for a small (~ 500 nm) space between each 5 X 5 μm area within the larger array. The overall result was a 50 X 50 μm pattern composed of separated 5 X 5 μm unit cells, each containing on average 6 – 9 particles/ μm^2 . The sizes of the nanoparticles composing the array were chosen as a result of previous work in our group where we saw good SERS enhancement from individual particles ranging in size from 250-300nm in size [143]. We wanted the size range of the particles to be centered there, so particles making up the original aggregate array range in size from 100 – 700 nm. In later studies involving the examination of the effects of aggregate density and nanoparticle size on SERS/SEF signal, particle size is cut in half to the range of 50 – 350 nm to make new aggregate arrays of varying particle density. The densities of these arrays range from 6 – 9 particles/ μm^2 to 22 – 30 particles/ μm^2 . In some cases, the 5 X 5 μm cells were separated from each other by 5 μm gaps.

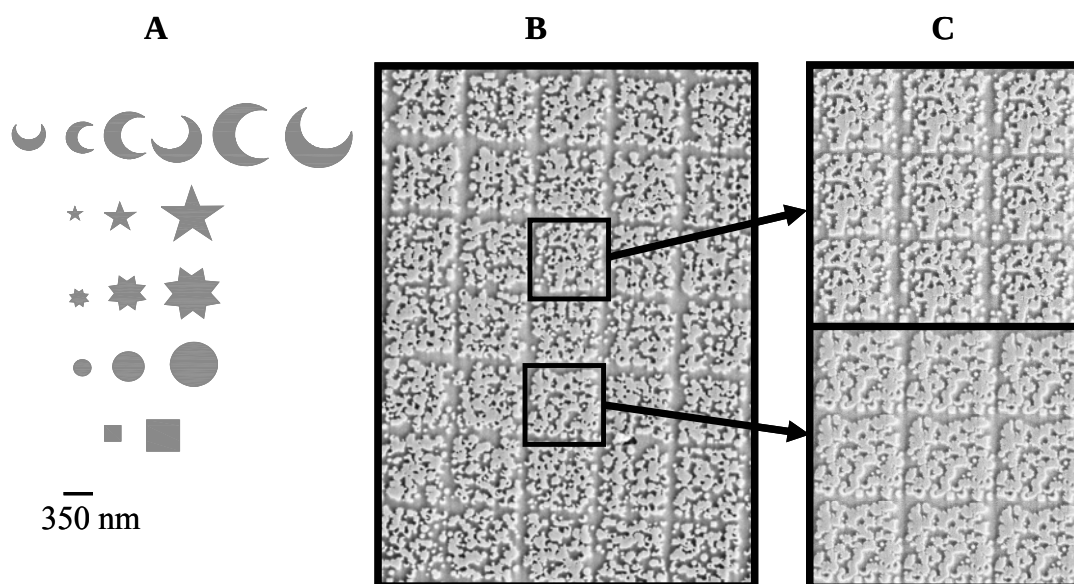


Figure 4-2. (A) CAD shapes used in generating the original 50 x 50 μm array. The shapes are randomly deposited into the array field at a density of approximately of 6-9 shapes/ μm^2 . (B) SEM image of a portion of the original aggregate array. The areas highlighted by the squares are cells yielding higher enhancement. (C) SEM images of portions of the cloned arrays from the original aggregate.

Once a “hot” cell was pin-pointed in any aggregate array via spectral data collection, described below, that 5 X 5 μm cell was found in the original AutoCAD drawing and cloned into a 50 X 50 μm pattern (called “cloned cell array”). The AutoCAD drawings were then converted to GDS-II format by using the LinkCAD conversion program. The files were transferred to the EBL system computer and converted to the format readable by the instrument. A 2-in. Si wafer (Wafer World, FL) was baked at 300°C for 1 hour to drive off any excess moisture adsorbed to the surface. Then, a 250 nm film of ma-N 2403, a methacrylate-based negative photoresist, was applied via spin coating at 3000 rpm for 30 s. Once coated, the wafer was baked at 90 °C for 60 s and placed under vacuum in the EBL system. Resist film thickness was estimated from a chart provided by the manufacturer and is based on spin rate.

A Jeol JBX-9300 FS/E electron beam lithography system with a 50 keV thermal field-emission gun was used for the fabrication of the nano-arrays. The resist film was exposed to a beam of 700 $\mu\text{C}/\text{cm}^2$ to produce the patterns. Each 50 X 50 μm array was spaced 100 μm in the y direction and 500 μm in the x direction to yield 3 – 5 rows of evenly spaced unique patterns. Once the exposure was complete, the patterns were developed in alkaline ma-D 332 developer for approximately 10 s, rinsed with deionized water (18 M Ω Barnstead E-Pure), and dried. Wafers were then exposed to an O₂ plasma for 15 seconds (Technics Reactive Ion Etching System) to remove any excess resist that may not have been removed completely during the developing step.

Substrates were made SERS-active by deposition of 99.999 % Ag (Alfa Aesar, MA) using a physical vapor deposition (PVD) chamber from Cooke Vacuum Products,

Inc. Samples were mounted 25 cm above and normal to the effusive source. Average mass thicknesses and deposition rates were monitored for each film using a quartz-crystal microbalance (QCM) mounted adjacent to the arrays. The ma-N 2403 arrays were coated with 20 nm of Ag at a deposition rate of 5.5 Å/s (unless a metal thickness study was being conducted).

