A Dual Nanostructured Approach to SERS Amenable to Large-Scale Production

Kory Brian Castro

Old Dominion University, kcast006@odu.edu

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A DUAL-NANOSTRUCTURED APPROACH TO SERS AMENABLE TO LARGE-SCALE PRODUCTION

by

Kory Brian Castro
B.S. May 2015, Old Dominion University

A Thesis Submitted to the Faculty of Old Dominion University in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

CHEMISTRY

OLD DOMINION UNIVERSITY
May 2019

Approved by:

John Cooper (Director)
Balasubramanian Ramjee (Member)
Guijun Wang (Member)
Richard Gregory (Member)
A SERS device was made using a dual-nanostructured surface comprised of silver nanoparticle and silver nanowires. The ability of each nanostructure to produce a uniform surface was characterized and the surface-enhanced Raman scattering (SERS) response of the resulting surfaces were examined using the reporter molecule 4-aminothiophenol (ATP) and a 638 nm excitation laser.

A synthetic method was developed to produce silver nanowires with lengths of ~20 μm and diameters of ~100 nm with a narrow size distribution. The method utilized a simple, one-pot synthesis that is amenable to large-scale production. A selective precipitation method was used to isolate the ~200 aspect ratio silver nanowires in a high purity. The wires were Mayer rod coated onto glass slides to produce uniform surfaces with ease. The SERS response was found to be highly variable depending on the sampled location. An enhancement factor of 2.2x10^5 was tentatively assigned using the 1600 cm^{-1} peak of ATP.

The silver complex \( \mu \text{oxolato-bis(ethylenediaminesilver(I))} \) was synthesized as reported in the literature. The aqueous solution of the complex was thermally decomposed to produce SERS-active silver surfaces on glass slides. The silver complex was unable to generate a uniform surface coating without the use of additives. By dropcasting the complex at the decomposition temperature, surfaces were
generated exhibiting ~75 nm, spherical nanoparticles. The surface was found to be SERS-active, however the poor processing characteristics of the complex prevent its applicability to large-scale device production.

Silver nanowire surfaces were used as substrates to Mayer rod coat the silver complex uniformly without the use of additives. This provided the proof-of-concept that the dual-nanostructured surface is amenable to large scale production through the use of roll-to-roll printing. A dual-nanostructured surface was produced by dropcasting the complex on silver nanowire surfaces to mimic the quality of surface achievable by an industrial production line. The dual-nanostructured surface produced a spatially consistent SERS response and an enhancement factor of $4.3 \times 10^5$ was tentatively assigned using the 1600 cm$^{-1}$ peak of ATP.
This thesis is dedicated to my family who supports me in everything I do.
ACKNOWLEDGMENTS

I would like to thank Alicia Herr and Tammy Subotich for their support throughout my graduate program. They continuously believed in me and offered countless opportunities for my growth.

I cannot express enough gratitude to Dr. John Cooper. His flexibility and tolerance of my constant juggle between school, work, and home life made my education possible. His guidance as an advisor was imperative to my success.
**NOMENCLATURE**

<table>
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<tr>
<th>Abbreviation</th>
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<tr>
<td>SERS</td>
<td>Surface-enhanced Raman scattering</td>
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<tr>
<td>ATP</td>
<td>4-Aminothiophenol</td>
</tr>
<tr>
<td>SPR</td>
<td>Surface plasmon resonance</td>
</tr>
<tr>
<td>NIR</td>
<td>Near-infrared radiation</td>
</tr>
<tr>
<td>EM</td>
<td>Electromagnetic field enhancement</td>
</tr>
<tr>
<td>CE</td>
<td>Chemical enhancement</td>
</tr>
<tr>
<td>NP</td>
<td>Nanoparticles</td>
</tr>
<tr>
<td>TEC</td>
<td>Thermoelectric cooler</td>
</tr>
<tr>
<td>CCD</td>
<td>Charge-coupled device</td>
</tr>
<tr>
<td>AgNP</td>
<td>Spherical silver nanoparticles</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet radiation</td>
</tr>
<tr>
<td>Vis</td>
<td>Visible radiation</td>
</tr>
<tr>
<td>AgNW</td>
<td>Silver nanowire</td>
</tr>
<tr>
<td>MTP</td>
<td>Multiply twinned particle</td>
</tr>
<tr>
<td>EG</td>
<td>Ethylene glycol</td>
</tr>
<tr>
<td>PVP</td>
<td>Polyvinylpyrrolidone</td>
</tr>
<tr>
<td>$C_{\text{Max}}$</td>
<td>The critical supersaturation concentration that results in homogeneous nucleation</td>
</tr>
<tr>
<td>$C_s$</td>
<td>The saturation concentration that results in heterogeneous nucleation</td>
</tr>
<tr>
<td>ESI</td>
<td>Electrospray ionization</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectroscopy</td>
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<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
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<tr>
<td>Abbreviation</td>
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<td>--------------</td>
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<tr>
<td>MPAA</td>
<td>4-Mercaptophenylacetic acid</td>
</tr>
<tr>
<td>ATR</td>
<td>Attenuated total reflectance</td>
</tr>
<tr>
<td>FT</td>
<td>Fourier transform</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>TGA</td>
<td>Thermogravimetric analysis</td>
</tr>
<tr>
<td>DLS</td>
<td>Dynamic light scattering</td>
</tr>
<tr>
<td>FE</td>
<td>Field emission</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>AFM</td>
<td>Atomic force microscopy</td>
</tr>
<tr>
<td>NPS</td>
<td>Nanoparticle surface</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolutions per minute</td>
</tr>
<tr>
<td>R2R</td>
<td>Roll-to-roll printing</td>
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<tr>
<td>NWS</td>
<td>Nanowire surface</td>
</tr>
<tr>
<td>DNS</td>
<td>Dual-nanostructured surface</td>
</tr>
<tr>
<td>EF</td>
<td>Enhancement factor</td>
</tr>
<tr>
<td>STD</td>
<td>Standard deviation</td>
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<tr>
<td>SNR</td>
<td>Signal-to-noise ratio</td>
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<tr>
<td>DMAB</td>
<td>4,4’-Dimercaptoazobenzene</td>
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<tr>
<td>RSD</td>
<td>Relative standard deviation</td>
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CHAPTER I

INTRODUCTION

Raman spectroscopy is a type of vibrational spectroscopy that provides information about the structure and environment of a molecule. As a result, the Raman spectrum of a molecule is a unique fingerprint that can be used to identify the molecule. Typically, the industrial applicability of Raman is limited to qualitative analysis due to the low sensitivity of the technique (part per thousand detection limit). The spectroscopic sensitivity is greatly enhanced by using surface-enhanced Raman scattering (SERS). The significantly lower detection limits (part per million to part per billion detection limit) allow the technique more opportunity to be applied as a sensitive quantitative method. The combination of quantitative and qualitative features makes SERS methodology highly desirable for analytical applications.

While SERS experimentation has gained a large amount of attention in the research field, the technique has failed to become widespread in industrial applications. This is a result of the complex procedures and protocols required to obtain reproducible SERS experiments as well as the high costs associated with SERS devices. The development of a SERS-active device that is fast, low-cost, and amenable to large-scale production is required to increase the applicability of SERS analysis for industrial process control. Progress towards developing a methodology to fabricate a dual-nanostructured, SERS-active device with these qualities is presented. The fundamental theory of Raman scattering, the theory of the SERS phenomenon, Raman instrumentation, relevant manufacture techniques, and the chosen nanostructures are

This thesis is formatted based on the Journal of the American Chemical Society.
presented in this chapter to establish the benefits of the dual-nanostructured SERS device.

RAMAN SCATTERING

When light strikes a molecule, the interaction of the photons with the electrons of the molecule scatter the incident light in all directions. An elastic collision produces scattered photons with the same frequency of the incident light, called Rayleigh scattering. Most incident photons are Rayleigh scattered, however approximately one in every ten million photons experience an inelastic collision, called Raman scattering. The Raman scattered photons experience a shift in frequency, or energy, equal to a vibrational frequency of the molecule. The shift in frequency is referred to as an anti-Stokes shift or Stokes shift if the emitted photon is higher or lower in frequency respectively.