Scanning electron microscopy (SEM) images were collected with a LEO 1525 microscope with a field-emission gun operating at 5.00 kV. Images were obtained in secondary electron detection mode. Sample damage and charge build-up were reduced under these conditions to yield high-resolution images of Ag-coated ma-N 2403 surfaces. Figure 4-2 shows SEM images of the original aggregate array (B) and cloned cell arrays 1 and 2 (C upper and lower, respectively), all fabricated via EBL and PVD.

Analyte Preparation and Data Acquisition. The analytes used in these studies were 1×10^{-7} M Crystal Violet (96%, Fisher), and 1×10^{-8} M Mitoxantrone dihydrochloride (97%, Sigma), both prepared in HPLC grade water (Fisher). 1×10^{-6} M Benzenethiol, prepared in 60% ethanol / 40% water, was used for the dielectric studies. Details of how data was collected and processed have been described previously [77, 143]. Very briefly, the wafer containing the rows of patterns was placed at the bottom of a plastic Petri dish that was filled with approximately 4 mL of analyte solution. SERS signal was optimized by fine-focusing the microscope objective, and the spectroscopic data was collected by rastering the laser beam across each pattern at 1-5 μm intervals (depending on the pattern being spectrally mapped or the study being performed, 1 spectral acquisition per step,) over the area containing the pattern. Spectra were collected on 3 – 5 arrays of the same

pattern and averaged. The average of these spectra (both SERS and SEF) was used in interpreting results for each pattern.

4.3 *Results and Discussion*

Low-Resolution Spectral Mapping. In order to determine whether the original aggregate array contained areas of enhancement, the array was spectrally mapped using low (5 μm intervals) and high (1-2 μm intervals) resolution with our LabRam spectrometer, and the SERS and SEF signals obtained from 1×10^{-7} M crystal violet and 1×10^{-8} M mitoxantrone were examined. The lower part of Figure 4-3A shows the observed signal map for crystal violet examined in 5 μm steps. Two areas of enhancement can be noted from the SEF (and/or SERS) map – one is denoted by a red star in the figure. For simplicity, Figure 4-3 diagrams the process of cloning only the starred cell. The two enhancing areas were found to be approximately 1.5 – 2 times hotter than the overall average signal from the original aggregate array itself. Once the cells of greatest enhancement were found spectrally, they were located in the original AutoCAD file. Subsequently, the cells were selected and made into their own 50 x 50 μm arrays (called cloned cell array 1 and 2), fabricated and made SERS-active via EBL and PVD, spectrally mapped, and examined under SEM. Figure 4-3B shows a cartoon of cloned cell array 1 on top as well as its SEF spectral map on bottom. On average, the cloned cell arrays had % RSD values ranging from 10% – 12% across their surfaces, yielding more reproducible analyte environments than the random original aggregate array (% RSD of

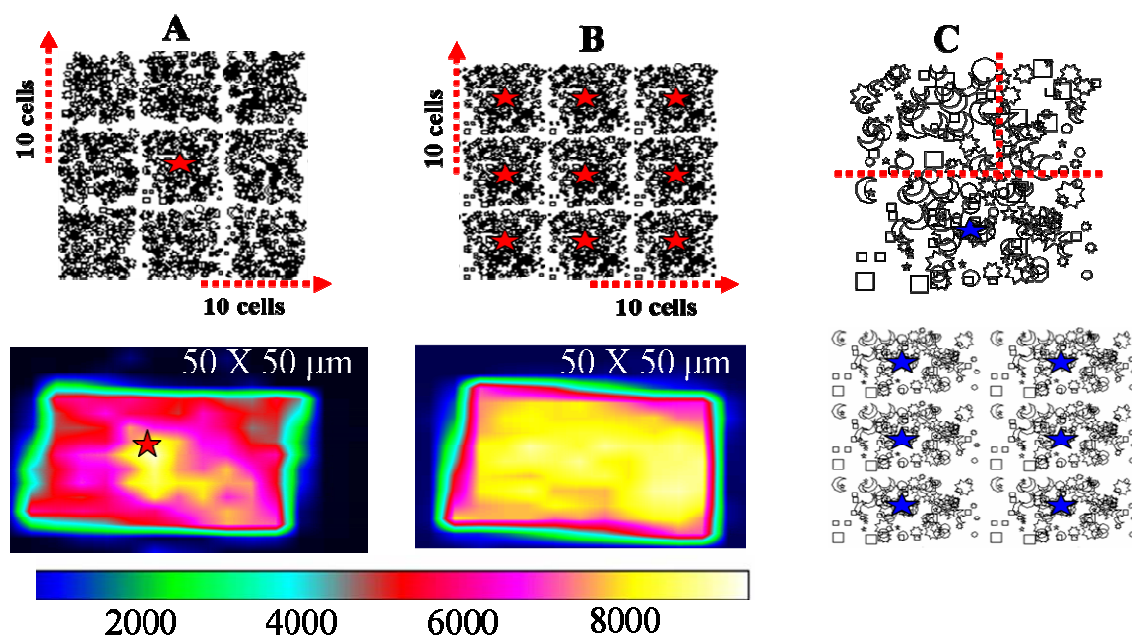


Figure 4-3. (A) Combinatorial inspection via SERS mapping ($\sim 5 \mu\text{m}$ resolution) of a 50 X 50 μm randomly generated substrate (original aggregate array) using different size/shape objects. For convenience the overall array is manually subdivided into separate and different 5 X 5 μm cells. The largest responding cell is denoted by the red star. (B) The high responding cell is *cloned* to create a cloned cell array containing identical 5 X 5 μm cells and similarly mapped. (C) The cloned cell can be further divided into aggregates that can be arrayed in order to pinpoint the most enhancing area of the cell. Alternately, the cell can be optically probed at higher resolution.

18%), and were 2.3 – 3.0 times hotter than the original aggregate array itself. Figure 4-4 shows typical spectra obtained for 1×10^{-7} M crystal violet – (A) raw data spectrum pointing out where SERS and SEF data are collected, and (B) background/baseline corrected spectrum. A discussion regarding the broad background seen in our spectra is discussed in the previous chapter.

We believe that the large fluorescence band we are obtaining from both crystal violet and mitoxantrone is surface enhanced fluorescence. Surface enhanced fluorescence, also called metal enhanced fluorescence, is one of the enhanced optical events that occur when large electromagnetic fields are created on the surfaces of metal nanostructures. Both SEF and SERS originate from the ability of metal nanostructures to support localized surface plasmons [9, 138, 144]. The main difference between the two phenomena is that they are optimized at different distances from the metal substrate surface. Maximum SERS signal is observed at the first adsorbed layer, while optimum SEF happens at a certain distance from the metal surface [145]. As a result, SEF is typically seen when a spacer layer is used that maximizes the signal of the target analyte by keeping the molecule just far enough away from the surface of the metal composing the substrate [145-148]. This is done in order to prevent the quenching of the fluorescence signal by interactions with the metal [146, 149].

We believe that we are observing SEF on our substrate surfaces for a few reasons. Since our analytes do not form a covalently attached or strongly adsorbed monolayer on the surface of the substrate, analyte molecules may “float” around near the metal substrate surface, just close enough to yield both SERS and SEF. Another scenario that

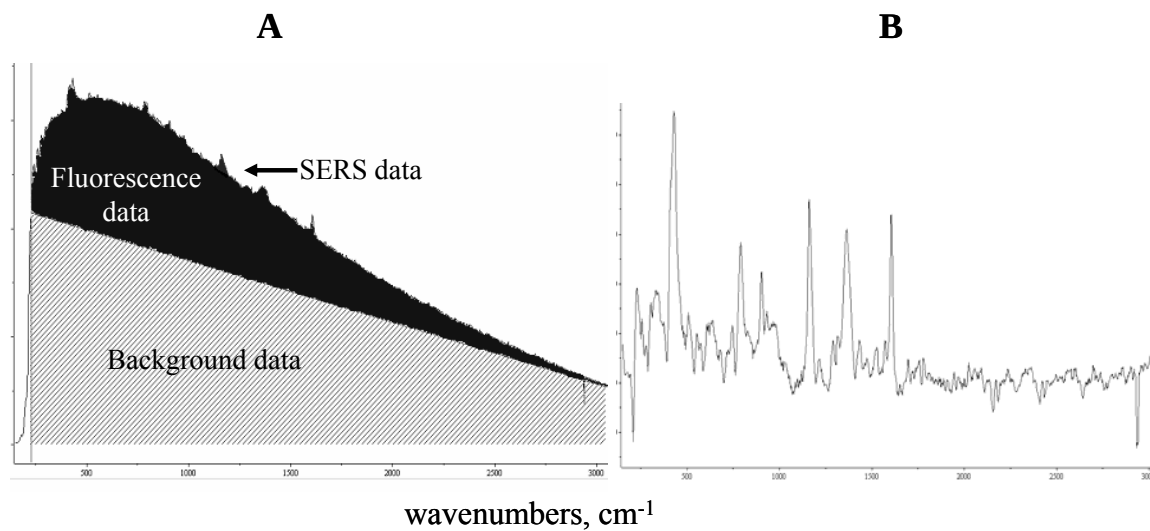


Figure 4-4. (A) Raw data spectrum for crystal violet. SERS data is from the area under the peak denoted by the arrow. Fluorescence data is the area above the baseline (including SERS peak area). Background data is the area under the baseline. (B) Background/baseline corrected SERS spectrum of 1×10^{-7} M crystal violet.

may be occurring at the same time is that the substrate surface is saturated by analyte molecules in such a manner as to pile up on top of one another. They could be acting as their own spacer to cause the appearance of surface enhanced fluorescence. Another indicator that the fluorescence is actually surface enhanced is that when the laser is focused off-pattern or in the bulk solution, no signal is observed.

Polarization Study. One experiment conducted using the lower resolution mapping involved looking at the effects of changing incident laser polarization. Local surface plasmon resonance is expected to be anisotropic across nanostructured SERS substrate surfaces [150]. This implies that enhancement should be dependent upon incident polarization [151]. Experiments in the literature have shown strong correlation between incident laser polarization that is parallel to longitudinal axes of closely spaced nanoparticle dimers or laser polarization that is diagonal across nanoparticles having sharp features, and increases in SERS enhancement [151-154]. However, when nanoparticle clusters composing substrates are made up of more than two particles, the polarization dependence appears to be more isotropic than for isolated dimers or individual particles having sharp features [151]. For these reasons, we expect that the random nature of our multi-particle aggregate substrates would lead to little difference in SERS signal with change in laser polarization. However, by examining the effects of laser polarization, we may be able to identify different localized “hot spots” at different incident polarizations if the “hot spots” have characteristics similar to the closely spaced dimers or sharp featured nanoparticles mentioned above.