Vibrational modes are Raman active only if the vibration results in a change in the polarizability of one or more molecular bonds. The polarizability, $\alpha$, of a bond refers to the susceptibility of the bonding electrons to the formation of a dipole, $P$, by the interaction with the oscillating electric field, $E_0$, of incident light as shown in Equation 1.

$$P = \alpha E_0 \quad \text{Eq. 1}$$

The induced dipole represents an energetically excited state, referred to as the virtual state. Figure 1 depicts the relaxation of the virtual state to produce Rayleigh scattered, Stokes shifted, and anti-Stokes shifted photons. Stokes shifted photons occur more often than anti-Stokes shifted photons because more molecules are in the ground vibrational states at room temperature.
Figure 1. The types of scattering. The bottom two lines represent a vibrational ground state and a vibrational excited state of a molecule. The top lines represent the virtual state caused by an induced dipole.
SURFACE-ENHANCED RAMAN SCATTERING

Surface-Enhanced Raman Scattering (SERS) occurs when a molecule exhibits an increase in the number of Raman scattered photons due to the interaction with a SERS-active surface. The Raman cross section of an analyte can increase by up to 11 orders of magnitude utilizing SERS. This huge amplification of signal allows SERS techniques to reach up to single molecule detection capabilities.\(^1\) In practice, the enhancement factor heavily depends on the nature of the interaction between the analyte and the SERS-active surface. For this reason, the average enhancement factor is more typically five to six orders of magnitude for a large sample area.\(^2\)

Noble metal nanostructures are the typical candidates for the fabrication of SERS-active surfaces. These nanostructures exhibit localized surface plasmon resonances (SPRs) that are necessary to generate the SERS enhancement. The enhancement factor is the highest when the SPR is in resonance with the incident wavelength of the laser, which are typically in the visible to NIR range for Raman instruments. The location of the SPR of noble metal nanostructures can be tuned by manipulating the shape, size, elemental composition, and surface chemistry of the material.\(^3\)\(^-\)\(^4\)

The total SERS enhancement originates from a blend of two physical phenomena.\(^1\) The first is an electromagnetic (EM) field enhancement due to the interaction of the photon with the nanostructure. This enhancement is independent of the analyte and is strongest at the surface. The EM field effect only extends ~5-10 nm from the surface and decays in intensity with the distance.\(^5\) The second effect is the chemical enhancement (CE) that occurs when an analyte chemisorbs on the active
surface. The charge transfer state generated intrinsically alters the polarizability of the analyte due to the direct interaction with the metal orbitals with the molecular orbitals of the analyte.\textsuperscript{1} The CE factor heavily depends on the chemistry of the analyte and metal surface; and is much more specific to the structure of the analyte.

The EM enhancement can be understood using the example of a spherical silver nanoparticle of a radius, $r$, in a vacuum. The polarizability of the particle is defined by Equation 2, assuming that the polarizability per volume is uniform, which is true as long as $4r < \lambda$.\textsuperscript{1}

$$\alpha = 4\pi \left(\frac{\varepsilon - 1}{\varepsilon + 2}\right) r^3 \quad \text{Eq. 2}$$

Where the term $\varepsilon$ is a wavelength dependent function representing the real and imaginary term of the dielectric constant of the metal, and $\lambda$ is the wavelength of the incident photon. Using Equation 1 and 2, the electromagnetic field of an incident photon, $E_0$, can be shown to result in a polarization dipole, $P$, by Equation 3.

$$P = 4\pi \left(\frac{\varepsilon - 1}{\varepsilon + 2}\right) r^3 E_0 \quad \text{Eq. 3}$$

The generated dipole creates a linear restorative force resulting in Rayleigh scattered light in all directions. A molecule sufficiently close to the surface experiences a higher intensity of incident photons due to surface reflectivity. In addition, the polarization dipole of the particle can directly couple to the polarization dipole of the molecule, simultaneously increasing the rate of Raman scattering.\textsuperscript{6} Equation 3 implies that larger particles will always produce a larger SERS enhancement since the magnitude of $P$ is proportional to $r^3$, however as the size of the particle increases the amount of optical retardation also increases complicating the simplified relationship. In addition, as the particle becomes larger, the surface plasmon resonance concomitantly
shifts to lower energy until the particle becomes so large that it approaches the bulk limit with delocalization of the plasmon.\(^7\)

The largest EM enhancement occurs through the generation of a SERS “hotspot” where a molecule is directly between two nanostructures that are separated by nm sized gaps.\(^1\), \(^8\) An example of an ideal case occurs when the incident light is perpendicular to a plane containing an analyte molecule between two spherical nanoparticles (NPs). The dipole in each particle affects the polarizability of the near edge of the neighboring particle, deviating the uniformity of the polarization per volume of each particle. The result is a large increase in the polarizability that is localized in the junction of the NPs.\(^8\) Theoretical enhancement factors of up to 11 orders of magnitude have been calculated and a large amount of work has been done to find the mathematical solutions to understand the optical phenomenon at the various types of nanostructure junctions.\(^4\), \(^7\)-\(^8\) These solutions are beyond the scope of this work and have been extensively reviewed in the literature.

The CE factor is much less straightforward and heavily depends on the structure of the analyte and composition of the SERS surface. Structural changes in the chemisorbed analyte can cause spectral shifts when compared to the Raman spectrum of the free analyte.\(^9\)-\(^10\) In addition, vibrational modes that have polarizability tensor components perpendicular to the surface are selectively enhanced.\(^5\) The chemisorbed state also opens the possibility of photochemical reactions due to various phenomena. For example, Förster resonance energy transfer between the molecule and the metal can result in redox reactions.\(^1\), \(^11\) In addition, the relaxation of the excited SPR of the
nanoparticle and increased photon flux result in localized heating\textsuperscript{12} which can also promote photocatalyzed reactions.\textsuperscript{1}

While the CE introduces much more variables, the phenomenon can be utilized to introduce many advantages in SERS analysis. By definition, the chemisorption process chemically bonds a molecule to the SERS surface. Since the EM enhancement is strongest closer to the surface, this can be utilized to introduce selectivity into an analytical method by enhancing the Raman signal of a desired analyte and suppressing the enhancement of potentially interfering background signals.\textsuperscript{13} The dependence of the SERS enhancement on the molecular orientation can be utilized and has proven to be effective in the detection of trinitrotoluene.\textsuperscript{14} Surface chemistries that have significantly slower surface dissociation than surface association can be utilized to further improve the solution detection limit of SERS techniques by taking advantage of the Langmuir adsorption isotherm.\textsuperscript{13, 15}

INSTRUMENTATION

While traditional SERS experiments typically utilize a confocal Raman microscope, their high cost and lack of portability lower their appeal for industrial applications. Their popularity in SERS applications is based on the confocal microscope's ability to acquire a precise plane of focus in the Z dimension with full control of the XY position of the focal point on the sample. Using a SERS-active surface allows the same feature can be incorporated into an instrumental setup that utilizes much cheaper components.
Figure 2. The Wasatch Photonics 638 nm Raman spectrometer. The pen was included to demonstrate the compact nature of the instrument.
A Wasatch Photonics 638 nm Raman spectrometer utilizing a backscattering geometry, a static grating, and a TEC cooled CCD detector is shown in Figure 2. This type of instrument is vastly lower in price than a typical Raman microscope and is extremely compact. The use of a static holographic transmission grating to produce a linear dispersion onto the CCD detector eliminates moving parts in addition to lowering the size requirement of the optical bench. This broadens the range of environments the instrument is able to handle by reducing its physical size, susceptibility to interference from vibrations, and improving portability. The design of this compact spectrometer was first developed at Old Dominion University Department of Chemistry and Biochemistry, and was first described in US Patent 6,373,567 by Wise, et al.16

The benefits associated with the instrument come at the cost of precision in the Z focal plane. In combination with a SERS-active surface, a similar effect can be accomplished through spatially controlling the enhancement of signal instead of the rejection of noise. A SERS surface also allows for the opportunity of an extremely quick sampling method for solution sampling. A small volume of solution can be deposited onto the surface and allowed to dry. This effectively concentrates the analyte relative to the spot size by compressing the 3D concentration of the analyte into a 2D plane. In addition, the drying process forces the analyte into the proximity of the SERS surface for analysis and potentially enhances the chemisorption process.

The benefits of the instrumental setup and sampling method depend heavily on a high-quality SERS-active surface. For quantitative application, the surface must produce a reproducible, concentration dependent signal while retaining high sensitivity. To achieve this, the device must have a large number of SERS hotspots within the focal
spot of the laser in order to average out the high spatial deviation inherent to SERS enhancements.² The distribution of SERS hotspots must also remain uniform on a spatial scale relative to the sampling area to prevent the influence of small variations in the sample position relative to the spectrometer. These parameters represent the experimental considerations for the performance of a SERS-active device.

MANUFACTURE TECHNIQUES

In addition to meeting the experimental requirements, the SERS device must be economically feasible to produce on a large scale. A large amount of laboratory techniques used in nanoparticle synthesis and device fabrication are not feasible for the use in industrial production. To ensure the plausibility of large-scale production, the existing industrial techniques of a whirlpool tank and Mayer rod coating were considered.