To complete this experiment, one original aggregate array was rotated from 0° to 90° , in increments of 30° , and spectrally mapped (crystal violet as the analyte) at each rotation. Each time a spectral map was collected, there was a $\sim 30\%$ drop in signal going from 0° to 90° rotation. The way the experiment is set up currently, we use the same array repeatedly for this study. We believe it may be due to substrate degradation from the repeated irradiation of the same pattern (this is discussed in greater detail in the next section). To solve this problem, we have planned an experiment where the same pattern is EBL-arrayed multiple times in the same row, but at different angles of rotation. This will allow us to examine the effects of changing laser polarization by precisely positioning the arrays at the proper angles of rotation reproducibly, while using a different pattern (that was fabricated under the same conditions) each time so pattern degradation does not interfere.

High-Resolution Spectral Mapping. Since the purpose of this work is to pinpoint “hot spots” or areas of greater SERS enhancement so they can be reproducibly fabricated, we needed to map our patterns in smaller steps. Specifically, aggregated hot clusters via fabricated synthetic methods occupy less than one μm^2 . By probing larger areas we are possibly diluting the sought after enhancement effects. Higher resolution was attempted by first dividing the cloned cell arrays into sections using AutoCAD, arraying those sections into $50 \times 50 \mu\text{m}$ patterns (see Figure 4-3C), and fabricating them for SERS detection using EBL and PVD. These patterns were originally mapped in $5 \mu\text{m}$ steps to see if one arrayed section gave better signal than others, and then mapped in $1 \mu\text{m}$ intervals to further narrow down the hot spots. In the first study ($5 \mu\text{m}$ intervals), the

results showed which areas of the cloned cell arrays were not good SERS enhancers. Table 4-1 shows data collected in this study. Only a few sections of the cloned cell arrays were as hot as the corresponding cloned cell array itself. When we were initially starting these studies, we wanted to use a higher magnification microscope objective (80X) to get a smaller laser spot for smaller interval steps. The problem with this was that the stage of the LabRam spectrometer has a minor amount of “jitter” that is more problematic under the 80X objective. Due to the movement in the stage, we were unable to raster across the surface of the arrays using this objective, so these studies were completed using the 50X microscope objective (spot size of $\sim 3\text{ }\mu\text{m}$). When we switched to using $1\text{ }\mu\text{m}$ intervals, it was determined that rastering across the surface of the substrate with such small steps seems to cause degradation of the substrate surface just as it did with the polarization study discussed previously. It seems that the same degradation of the pattern surface occurs whether using the same pattern repeatedly or stepping across its surface in small intervals. Figures 4-5A – C show evidence of this, where 4-5A shows the $1\text{ }\mu\text{m}$ steps taken across the substrate surface and 4-5B shows the same pattern when mapped at a larger interval, after mapping with the $1\text{ }\mu\text{m}$ steps. In the figure, the upper left corner of the pattern was mapped at the smaller interval. Figure 4-5C is an SEM image of damage to a pattern after larger interval steps, with the circles across the pattern being where it was exposed to the laser. We saw pattern degradation regardless of which analyte was being used. The substrate damage may be due to the fact that each small interval step has some overlap of the laser spot when moved to the next step. We tried to overcome this drawback by combining a high neutral density filter (NDF 3.0) with intervals that may

Table 4-1. How much “hotter” each pattern is in comparison to each cloned cell array.

Pattern	Fluorescence	SERS
Cloned Cell Array 1 - Section 1	0.88	0.85
Cloned Cell Array 1 - Section 2	0.64	0.74
Cloned Cell Array 1 - Section 3	0.75	0.75
Cloned Cell Array 2 - Section 1	0.78	0.86
Cloned Cell Array 2 - Section 2	0.81	0.95
Cloned Cell Array 2 - Section 3	0.97	1.03
Cloned Cell Array 2 - Section 4	0.87	1.03

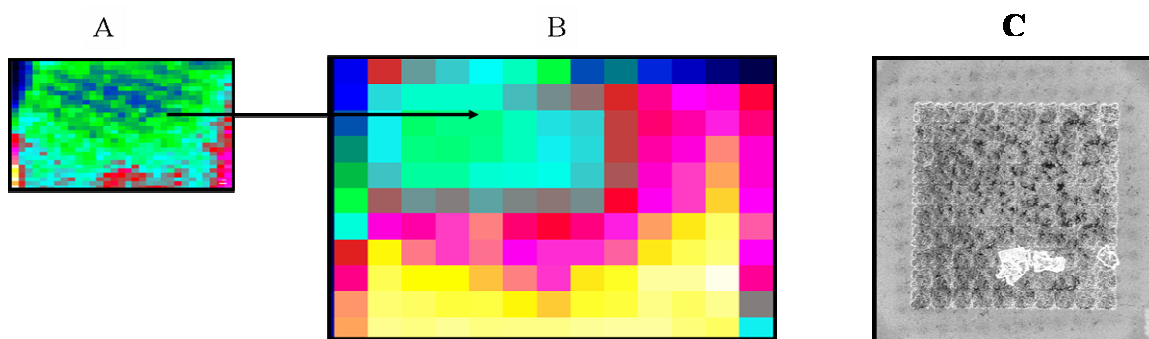


Figure 4-5. (A) Map of section 1 from cloned cell array 1 using 1 μm steps. (B) Map of the same section, using 5 μm steps *after* the 1 μm mapping. Note the damage to the upper left corner of the pattern. (C) SEM image showing where lasers damaged Ag on a random aggregate pattern.

not overlap as much (2 μm steps) to possibly reduce the effects of overlapping laser spots while still obtaining SERS signal. In some cases, this was successful, in others it was not. We are planning to try this experiment again. Currently, we do not have a solution to these issues and are looking into other methods that could be used for the characterization of our SERS substrate surfaces.