A whirlpool tank takes advantage of fluid dynamics to separate liquid from solid particulates.¹⁷ The separation method uses a tank with a cylindrical body and a cone bottom. A drawing of a whirlpool tank is shown in Figure 3. The solid-liquid mixture is pumped into the tank via an entrance pipe that is tangential to the circular cross section to produce a whirlpool. The solid particulate accumulates at the bottom of the cone significantly quicker than it would by natural settling.¹⁷ An upper exit pipe is located on the cylinder directly above the top of the cone and a lower exit pipe is located on the bottom at the tip of the cone. The liquid or the solid can be isolated by opening the upper or lower exit pipe respectively.
Figure 3. A drawing of a whirlpool tank. The direction of fluid flow is depicted by the blue arrow. Pipes labeled with black arrows are controlled by valves.

Figure 4. A drawing of the contact between a surface and the wire windings of a Mayer rod.
Centrifugation is the most common method of isolating nanomaterials from solution in a laboratory setting. However, large scale centrifugation is time and energy intensive. The incorporation of particle precipitation coupled with a whirlpool tank significantly reduces or eliminates the need for centrifugation isolation.

Mayer rod coating is a method of depositing a liquid layer onto a substrate in an even, controllable thickness. The Mayer rod itself is typically a stainless steel rod with a stainless steel wire wound tightly around its circumference. A substrate is wet by an excess of the liquid to be coated and then the Mayer rod is quickly drawn across the surface. The majority of the liquid is pushed off of the substrate while the amount of residual liquid is controlled by the open space between the rod and the surface. As shown in Figure 4, the diameter of the wire winding dictates the size of the open space and ultimately thickness of the coat. The process is cheaper than printing or spray coating and is highly amenable to automation. However, the quality of the coat is heavily dependent on the wettability of the coating material with the substrate.

SPHERICAL SILVER NANOPARTICLES

Spherical silver nanoparticles (AgNPs) are an attractive nanostructure to fabricate a SERS-active surface. They exhibit a diameter dependent SPR in the UV-Vis spectrum and are easily synthesized in a large number of ways. However, a large majority of literature techniques utilize colloidal solutions of presynthesized AgNPs and generate SERS hotspots by using various aggregation methods after the introduction of an analyte. This methodology has a large number of drawbacks that can be addressed by using a presynthesized surface of AgNPs.
The colloidal aggregation method heavily depends on the colloidal stability of the presynthesized AgNPs. To achieve this, the silver surface is passivated with either ionic stabilizers like citrate anions, polymeric capping agents, or covalently bound stabilizing ligands. This inherently lowers the ability of an analyte to come close enough to the surface to experience the EM enhancement and lowers the free energy of the surface for CE through chemisorption. The SERS signal generated from the capping agent itself can also complicate the interpretation of a spectrum due to spectral overlap. Another issue is that the aggregation step introduces a large amount of variation between different measurements. The number of particles, the interparticle distances, and analyte distribution in each aggregate is highly variable and is difficult to control or reproduce. In addition, the orientation of the excitation laser and the detector relative to each generated hotspot heavily influences the intensity of the collected signal.

Many of the aggregation-based issues can be partially alleviated by prefabricating a solid surface of AgNPs prior to the introduction of an analyte. Various methods have been established to generate surfaces with controllable statistical distributions of SERS-active hotspots, however most of them utilize presynthesized AgNPs. The use of presynthesized AgNPs to fabricate a SERS surface introduces unnecessarily complicated processing steps. Colloidal stability is important to produce an even deposition, and the capping agents required for this stability produce a background signal. Additional background signals are produced by the additives that physical deposition techniques require to avoid the coffee stain effect (uneven distribution resulting in concentrated rings on the surface). Methods have been proposed to remove these interfering background agents, however the majority of
removal steps utilize harsh chemical treatments\textsuperscript{21}, high temperature exposure\textsuperscript{23}, or photonic treatments\textsuperscript{24} that are undesirable from the perspective of industrial production.

Silver ion molecular complexes offer an attractive alternative to the fabrication of a AgNP surface\textsuperscript{25}. The identity of the ligands, concentration, and decomposition method influence the morphology of the resulting AgNP surface\textsuperscript{25-26}. The methods take advantage of a modified version of the chemistry infamously utilized by Tollens’ reagent. Traditionally, a solid silver surface is rapidly produced when a diamminesilver(I) complex is exposed to an aldehyde functional group. By utilizing a carboxylic acid as a ligand, gaseous CO\textsubscript{2} is produced upon decomposition, and the decomposition reaction is slower. Since the reaction produces all gaseous organic species, a clean silver surface is produced absent of background interference.

The silver complex $\mu$-oxolato-bis(ethylenediaminesilver(I)), hereby referred to as Ag complex, is shown in Figure 5 and was developed by Williams \textit{et al.} for use in printable electronics\textsuperscript{27}. While the SERS response of the complex was not reported, the thermal decomposition the Ag complex was shown to produce highly uniform AgNP surfaces with high mass loadings. In addition, the complex was proven to have an extended shelf life as a solid or aqueous solution\textsuperscript{27}. Existing literature utilizing the decomposition of silver ion complexes for SERS experimentation failed to produce the nanoparticle morphology (and therefore SERS response) under a high mass loading of silver\textsuperscript{25}. The consistent formation of a nanoparticle surface with shelf stability make the Ag complex an ideal choice for the incorporation into a SERS-active device.
Figure 5. The structure of the silver complex μ-oxolato-bis(ethylenediaminesilver(I)).
SILVER NANOWIRES

Silver nanowires (AgNWs) represent another attractive nanostructure to fabricate a SERS-active surface. Using AgNW substrates, detection limits as low as $1 \times 10^{-11}$ M have been reported in the literature. Each individual wire is a multiply twinned particle (MTP) of face-centered cubic silver crystals with a five-fold rotation symmetry around a central axis. The structure is considered a one-dimensional material because the pentagonal cross section of the wire is on the nanometer scale while the length of the wire exists on the micrometer scale. The diameter, length, and aspect ratio can be fine-tuned experimentally. AgNWs with aspect ratios above 1000 have been successfully synthesized with diameters less than 20 nm and lengths exceeding 20 μm. The large aspect ratio results in unique optical, electrical, and thermal properties. In addition to SERS applications, AgNWs have received a large amount of attention for transparent conductive films, printable electronics, and catalysis applications.

Since the longitudinal and the transverse axis of the wires are remarkably different in dimensions, they each exhibit different SPRs. The longitudinal plasmon is red-shifted as the aspect ratio of the wire is increased. Typically, this plasmon mode is far into the NIR while the transverse plasmon occurs in the UV-Vis region. For extremely thin wires, it starts in the UV and is continually red-shifted as the diameter of the wire grows. The resonance is relatively broad and typically exhibits a characteristic shoulder due to the quadrupole nature of the resonance. The tunable, broad SPR in the UV-Vis spectrum makes AgNWs attractive for the fabrication of a SERS-active device.

The “polyol” method of synthesis is the most popular synthetic route to AgNWs,
and a large amount of literature exists on the various synthetic factors effecting the final dimensions of the wire. Conversely, the mechanisms of the reaction and wire growth are an arena of debate and continued research.\textsuperscript{36} In short, the polyol method utilizes hot ethylene glycol (EG) to reduce Ag\textsuperscript{+} in the presence of polyvinylpyrrolidone (PVP). EG is also the solvent and a small amount of halide ions, either Cl\textsuperscript{-} or Br\textsuperscript{-}, is included to promote wire formation. The method is popular due to the use of environmentally friendly reactants and relatively mild reaction conditions compared to other thermal solvolysis methods. Despite the widespread use, the precise reaction mechanism is still an area of active research. The LaMer nucleation and growth theory can be used as a basis to understand the mechanism.\textsuperscript{37-38}

The general LaMer nucleation and growth mechanism of nanoparticle formation follows the trend shown in Figure 6. During a nanoparticle synthesis, monomer units are produced until a critical supersaturation concentration, $C_{\text{max}}$, is reached and an initial nucleation event occurs. This form of nucleation, referred to as homogenous nucleation, produces seed particles. The seed particles produce sites for heterogeneous nucleation, where the available surface lowers the energy of the nucleation event and the monomers further nucleate onto the surface of the seed particle. This results in a growth phase following the original nucleation event because the new saturation concentration, $C_s$, is lower.\textsuperscript{38}

In the case of silver, a portion of the seed particles produced by the initial nucleation event can coalesce into a MTP with five-fold symmetry\textsuperscript{37} that is necessary for AgNW growth. The portion of seed particles that do not coalesce compete for silver atom deposition and ultimately grow into spherical nanoparticles. Several key factors of
Figure 6. The LaMer theory of nucleation and growth. The concentration of an arbitrary monomer vs time during an arbitrary nanoparticle synthesis is shown above.