Advanced Methods of Substrate Characterization. Instead of using our LabRam spectrometer to spectrally map the surface of our random substrates, we could use another method that will provide both topographic/surface characterization of our substrates as well as SERS signal at each interrogated spot. The general name of this method is tip-enhanced Raman spectroscopy (TERS). This technique uses a sharp tip (either made of metal or coated with metal – Au or Ag for example – whose surface plasmons can be excited with a visible wavelength laser) that can be positioned using scanning probe microscopy methods to examine a region of interest. The interaction of the laser and the metallized tip can lead to enhancements of the local electric field near the point of the tip [155]. Several research groups are working with this technique and resolution in TERS has been reported on the sub-100 nm level [155-158], so it has the potential to provide the resolution and the surface sensitivity needed for our application. However, we are unsure about the information we will obtain from this technique. We wonder whether the signal we will acquire will be from the fact that areas of our arrays are intrinsically “hot”, or if it will arise just because we are bringing a metal-coated probe into contact with our sample. There will need to be extensive work done to determine what is causing the signal if we are able to try the TERS technique with our substrates.

Robert Shaw's group at Oak Ridge National Laboratory has recently developed a probe that combines both AFM and near-field scanning optical microscopy (NSOM). They have used it to image the 1330 cm^{-1} Raman mode of a polycrystalline diamond thin film [159]. Our research group has been in contact with Dr. Shaw and we hope to collaborate with his group at ORNL to characterize our substrates to better pin point "hot spots."

Effects of Nanoparticle Density. Even though we have not yet solved the problem of high-resolution mapping, we did demonstrate that areas of greater SERS enhancement can be found and cloned, to fabricate reproducible substrate surfaces/analyte environments. With the proof of concept established, we wanted to examine the effect of nanoparticle density (e.g. number of nanoparticles making up the aggregates / extent of particle aggregation) on SERS enhancement. To do this, an additional four random aggregate arrays were designed in AutoCAD. One array used the exact same shapes (in the same sizes) as the original aggregate array used in the initial studies, but was less dense ($3 - 6\text{ particles}/\mu\text{m}^2$). The other three arrays were also made of the same shapes, but the shapes were half the size of those used in the original aggregate array. These three arrays ranged in density from $6 - 9\text{ particles}/\mu\text{m}^2$ to $22 - 30\text{ particles}/\mu\text{m}^2$. Figure 4-6 shows SEM images of several $5 \times 5\text{ }\mu\text{m}$ unit cells from each of the new aggregate arrays as well as cells from the original for comparison.

Initial studies conducted with these varying density patterns (as well as all studies described above) used previously determined deposition conditions for substrates fabricated via EBL and PVD [143]. Table 4-2 shows data obtained for different density

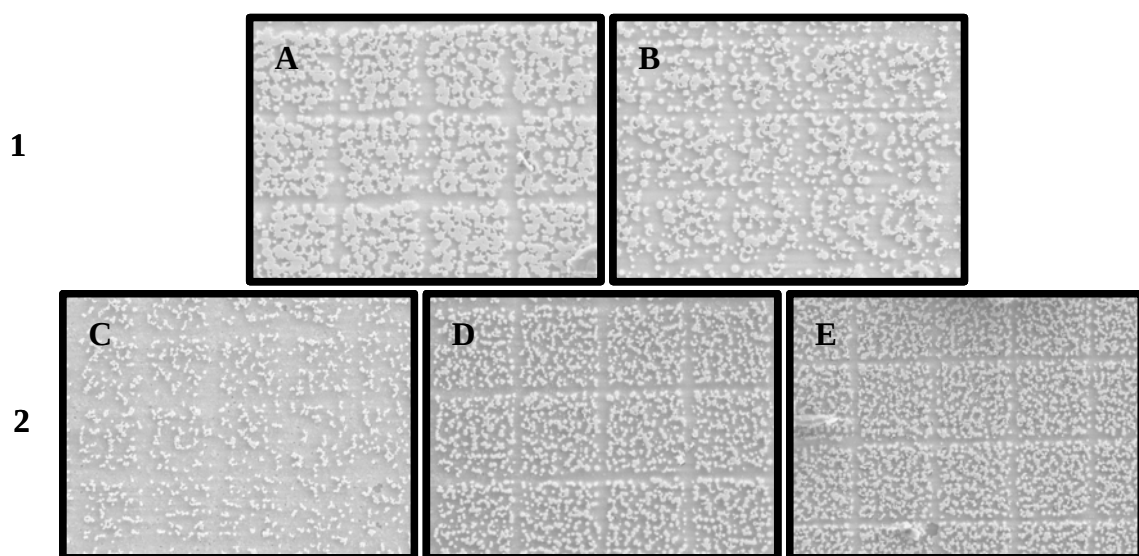


Figure 4-6. Aggregates made for the study of particle density and size. Row 1: (A) original aggregate array, (B) same shapes as original aggregate array, but less dense. Row 2: smaller shapes than those used in original aggregate array, with increasing particle density from (C) to (E).

patterns as well as data from the cloned cell arrays discussed previously. The data presented tells how much “hotter” each pattern is in comparison to the original aggregate array using the average SEF/SERS signal obtained for each. It is seen here that the cloned cell arrays are ~ 2 -3 times hotter than the original aggregate array (as reported above), and that the patterns made of smaller particles with middle to high density/aggregation show a greater hotness on average when compared to the original aggregate array. However, this could be due to the fact that there is greater substrate surface area for the aggregates containing more particles.

Effects of Metal Thickness. Though the concept was proven using previously determined deposition conditions for substrates fabricated via EBL and PVD, these substrates are random in morphology and thought to perform differently than the ordered, uniform morphology substrates in our previous work. We completed another study using the varying density patterns to determine optimum noble metal thickness. This experiment involved depositing Ag onto three separate rows of patterns at three different thicknesses at one deposition rate (20 nm, 40 nm, and 70 nm, all deposited at 5.5 Å/s). Results of this study revealed that greater metal thickness yields greater SERS/SEF signal. This may be due to changes in certain features of the substrates that occur with the deposition of greater amounts of metal. Under SEM it can be seen that as metal thickness is increased, the nanoparticles making up the patterns lose their sharp features and take on more rounded edges and surfaces. In addition, gaps between nanoparticles close-in forcing nanoparticles closer together. These observations are shown in Figure 4-7. In addition, with greater metal thickness, analyte accessible surface area is increased – at

Table 4-2. How much “hotter” each density pattern is in comparison to the original aggregate array.

Pattern	Fluorescence	SERS
less dense, same shapes as original	0.74	0.83
Cloned Cell Array 1	1.97	1.97
Cloned Cell Array 2	1.87	2.60
small shapes, least dense	0.96	0.81
small shapes, medium density	1.23	1.04
small shapes, high density	1.57	1.46

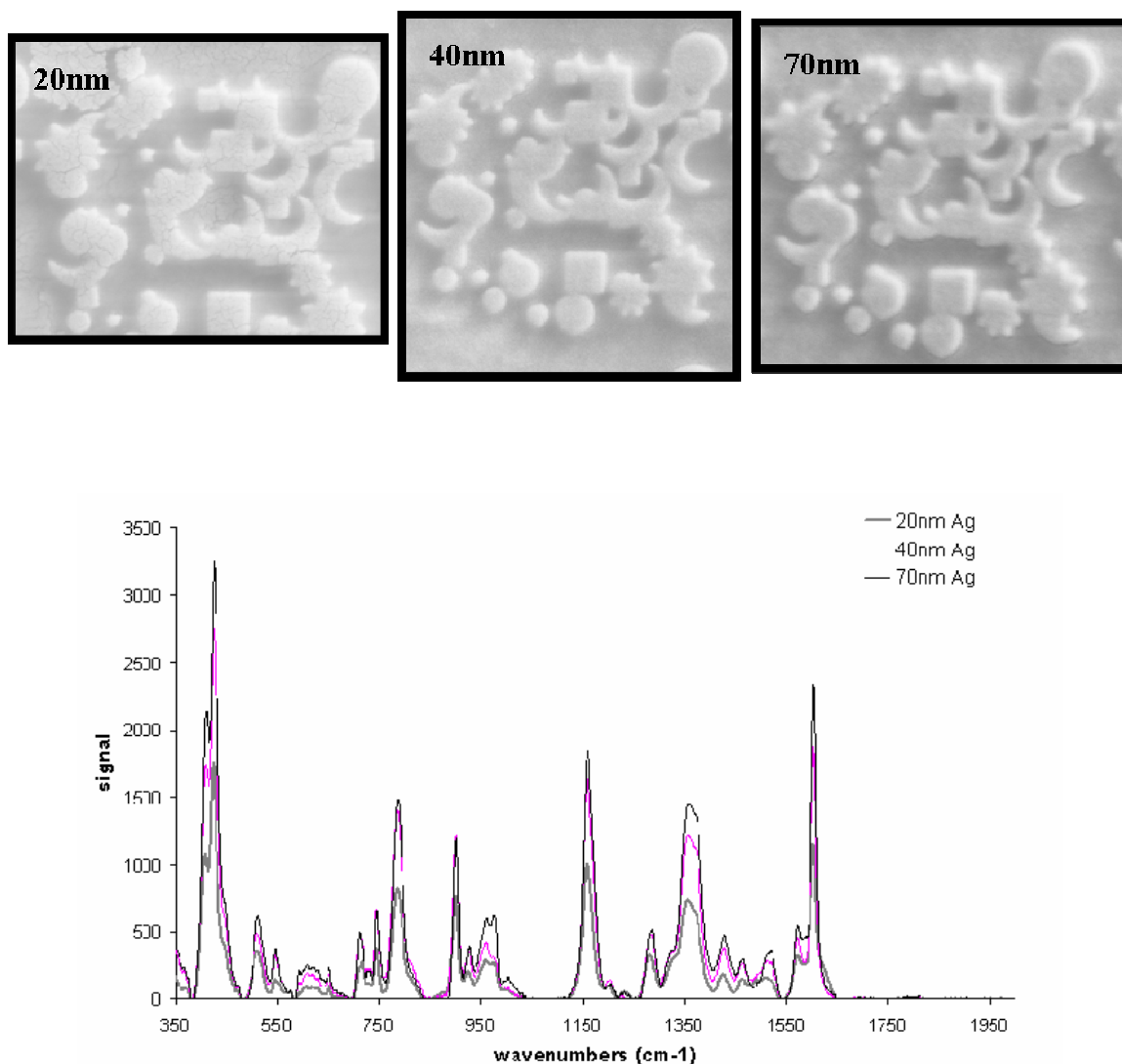


Figure 4-7. (top) SEM images of one 5 x 5 μm cell for comparison of varying thicknesses of Ag. Note the loss of sharp features and the closing in of space around particles as thickness is increased. (bottom) Spectra of 1x10⁻⁷ M crystal violet on the original aggregate array pattern coated with three thicknesses of Ag.

least as far as disc thickness on top of the pillars is concerned. This may account for some of the increase in the SERS/SEF signal as well.