Figure 7. The structure of polyvinylpyrrolidone.
the polyol synthesis affect the balance of these competing processes.

The first key factor in these competing processes (nanowire vs. nanoparticle) is the role of EG. The ability of it to act as a solvent and reducing agent simultaneously simplifies and complicates the reaction. It can solubilize all the reactants at room temperature, however it will only reduce silver at temperatures above 130 °C. This allows the reaction mixture to be easily prepared but ultimately obscures the direct mechanism and therefore rate of the Ag⁺ reduction event. This makes it difficult to control the concentration of the initial seed particles and their MTP counterparts. The possible thermal degradation products of EG, acetaldehyde³⁹ and glycolaldehyde⁴⁰, as well as EG itself have been proposed to play a reductive role within the temperature range of the polyol synthesis.

The next key factor is the role of PVP in the reaction. The structure of PVP is shown in Figure 7. The carbonyl carbon of the lactam moiety is able to coordinate Ag⁺, Ag⁰, and simultaneously acts as a capping agent, dispersion agent, and directing agent for the 1D growth of AgNWs.³⁶ The surface of AgNWs produced by the polyol method have been proven to retain at least a monolayer of PVP.⁴¹ The protruding lactam moieties or freely rotating ends of partially bound polymer chains confer a colloidal stability that parallels the solubility characteristics of the free polymer.²⁹

The polymer is believed to direct 1D growth by preferentially binding silver on the [100] face, therefore promoting the attachment of supersaturated Ag⁰ atoms to the exposed [111] face during the growth phase.³⁶ Typically, high molecular weights of PVP (Mₘ ≥55,000) are used in the polyol synthesis. Increasing the molecular weight, and therefore number of coordinating lactam units, results in AgNWs with much larger
aspect ratios and higher yields.\textsuperscript{42} The polydentate effect of longer polymer chains increases the binding affinity for the silver surface. The thermal reversibility of capping agents during high temperature reactions is well documented\textsuperscript{21}, and the higher binding energy lowers the amount of time the [100] surface is exposed directly to the solution and suppresses the attachment of supersaturated Ag\textsuperscript{0} atoms. The lowered kinetics for [100] face deposition results in smaller diameter wires that increase significantly in length.\textsuperscript{43} The kinetics for the [111] face deposition are not effected nearly as much due to either the low affinity for the lactam coordination, a lower efficacy of the polydentate effect due to the comparable sizes of the length of the polymer and any linear cross section of the face, or a combination of both.\textsuperscript{36}

Literature discussing the chemical influence of PVP on the polyol reaction is scarce, however the polymer may contribute a much larger role than it is currently credited for. It has been proposed that the polymer may coordinate multiple Ag\textsuperscript{+} ions in solution, forming a coordination complex with the ability to orient in a linear fashion to promote linear growth.\textsuperscript{42} While this has never been proven, argentophilic interactions between silver atoms are well documented and could stabilize the linear structure in solution.\textsuperscript{44} A silver complex with cycloserine, a molecule very similar in structure to the pyrrolidone side chains of PVP, exhibited a pseudo-polymeric linear X-ray crystal structure that was proven to self-associate with various chain lengths in solution by ESI-MS.\textsuperscript{45} PVP has also been shown to be able to reduce Ag\textsuperscript{+} without the aid of other reducing agents.\textsuperscript{46} This may be due to residual polymerization initiators, residual monomers, or coupling to the oxidative cross-linking of the polymer.\textsuperscript{47} Thermal studies of solid PVP show that the polymer begins to crosslink at or above the glass transition
point. This occurs at temperatures as low as 120 °C and future studies may find it to play an important role in the polyol reaction.\textsuperscript{47}

The last key factor is the role of the halide ion in the reaction. While AgNWs can be synthesized in the absence of the halide ions, the reaction will only proceed with very stringent control of experimental conditions. Both AgCl and AgBr are insoluble in solution and form PVP capped nanocubes in polyol conditions.\textsuperscript{48} The largest role of the halide is in the formation of these nanocubes that act as a heterogeneous nucleation sites, promoting the formation of the MTPs that grow into wires.\textsuperscript{49} TEM studies have shown that the initial wire nucleation occurs primarily at the corners of the nanocubes\textsuperscript{48}, therefore the initial number of nanocubes ultimately dictates the amount of nucleated particles and the final dimensions of the wires produced. This is supported by the fact that the size of the nanocubes has been shown to be independent of the final dimension of the synthesized wires.\textsuperscript{49}

The effect of halide addition on the final wire dimensions has also been attributed to kinetic factors. The halide ions lower the amount of initially solubilized Ag\textsuperscript{+} and consistently replenish it throughout the reaction through the dissolution of the AgCl nanocubes.\textsuperscript{36} In addition, the liberated halide ion may act as an etching agent against silver. This is believed to selectively promote the growth of AgNWs through Oswald ripening because the PVP coating protects the AgNWs more than the spherical particles.\textsuperscript{36} The ability to control the amount of nucleated multiply twinned particles, lower the initially available Ag\textsuperscript{+}, and replenish availability of Ag\textsuperscript{+} after the original nucleation event allows for much higher yields of AgNWs with smaller distributions.
The large amount of synthetic methods that exist in the literature utilize these key factors but typically require meticulous control over several other experimental factors. Common examples of these include stirring speed, injection order, rate and method of reactant addition, temperature ramps and ranges, and the use of blanket gases.\textsuperscript{43} In addition, temperatures of 170 °C\textsuperscript{28-29, 43} and reaction times of several hours\textsuperscript{28, 31} are common place in these methods. For an industrial scale production, a simple method of synthesis with a fast reaction rate and low temperature is desired to minimize production costs.

Transition metal cations that are redox active have been shown to catalyze the reduction of silver during the polyol synthesis. Various cations, such as Cu\textsuperscript{2+} and Fe\textsuperscript{3+}, produce larger diameter wires with shorter overall lengths under otherwise identical reaction conditions.\textsuperscript{31} The catalytic property makes FeCl\textsubscript{3} an ideal candidate to lower the necessary reaction temperature and time while simultaneously providing the halide ions that promote wire formation.

Regardless of the conditions used, the polyol method produces AgNWs with a distribution of diameters and lengths, a portion of truncated wires, and a portion of spherical AgNPs.\textsuperscript{29} For the production of a SERS-active device, the unwanted side products must be removed and the size distribution of the wires must be minimized. On a laboratory scale, centrifugation is the most common product isolation method to purify the desired wires. On an industrial scale, centrifugation is a costly process and must be minimized. Recently, Wiley \textit{et al}. demonstrated a selective precipitation method\textsuperscript{29} that is readily adaptable to large scale separation techniques, such as the use of a whirlpool tank.
The ability of AgNWs to produce significant SERS signal and comparative ease of their synthesis make them an ideal candidate for a SERS-active device. However, the current synthetic methods utilize reaction conditions that have undesirable characteristics for large scale production. An easy, scalable synthetic method that produces a narrow size distribution of AgNWs with high purity was designed by combining the key factors influencing the polyol method, incorporating a transition metal cation catalyst, and utilizing a selective precipitation purification method.
CHAPTER II

OBJECTIVES

The objective of this research is to establish a framework for the fabrication of a low-cost, SERS active device. A dual-nanostructured SERS surface is proposed utilizing AgNPs and AgNWs. The hypothesis is that a superior SERS device can be realized by fabricating a dual-nanostructured surface utilizing both long-range ordering of silver nanowires and dense non-ordered decoration via silver nanoparticles. The superiority of the device lies in its amenability to large-scale production with improved SERS response characteristics compared to surfaces of silver nanoparticles or silver nanowires alone.
CHAPTER III

EXPERIMENTAL

MATERIALS

Silver nitrate (99.9%), ethylenediamine (99.8%), ethylene glycol (EG) (99.5%), iron chloride hexahydrate (99.9%), 4-mercaptophenylacetic acid (MPAA) (97%), 4-aminothiophenol (ATP) (97%), and PVP with $M_w$ of $\sim$10,000, $\sim$29,000, and $\sim$55,000 were purchased from Sigma Aldrich. Oxalic acid dihydrate (99.9%), acetone (99.9%), and isopropanol (98%) were purchased from Fischer Chemical. Ethanol was purchased from Decon Laboratories, Inc. and was 95% by volume. All chemicals were used as received. All the water used was 18.2 MΩ generated from a Milli-Q purification system. All silver surfaces were made using J. Melvin Freed brand precleaned microscope slides as the substrate. Mayer rod coatings were performed by hand using a Gardco #2.5 or #10 Mayer rod. A Laurell model WS-650 spin-coater was used to perform all spin coatings. An Eppendorf 5430R centrifuge was used for centrifugation and the term oven refers to a modified Hewlett-Packard 5890 GC oven.

SILVER COMPLEX SYNTHESIS

The silver complex was synthesized as reported in literature. First, aqueous solutions of silver nitrate and oxalic acid were separately prepared (0.5 M each). The solutions were combined in a $\text{Ag}^+/\text{oxalate}$ volume ratio of a 5/3 (30 ml/18 ml) and the white precipitate of silver oxalate was collected by filtration, washed with water, and dried at room temperature under vacuum. Next, 1 ml of an aqueous solution of ethylene diamine (20% v/v) was freshly prepared in a 5 ml vial. While stirring, small increments of
silver oxalate were added, allowed to dissolve, and then repeated until a total added mass of 300 mg. The dissolved complex was precipitated with an excess of isopropanol (4 ml) under vigorous stirring and was subsequently filtered. The white precipitate was washed with cold isopropanol (~2-3 ml) and vacuum dried at room temperature for 6 hours. The final product was stored at -20 °C. Care was taken to avoid light exposure during all synthetic steps and storage. The structure of the complex was confirmed by taking an IR spectrum of the solid silver complex using a Bruker Platinum ATR Alpha FTIR spectrometer and by taking the $^{13}$C NMR spectrum of the complex in D$_2$O (~78 mg/ml) using a Bruker Ascend 400 MHz NMR. The thermal behavior of the complex was analyzed using a TA Q5000 TGA.

IR: Ag complex, 1552 (s) $\nu_{as}$ (O-C=O), 1355 (m) $\nu_s$ (C-C), 1294 (s) $\nu_s$ (C-O) $\delta$ (O-C=O), 754 (m) $\delta_{as}$ (O-C=O) $\nu_s$ (Ag-O), 340 (m) $\nu$ (Ag-O).

$^{13}$C NMR (100 MHz, D$_2$O): Ag Complex, $\delta$ 173.5 (s), $\delta$ 44.6 (s).

SILVER NANOWIRE SYNTHESIS

All the glassware for the polyol reaction was cleaned with freshly prepared aqua regia, rinsed thoroughly with water, and oven dried at 150 °C overnight. A stock solution of FeCl$_3$ (11 mM) in EG was prepared separately and used in each reaction. In a typical synthesis, of PVP (~150 mg) was dissolved in EG (20 ml) with vigorous stirring in a round bottom flask. The flask was then protected from light exposure, and silver nitrate (~180 mg) was dissolved directly into the same reaction vessel. The iron chloride stock (100 μl) was added with gentle swirling, and the round bottom was put into the oven at room temperature and equipped with a condenser tube. The oven was set to 100 °C.
using a single step ramp and held there for 45 minutes. The temperature was then increased to 140 °C using a single step ramp and held there for 70 minutes. Afterwards the oven was set back to room temperature using a single step ramp and allowed to cool.

Samples of the reaction mixture were removed by pipette after the initial 100 °C step (t=0) and at various time points throughout the reaction at 140 °C. The samples were diluted in EG and the UV-Vis spectra were taken with a Thermo Scientific Evolution 201 UV-Vis spectrometer. The sample at t=0 was centrifuged at 5000 rpm for 5 minutes, the supernatant was removed, and the resulting red solid was re-suspended in EG. The process was repeated 4 times and the particle size of the final colloid was estimated using a Brookhaven Nanobrook OMNI DLS instrument.

The reaction products were then transferred to a new round bottom flask that had been cleaned identically to the reaction glassware. Acetone (40 ml) was added immediately with gentle swirling until the reaction mixture returned to a homogenous dispersion. Acetone was then added slowly, dropwise with swirling until a visible granularity was observed and the mixture was allowed to settle for 15 minutes. The supernatant was pipetted off, and water (~20 ml) was added to resuspend the AgNWs. The selective precipitation was repeated 3-5 times as necessary. The residual acetone and water were then removed by centrifuging the solution at 3000 rpm for 3 minutes, removing the supernatant, redispersing in ethanol, and repeating the procedure 4 times before suspending the purified wires in a stock solution of ethanol (~5 ml).

The UV-Vis spectrum of the wires was taken by preparing a dilute sample of the wires in ethanol. The composition of the wires was examined using TGA. Qualitative
analysis of the wire size distribution and purity was determined by bright-field optical microscopy using an Olympus BX60 microscope equipped with a DP73 color camera and polarization filters. SEM images were taken using a Hitachi SU8000 UHR FE-SEM.

SINGLE-NANOSTRUCTURE SURFACE FABRICATION

Solutions of aqueous inks for rod coated surfaces were first prepared by creating a stock solution of the Ag complex in water (1500 mg/ml). A serial dilution was performed to create test ink concentrations of 300, 60, 12, and 2.4 mg/ml. An 11 mg/ml Ag complex ink was also prepared using a 1.5% w/w PVP (Mw ~10,000) aqueous solution. The inks were pipetted (25 µl) into a line on the glass slides and spread by hand using a #2.5 and #10 Mayer rod. The Mayer rod was cleaned by an excess of water using a homemade rod cleaner in between uses. In addition, spin casting was attempted with the inks using dynamic and static techniques with speeds of 500, 1000, 2000, and 3000 rpm. The deposited ink layers were dried at 100 °C for 5 minutes and then decomposed at 125 °C for 5 minutes.

Aqueous inks for dropcasted surfaces were prepared by dissolving the silver complex in water (11 mg/ml). All dropcasted surfaces were prepared using 25 µl of the ink. Two methods of deposition and decomposition were tested. For the first method, the aqueous ink was dropcasted onto a room temperature slide, dried for 1 minute at 100 °C, then decomposed at 125 °C for 5 minutes. For the second method, the glass slide was preheated to 125 °C and the ink was dropcasted directly onto the hot slide and decomposed for 5 minutes.
Three AgNW stock solutions for spincasting were prepared by suspending the centrifuge-washed wires in ethanol, water, and an aqueous solution of 3% w/w PVP respectively to a silver concentration of 20 mg/ml. Serial dilutions were performed to create solutions with silver concentrations of 15, 10, 5, and 1 mg/ml in each solvent. Spin casting was attempted with all of the solutions using dynamic and static techniques with speeds of 500, 1000, 2000, and 3000 rpm.

Mayer rod coating was used to produce AgNW surfaces, herein referred to as NWS, for the direct use as a SERS substrate and for the fabrication of dual-nanostructured surfaces. In short, the stock AgNW solution was pipetted (25 μl) in a line on glass slides and spread by hand using either a #2.5 or a #10 Mayer rod. The Mayer rod was cleaned by an excess of water using a homemade rod cleaner in between uses. The slides were dried at room temperature for ~30 minutes.

The uniformity of the fabricated surfaces was examined using an optical microscope. The morphology of the nanostructures on the surfaces were characterized using a Veeco Dimension 3100 AFM operating in tapping mode.

SINGLE-NANOSTRUCTURE SERS

Standard solutions of MPAA and ATP were made by dissolving the solid analyte in ethanol under an argon blanket. The standard samples were drop-casted in either 5 or 10 μl aliquots onto the respective silver surfaces. The spot was air dried at room temperature for 2 minutes and it’s was diameter was measured using a micrometer. Raman spectra were acquired with a Wasatch Photonics 638 nm Raman system using a spring-loaded slide holder.
DUAL-NANOSTRUCTURE FABRICATION

Dual-nanostructured surfaces (DNS) were produced using two methods. The first method produced full-scale surfaces of low-quality while the second method produced small-scale surfaces of SERS quality. Low-quality surfaces were produced by Mayer rod coating 25 μl of the stock AgNW solution onto a glass slide using a #2.5 Mayer Rod. After drying the slide at room temperature for 30 minutes, it was used as a substrate to Mayer rod coat 25 μl of the Ag complex (11mg/ml) using a #10 Mayer rod. The slide was then immediately heated to 125 °C and decomposed for 5 minutes.

SERS quality NWS and DNS were produced by first Mayer rod coating 25 μl of the stock AgNW solution onto a glass slide using a #10 Mayer rod. After drying at room temperature for 30 minutes, the slides were stored in covered containers. Prior to use, the slide was preheated to 125 °C. On the central region of one half of the slide, 25 μl of a 11 mg/ml Ag complex ink was dropcast and allowed to decompose for 5 minutes. The slide was allowed to cool to room temperature and was gently rinsed with ethanol and allowed to dry.