Dielectric Study. So far, in our work, we have examined the effects of changing solvent on the signal of benzenethiol adsorbed on the surface of our cloned cell array 2 pattern. Benzenethiol was chosen as the analyte in this experiment because it forms a monolayer on the silver surface. Substrates were treated with the analyte for approximately ten minutes to ensure monolayer formation. Four different alcohols were used ranging in alkane chain length, ethanol, butanol, pentanol and octanol. Our results showed that as the alkane chain increased in length, analyte signal dropped. This means one of two things, analyte is being washed off the substrate surface each time the solvent is changed, or the dielectric properties of ethanol combined with the properties of the substrate work together to enhance the SERS signal. Figure 4-8 is a bar chart showing the 1575 cm^{-1} band area of benzenethiol collected using with the different dielectric environments of the varying length alkane chain alcohols.

Conclusions and Future Work. So far, in these studies, we have successfully demonstrated that it is possible to reproducibly create uniform analyte environments via cloning “hot spots” found within randomly generated arrays of nanoparticles fabricated via EBL and PVD. Future studies will involve the use of advanced methods to characterize the random substrate surfaces to pin point “hot spots”. We will also determine better ways to spectrally map the substrates using our LabRam system. Polarization and dielectric experiments will be repeated once mapping procedures are optimized, to see if indeed there are enhancing areas that appear at different incident laser

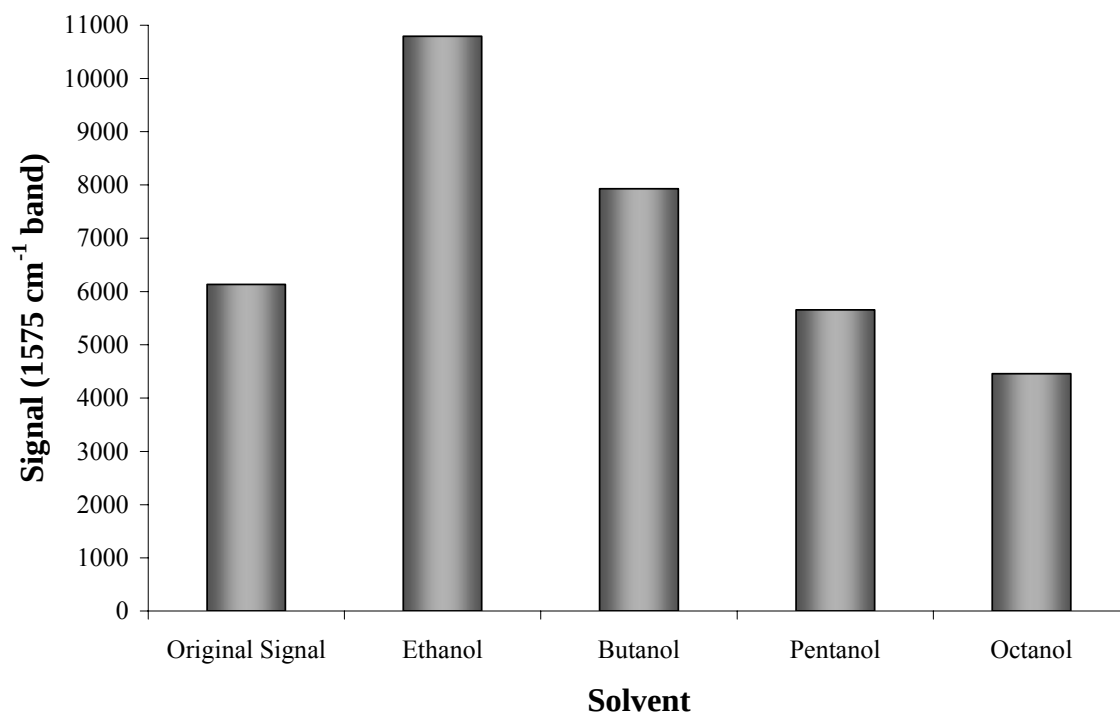


Figure 4-8. Results of first dielectric study using cloned cell array 2 pattern.

polarizations and which solvent environment is the best for exciting surface plasmons (and yielding better SERS/SEF signal) within these random substrates.

Once areas of greater enhancement are found spectrally and viewed under SEM, they can then be found in the original AutoCAD file, cloned, arrayed, and fabricated via EBL and PVD. The ultimate goal of this work is the creation of SERS substrates that offer enhancement capabilities comparable to (if not better than) traditional random substrates with the added advantages of being able to reproduce the “hot” substrate while at the same time providing a homogeneous analyte environment. Recently, we have used EBL in conjunction with reactive ion etching to create our random aggregate array patterns in silicon that we are hoping will be more robust and not subject to as much substrate degradation. In the future, once fabricated, these substrates, whether polymer or silicon-based, can be used as a stamp in the nanotransfer printing process where they can be used multiple times, thus addressing the scale-up and cost issues associated with EBL [160].