The uniformity of the fabricated surfaces was examined using an optical microscope. The morphology of the nanostructures on the surfaces were characterized using a Veeco Dimension 3100 AFM operating in tapping mode.

DUAL-NANOSTRUCTURE SERS

A set of standard solutions of ATP in ethanol were made with concentrations of 847, 423.5, 42.35, 8.47, and 0.5 μM. The standard samples were drop-casted in 5 μl aliquots onto both the NWS and DNS sides of the SERS quality surfaces. The spots
were air dried at room temperature for 2 minutes and their diameters were measured using a micrometer. Raman spectra were acquired with a Wasatch Photonics 638 nm Raman system using a Thorlabs MT3 XYZ stage controller equipped with micrometers to control the positioning (XY) and focus (Z) of the laser on the slide surfaces.
CHAPTER IV

RESULTS AND DISCUSSION

SILVER COMPLEX SYNTHESIS

The IR spectrum was taken in transmission mode and is shown in Figure 8. The $^{13}$C NMR spectrum was obtained with 1024 scans and is shown Figure 9. Both spectra exhibit peaks that match the reported spectra of the complex. The TGA of the Ag complex was performed in an aluminum pan with a linear heating ramp of 10 °C/min from 50 °C to 500 °C. Nitrogen was used as the purge gas with a flow rate of 25 ml/min. The TGA curve obtained is shown in Figure 10 and the complex was calculated to be 47.01% w/w percent silver. The deviation from the theoretical value of 50.8% is due to the mass loss prior to 80 °C. The loss of water and the release of residual reactants is believed to have caused the initial mass losses. The thermal decomposition of the complex begins to occur above 90 °C and is fully complete just past 130 °C.

SILVER NANOWIRE SYNTHESIS

Various synthetic conditions were tested to establish a method that is effective and amenable to industrial scale up. A ratio of 100 and 300 mole/mole of Ag$^+$ to Fe$^{3+}$ and Cl$^-$ respectively was held constant at for each reaction. The tested experimental conditions are summarized in Table 1. Images of the reaction mixture throughout the progression of a typical reaction are shown in Figure 11.
Figure 8. The IR absorbance spectrum of the solid Ag complex.

Figure 9. The $^{13}$C NMR spectrum of the Ag complex. The concentration of the Ag complex was 78 mg/ml in D$_2$O.
Table 1. A summary of the reaction conditions used to optimize the synthetic method

<table>
<thead>
<tr>
<th>Reaction Condition</th>
<th>PVP M&lt;sub&gt;w&lt;/sub&gt; (g/mole)</th>
<th>PVP*/Ag* (Mole/Mole)</th>
<th>145 °C Step time (Minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29,000</td>
<td>1</td>
<td>210</td>
</tr>
<tr>
<td>2</td>
<td>29,000</td>
<td>1.4</td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td>55,000</td>
<td>1.4</td>
<td>70</td>
</tr>
<tr>
<td>4</td>
<td>55,000</td>
<td>2</td>
<td>70</td>
</tr>
</tbody>
</table>

*Here PVP refers the moles of monomer units

Figure 10. The TGA curve of the Ag complex. Since no mass loss occurred past 145 °C, values after 150 °C are omitted for clarity.
Figure 11. Camera images of the time points in the polyol reaction. The time points are after the addition of FeCl$_3$ (A), after the completion of the 100 °C step (B), and after the completion of the full reaction (C).

Figure 12. The UV-Vis spectra of the raw reaction mixture at various time points. The spectra are normalized to the highest absorbance past 320 nm. The spectra at time points past t=30 are omitted for clarity.
The reaction mixture is clear and colorless until the addition of the FeCl₃ solution. After the addition, the solution becomes slightly cloudy and faint yellow. After the 100 °C step, the solution changes to a cloudy red with less transparency and turns into an opaque off-white composition upon completion. The UV-Vis spectra taken from various time points during a reaction are shown in Figure 12. The peak absorbance at 403 nm at t=0 in Figure 12 is consistent with the value reported for AgCl nanocubes synthesized in literature. In addition, the peak position, Gaussian shape, and the lack of a quadrupole moment shoulder indicate that little or no AgNW formation has occurred. Therefore the transition from pale yellow to red in the 100 °C step occurs because of the independent formation of AgCl nanocubes. This is consistent with the addition of chloride ions to the silver solution.

The shift in the peak position, peak shape, and appearance of the quadrupole moment shoulder at 15 minutes is consistent with the formation of AgNWs. As shown, at 30 minutes, the position of the peak maximum remains constant but the peak is much broader. This behavior indicates that the reduction of silver is continuing to occur between 15 and 30 minutes. From 30 minutes up to 210 minutes, no change was observed in subsequent spectra, indicating the completion of the reaction.

The shift in the peak position, peak shape, and appearance of the quadrupole moment shoulder at 15 minutes is consistent with the formation of AgNWs. As shown, at 30 minutes, the position of the peak maximum remains constant but the peak is much broader. This behavior indicates that the reduction of silver is continuing to occur between 15 and 30 minutes. From 30 minutes up to 210 minutes, no change was observed in subsequent spectra, indicating the completion of the reaction.
The average particle size of the isolated and washed nanocubes obtained at t= 0 was determined to be 184 nm (with a 0.171 polydispersity index) by the DLS analysis, shown in Figure 13. The results were obtained assuming a spherical particle and therefore the measured diameter only approximates the true size of the nanocubes. However, the low polydispersity index of 0.171 supports that the method produces high uniformity nanocubes at this stage of the reaction.

The microscope image in Figure 14 shows that reaction condition 1 produces a low yield of wires. A large amount of big, spherical nanoparticles is present compared to the number of wires. In addition, there is a large disparity in the diameter and length of the wires. The UV-Vis spectrum of the purified wires, shown in Figure 15, displays an extremely broad SPR and is also indicative of the poor quality of the products. Since the transverse plasmon of AgNWs displays a diameter dependent peak wavelength, a polydisperse sample is expected to display the observed very broad SPR. The shoulder associated with the quadrupole moment of the transverse plasmon is also completely obscured by the overlapping SPR of the large amount of NP side product.

The large amount of side product and the variation in the sizes of the wires produced by reaction condition 1 indicate that the reduction of silver occurs too rapidly under these reaction conditions. The poor quality of the product can be understood by considering the reaction in the terms of the LaMer nucleation and growth theory presented in the introduction. In the context of the polyol synthesis, the monomer of Ag⁰ is produced by the reduction of solubilized Ag⁺. Homogenous nucleation refers to the formation of silver atom clusters from individual Ag⁰ monomers. Heterogeneous nucleation refers to the deposition of individual Ag⁰ monomers onto any existing
Figure 13. The size distribution obtained by the dynamic light scattering analysis for the AgCl nanocubes.

Figure 14. The microscope image of the products obtained by reaction condition 1.
Figure 15. The normalized UV-vis spectrum of the wires produced by reaction condition 1 after cleaning.
in the absence of AgCl nanocubes, the reaction would proceed as shown in Figure 6, where the \([\text{Ag}^0]\) would rise to \(C_{\text{Max}}\) and homogenous nucleation would occur to produce seed AgNPs in the bulk solution.\(^{38}\) The initial seed AgNPs then act as a surface for heterogeneous nucleation, resulting in the rapid growth of the particles as the \([\text{Ag}^0]\) drops from \(C_{\text{Max}}\) to \(C_s\).

To produce AgNWs, MTPs must be formed from the coalescence of icosahedral and cuboctahedral AgNP seeds, which is a diffusion-limited process.\(^{50}\) However, the formation of MTPs is only thermodynamically favorable in small particles with extremely high surface area to volume ratios.\(^{37}\) If particle growth occurs faster than diffusion-limited collisions, MTPs, and therefore AgNWs, will no longer be produced. The rapid growth that occurs following homogenous nucleation as \(C_{\text{Max}}\) drops to \(C_s\) occurs fast enough to make the formation of MTPs unlikely. As stated previously, AgNWs can be grown in the absence of the addition of chloride ions, however the reactions require very stringent control of stirring speeds, injection rates, etc. to prevent the initial seed AgNPs from growing faster than the coalescence of MTPs can occur.\(^{51}\)

The AgCl nanocubes promote the formation of MTPs by providing a surface for heterogeneous nucleation, allowing seed particles to be produced at a \([\text{Ag}^0]\) = \(C_s\) and bypassing the need of homogenous nucleation.\(^{48}\) In addition, the production of seed particles is spatially concentrated in the proximately of the AgCl nanocubes, instead of distributed throughout the bulk solution. The lower growth rate experienced by the initial seed particles and the spatial concentration of a small number of seed particles increases the likelihood of interparticle collisions to produce MTPs. A portion of non-
twinned particles will always be produced and will eventually grow into spherical particles.

However, if Ag\(^0\) atoms are produced faster than heterogeneous nucleation can deposit them, the [Ag\(^0\)] continues to rise above C\(\text{S}\) and the seed particles experience faster growth. If the [Ag\(^0\)] reaches C\(\text{Max}\), homogenous nucleation events produce seed particles in the bulk solution.\(^{38}\) These particles compete for the heterogeneous nucleation of silver atoms and result in a large portion of spherical particles.

Under reaction condition 1, Ag\(^0\) was produced too quickly and likely reached C\(\text{Max}\), producing a large number of seed particles through homogenous nucleation. The seed particles produced in the bulk of the solution experienced rapid growth and resulted in the large amount of spherical AgNPs. In addition, the seed particles produced by heterogeneous nucleation at the surface of AgCl nanocubes experienced rapid growth, making the formation of MTPs unfavorable early in the reaction. However, heterogeneous nucleation of new AgNP seed particles at the AgCl nanocube surface competes with the growth of existing particles and continues to occur. This gives rise to a wide range of time periods for the growth-phase experienced by the final products— including spherical AgNPs and AgNWs.

Some AgNWs were still produced because the likelihood of MTP events increased as the reaction proceeded and the depletion of free Ag\(^+\) lowered the rate of Ag\(^0\). The resulting wires were a large variety of lengths and diameters. Normally, AgNWs are formed from the one-dimensional growth of MTPs on the exposed [111] face.\(^{36}\) When silver atoms are being produced at a higher rate than the rate at which [111] nucleation can occur at MTPs, large diameter wires occur due to the deposition of
silver on the competing [100] face of the MTP. This gives rise to latitudinal growth at the expense of longitudinal growth yielding shorter, fatter wires. Likewise, the formation of an abundance of nucleation sites results in a rapid decrease in silver atom concentration as the particles grow in size, with longitudinal growth having to compete with both spherical particle growth and with latitudinal growth of wires.

Reaction temperature, the use of Fe$^{3+}$ as a catalytic ion, or the initial Ag$^+$ concentration are all factors that affect the kinetics of Ag$^0$ production. The concentration of Ag$^+$ was chosen to be lowered for reaction condition 2 because of the previously discussed benefits of the temperature and catalyst to industrial scale up. The mole ratio of PVP monomer to silver was also increased to 1.5 to help direct one-dimensional growth.

The microscope image in Figure 16 shows the typical products of reaction condition 2. By comparing Figure 16 and Figure 14, it is clearly shown that the number of large spherical AgNP produced drastically decreased. This supports that the reduction of the initial [Ag$^+$] lowered the reduction kinetics enough to avoid an excess of seed particles produced through homogenous nucleation. Since no additional silver is added after the reaction begins, homogenous nucleation events can only occur at the beginning stages of the reaction. The early formation, low chance of MTP formation due to rapid growth, and long growth phase produce large, spherical particles.

In addition, Figure 16 also shows that a larger number of wires were produced with a length over 5 μm. Wires with short lengths are also present and the wires still exhibit a large variation in their diameters. However, wires with small diameters are mechanically weaker, so the appearance of curvature in some of the long wires
Figure 16. The microscope image of the products obtained by reaction condition 2.
Figure 17. An AFM image of the products obtained from reaction condition 2. The reported diameter was measured from the wire indicated by the arrow. The image was acquired using tapping mode AFM.
suggested that much smaller diameters were achieved.\textsuperscript{35} The AFM image shown in Figure 17 depicts the smallest diameter, flat wire found from reaction condition 2. It was measured to be 121 nm and confirms that small diameter wires were present in the products. Diameters under 120 nm were measured in wires that were not flat in the Z direction, however a variable diameter was consistently measured along their lengths so the results could be an artifact produced by the method. In addition, small AgNPs are visible on the surfaces of the wires that could not be seen in the microscope images due to the diffraction limit of light.

Reaction condition 3 utilized PVP with a $M_w$ of $\sim55,000$ and the selective precipitation procedure\textsuperscript{29} was incorporated to obtain AgNWs in a high purity. The microscope image in Figure 18 shows the purified product of a typical synthesis. A marked increase in the frequency of wires longer than 5 $\mu$m can be seen. In addition, most wires exhibit the curvature that is characteristic of thin wires. Using AFM images, such as the one shown in Figure 19, the diameter of wires over 5 $\mu$m long were determined to be 120-230 nm. Once again, only wires that are lying flat in the Z plane were considered for accurate diameter measurements.

The higher molecular weight PVP lowered the distribution of diameters by increasing the binding energy to the [100] face of the MTPs.\textsuperscript{52} The increased passivation of the [100] face prevented significant latitudinal growth from occurring, even during the early stages of the reaction. The increase in length was a result of the increase in silver mass available for longitudinal growth. The decreased average diameter and increased average length demonstrates the ability of the higher molecular weight PVP to improve the yield of high aspect ratio AgNWs.
Figure 18. The microscope image of the products obtained by reaction condition 3.
Figure 19. An AFM image of the products obtained from reaction condition 3. The image was acquired using tapping mode AFM.
Due to the portion of non-twinned particles and constant heterogeneous nucleation of new seed particles, the formation of side products cannot be avoided in the polyol synthesis. Reaction condition 3 also incorporated a selective precipitation method\textsuperscript{29} to increase the purity of the final product obtained. By comparing Figure 18 to Figure 16, a drastic decrease in truncated wires and nanoparticles is seen, demonstrating the effectiveness of the selective precipitation.

Acetone was chosen as the precipitation agent during purification because PVP in insoluble in it.\textsuperscript{29} To determine the presence of a PVP coating, the TGA of the AgNWs was performed in an aluminum pan with a linear heating ramp of 5 °C/min from 50 °C to 600 °C. Nitrogen was used as the purge gas with a flow rate of 25 ml/min. The TGA curve obtained is shown in Figure 20 and the wires were calculated to be 0.8% PVP by weight. The presence of PVP on the surface of the AgNWs was also confirmed by the SERS spectrum of PVP, shown in Figure 21.

The C-C aliphatic vibrations of the polymer backbone of solid PVP is seen at ~500 cm\textsuperscript{-1} while the region from ~1000 to 1150 cm\textsuperscript{-1} contains contributions from CH vibrations of the polymer backbone and lactam ring chains.\textsuperscript{52} The intense peak at ~1450 cm\textsuperscript{-1} is from CC aliphatic vibrations in the backbone and lactam ring. The peak at 890 cm\textsuperscript{-1} in solid PVP is the ring breathing mode of the pyrrolidone side chain’s lactam ring. Figure 22 depicts a higher magnification of the Raman spectrum of solid PVP, where a small peak at 1600 cm\textsuperscript{-1} is present due to carbonyl vibration of the lactam ring. The CN vibration of the lactam ring is also visible as a shoulder at 1400 cm\textsuperscript{-1}.\textsuperscript{52} The SERS spectrum show significant enhancement for the carbonyl stretch at 1600 cm\textsuperscript{-1} and the CC aliphatic vibrations at 500 cm\textsuperscript{-1}. These enhancements in these regions have been
Figure 20. The TGA of AgNWs from room temperature to 700 °C. Outside the range of 100 °C to 550 °C, no change in weight was observed.

Figure 21. The Raman spectrum of PVP and the SERS spectrum of PVP on a AgNW surface.
Figure 22. A higher magnification of the Raman spectrum of solid PVP.

Figure 23. A camera picture of the selective precipitation process after settling.
attributed to the orientation of the lactam ring, which coordinates through the carbonyl oxygen and maintains the aliphatic backbone of the polymer parallel to the silver surface. The disappearance of the lactam breathing mode at 890 cm\(^{-1}\) has been attributed to a poor enhancement due to a tilted orientation of the lactam ring relative to the silver surface.

The selective precipitation of the desired AgNW product typically requires the addition of acetone until the solution is \(~70\%\) v/v acetone. Figure 23 depicts the granularity that was used as the visual indicator for the selective precipitation after settling. Care must be taken during this step because the unwanted side products will also precipitate if too much acetone is added. The microscope image in Figure 24 shows the supernatant taken from the first precipitation performed. Small and large spherical AgNPs are visible as well as a small number of wires. However, the method is highly selective because the number of particles is several orders of magnitude higher than the number of wires.