Chapter 5 Concluding Remarks

Surface enhanced Raman spectroscopy is a phenomenon based on the enhancement of analyte signal at or near a metallic nanostructured surface. The optimization of the performance characteristics of substrates used for SERS is vital to the improvement of the technique analytically. Over the years, there has been an increase in the number of SERS applications for chemical analysis because of the ability of the technique to provide structural information about compounds. It has even been used to detect molecules at ultra-trace and single-molecule levels.

However, there are several drawbacks to typical SERS substrates, which are debilitating to SERS as an analytical method. Substrates often suffer from the following: inhomogeneity of enhancement sites across their surfaces leading to irreproducible analyte signal, quick oxidation when exposed to air or water preventing reliable use of the substrate, and degradation in sensitivity when photolytic or thermal effects occur making it difficult to detect molecules at low concentrations. These issues produce significant qualitative and quantitative problems that prevent SERS from being as widely used as it could be. As a result, it is important to improve the figures of merit of the SERS method by developing substrates that rectify these concerns. In the preceding chapters, the lithographic fabrication of both uniform and random morphology substrates was presented as an approach to address and overcome some of the issues associated with conventional SERS substrates, thus helping to improve the technique itself. This chapter

discusses some conclusions and includes comments regarding future work with our substrates.

The rational design and control of variables such as shape and size, and distribution, packing density, and spacing of nanostructures composing a SERS substrate can be used to create ordered substrate surfaces that can yield reproducible enhancement of signal and greater substrate sensitivity. Our work used *direct* electron beam lithography to fabricate densely packed polymeric arrays of pillars that were subsequently coated with metal via physical vapor deposition to create isolated metal nanoparticles as SERS substrates. The direct approach was used because it was simple and did not include more complicated and/or instrumentally involved nanofabrication procedures such as reactive ion etching and lift off techniques. Using this approach, we surveyed different conceptual substrate designs (discussed in chapters 2 and 3), including ordered arrays of: nanoparticles shaped as squares, hexagons, and ellipses having different densities; nanoparticle ellipses having different point symmetries; and nanoparticle ellipses having different aspect ratios. In general, when examining the signal from 1,10-phenanthroline on the square pillar arrays discussed in chapter 2, a trend was seen that showed an increase in SERS signal as particle density increased. Another study showed the effects of changing nanoparticle shape and determined that ellipses were a better shape for SERS enhancement when compared to squares and hexagons. Results of the experiments involving nanoparticle ellipses arranged in different symmetry orientations demonstrated that ellipses of a C_2 geometry yielded the best SERS signal out of the arrangements fabricated and examined.

The above-mentioned findings led to the studies that were completed in chapter 3 where arrays of ellipses were created having different aspect ratios in the range of 6:1 (300:50 nm) to 6:6 (300:300 nm). With these arrays, deposition conditions were optimized and several different studies were completed. The effects of surface roughness and surface area of the substrates in relation to SERS enhancement were examined. In either case, roughness or surface area, it was found that when they are greater, SERS signal is increased for these elliptical arrays. During this work, the homogeneity of the substrate surface of different patterns of the same array as well as in comparison to a random morphology substrate was addressed. It was found that reproducibility between patterns of the same array varied between 12% and 28%, and that in comparison to random morphology Ag-PDMS substrates, the EBL fabricated substrate is considerably more homogeneous. With these ordered arrays of ellipses, raw and normalized SERS data was correlated with data from simple electrostatic calculations as well as with the broad background continuum underlying each spectrum collected.

In chapter 4, our EBL fabrication approach was used to fabricate random aggregate arrays. Even though they were random, because we used CAD and EBL, we still knew the precise morphology of our substrates. Borrowing from biological and combinatorial chemistry and combining it with the fact that metal colloid is traditionally a widely used successful SERS substrate, we created random aggregate substrates; spectrally mapped them, located “hot spots” within the aggregates, and cloned them to create larger, homogeneous, enhancing substrates. Studies were done to look at the effects of laser polarization, particle density, particle size, metal thickness, and dielectric

environment on SERS signal. So far, we have been able to fabricate a substrate that is ~3.0 times hotter than its original aggregate array. While not wonderful, we feel these results are promising and have demonstrated the concept.

These lithographically fabricated substrates, whether ordered or random, have several advantages over conventional, non-precision made substrates. With substrates fabricated via EBL-PVD, a researcher knows the precise morphology of the substrate. With this knowledge, substrate morphology can be changed with control of particle shape, size, spacing, etc., or “hot spots” can be found and cloned, to yield better SERS enhancing substrates. Both random and ordered substrates can be made quickly using computer aided design software, and both have been shown to offer homogeneity across the substrate surface. This gives reproducible analyte environments, which help improve substrate sensitivity across extended areas.

Although progress has been made in improving our lithographically fabricated SERS substrates, there is still much to be done. The work presented herein shows the initial steps taken in the development of SERS substrates of both ordered and random Ag nanoparticle arrays having precisely known morphologies. The next steps involve finding a “hot spot” on a random aggregate pattern that once cloned will yield giant SERS enhancement. It would be ideal if we could create this cloned pattern in such a way as to be able to combine it with nanotransfer printing to use it as a stamp as well as figure out a way to reliably map our substrate surface over a large area. This would lower the cost of fabrication associated with EBL while creating a reusable, extended area substrate that

yields excellent SERS enhancement. Our access to CNMS provides us with additional fabrication possibilities to reach this goal.

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Vita

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