The mole ratio of PVP/Ag was increased to 2 for reaction condition 4 with the intention of lowering the amount of side product formation. The reaction failed to produce less side product, however the higher ratio of PVP increased the aspect ratio of the final wire product to \(~200\). The SEM images of typical reaction products are shown in Figure 25. The AgNWs exhibit a narrow distribution of diameters from 80 to 120 nm with lengths of 10 to 30 \(\mu\)m. The UV-Vis spectra of wires produced by reaction condition 1 and 4 are shown in Figure 26 to exemplify the drastic increase in the quality of the products obtained under the optimized conditions.
Figure 24. The microscope image of the supernatant removed by the first selective precipitation performed on the products of reaction condition 3.
Figure 25. SEM images of the wires produced by reaction condition 4 at a low magnification (A) and a high magnification (B).

Figure 26. The UV-Vis spectra obtained for the cleaned products of reaction conditions 1 and 4.
The higher concentration of PVP further increased the passivation of the [100] face of MTPs, allowing even less latitudinal growth to occur. The failure to produce less side product indicated that the additional PVP did not increase the proportion of MTP events compared to non-twinned events. This is also supported by the simultaneous increase in the length of the obtained wires. If the diameter of MTPs is reduced but the number of MTP events is held constant, a larger mass of silver is available for longitudinal growth. This is consistent with the observed reduction in diameter and increase in length of wires produced by reaction condition 4 compared to reaction condition 3.

The optimized reaction condition 4 contains many desirable qualities for industrial scale up. The two-step temperature ramp synthesizes uniform AgCl nanocubes prior to the synthesis of AgNWs in one pot. Since only the number of heterogeneous nucleation sites is relevant to the final wire product, the sampling and characterization of the AgCl nanocubes offers a quality control checkpoint. A highly uniform size distribution with an average aspect ratio of ~200 was achieved with reaction conditions that require no control over stirring, injection rate, or other complicated factors that would be difficult or expensive to incorporate into industrial scale reactions. The mechanical strength of the ~100nm wires is enough to remain mostly straight with only a small amount of curvature. This mechanical robustness is important to the processability of the wires for device manufacture and broad applicability. The selective precipitation successfully isolates the desired AgNWs in a high purity and is readily adaptable to large scale execution in a whirlpool tank.
SINGLE-NANOSTRUCTURE SURFACE FABRICATION

Aqueous inks composed of only the Ag complex failed to produce uniform coatings with both Mayer rod coating and spin casting. Regardless of the concentration of the Ag complex, the ink rapidly coalesced into droplets in every deposition attempt. The failure of the ink to coat the glass slide is a result of the extremely hygroscopic nature of the complex. The behavior was first seen during the synthesis of the complex when the excess of isopropanol was added to precipitate is as a solid. The complex was so hygroscopic that without rapid stirring, the solid complex would pull the water out of the isopropanol. The mixture would then separate into an aqueous bottom layer of the dissolved complex and a top layer of isopropanol.

During these coating processes, the hygroscopic behavior caused the initially thin liquid layer to bead up into a droplet. The inability to uniformly coat the precursor Ag complex inherently prevents the formation of an even AgNP coating through its decomposition and therefore SERS response. A uniform, reproducible SERS response is necessary to produce a SERS-active device with a potential for quantitative application. Therefore, the poor processability of the Ag complex limits its application as a standalone nanostructure production technique for the largescale manufacture of SERS-active devices.

When the ink complex was developed by Williams et al., the group used the surfactant BYK 333 to improve the coating characteristics of their Ag complex ink. For SERS application, the use of additives is undesirable because they produce excess background signal that can interfere with analysis. As shown previously, the AgNWs synthesized by the polyol method have a surface coating of PVP. Therefore, if PVP can
improve the coating ability of the Ag complex, then an AgNW substrate could be utilized to increase processability of the Ag complex without the need of additives.

The viability of PVP to increase the coating characteristics of the Ag complex was tested by incorporating ~10,000 Mₗ PVP into an aqueous ink of the Ag complex. The ink, referred to as PVP-ink, was 11 mg/ml of the Ag complex and 1.5% w/w of PVP. Spin casting also failed with the PVP-ink, however a relatively uniform coating was successfully made with the #10 Mayer rod. The ink was dried at 100 °C for 5 minutes and then decomposed at 125 °C for 5 minutes resulting in the formation of a silver surface, shown in Figure 27.

The banded pattern of the surface in Figure 27.A formed during the drying step because the slide was placed on a wire rack of an oven during the drying step. The areas in between the bands were in direct contact with the metal wire and therefore heated faster than the rest of the slide. The Ag complex remained dissolved in the receding meniscus, resulting in little to no complex deposition in these areas. The areas of the slide that were not in direct contact with the rack experienced a more even heat flow and the Ag complex was deposited as a clear, crystalline layer.

During the decomposition step, the clear layer turned faint yellow over the first 10-20 seconds, followed by a rapid change to red, purple, and ultimately the silvery pink color shown in Figure 27.A. The color changes occurred as the reduction of silver produced AgNPs and as their size increased, the SPR continually red shifted.⁴ Vapor was observed as the complex decomposed and stopped visually occurring ~3 minutes. The decomposition temperature was held for 5 minutes to ensure the reaction continues to completion.
Figure 27. A camera picture (A) and a microscope image (B) of a silver surface produced by Mayer rod coating the Ag complex with PVP.
The microscope image in Figure 27.B was taken from the center of one of the silver bands. The flower like pattern is the result of the coffee stain effect causing the uneven deposition of the silver complex during the drying stage. The pattern could not be caused during the decomposition stage, because as silver is reduced it results in solid silver and gaseous organic species. Without a liquid medium, the spatial migration of the solid silver is highly restricted. Together, the banding pattern and the coffee stain effects within the bands demonstrated that the uniformity of the surfaces suffered from incorporation of a separate drying stage. Regardless, the addition of PVP significantly improved the ability of the PVP-ink to coat the glass surface compared to the Ag complex alone. Therefore AgNWs, that inherently have a PVP surface layer, have the potential to act as a substrate to produce a uniform AgNP coating from the Ag complex without additives.

The influence of separating the drying and decomposition steps of the Ag complex was examined using a simplified dropcasting system. The surface in Figure 28, referred to as nanoparticle surface-hot (NPS-H), was created by dropcasting 25 μl of an aqueous solution of 11mg/ml of the Ag complex onto a glass slide that was preheated to 125°C and held at that temperature for 5 minutes to decompose. The surface shown in Figure 29, referred to as nanoparticle surface-cold (NPS-C), was dropcasted on a room temperature glass slide, dried at 100 °C for 5 minutes, and then decomposed at 125 °C for 5 minutes. The insets of Figure 28 and Figure 29 depict the camera images of NPS-H and NPS-C respectively. The outer rims of both surfaces display a significant coffee stain effect which is expected when using a dropcast method. However, NPS-H visually has a much better coverage of silver in the central region compared to NPS-C.
Figure 28. A microscope image of the middle region of NPS-H. It was produced by dropcasting the Ag complex directly onto a hot glass slide. The inset is a camera picture of the entire spot.
Figure 29. A microscope image of the middle region of NPS-C. It was produced by dropcasting, drying, and then decomposing the Ag complex on a glass slide. The inset is a camera picture of the entire spot.
The microscope images of the central regions of NPS-H and NPS-C, Figure 28 and Figure 29 respectively, both exhibit black spots that are craters due to the formation of bubbles during decomposition. NPS-H displays a dense covering of silver over the entire surface area of the dot, while NPS-C only has sporadic islands of dense coatings with no regular patterning. On NPS-C, the craters only appear in areas where silver is deposited. In addition, the size of the crater corresponded to the size of the island. Large craters are only seen at large islands of silver coating, while small islands have small or no visible craters. This relationship supports that the craters are formed from bubbles by the gaseous decomposition products of the Ag complex. The sporadic nature of the silver islands on NPS-C were a result of the uneven deposition of the Ag complex during the drying step due to the coffee stain effect.

The uniformity of NPS-H shows that the direct decomposition of the ink successfully eliminated coffee stain effects in the internal region. The internal effects are presumably avoided by the immediate deposition of a layer of nanoparticle upon the contact of the ink droplet. The initial deposition then provides a framework of nucleation sites, increasing the uniformity of the surface. The persistence of the coffee stain effect at the border demonstrates the need to couple a uniform coating method with direct decomposition to accomplish an even deposition of silver.

The middle section of each surface was imaged by AFM at various locations to confirm the formation of a AgNP structure on the surface. The presence of a nanostructured surface is important because it is what produces a large number of SERS hotspots, and therefore signal enhancements. The large craters were avoided to prevent extreme changes in Z height from crashing the tip or causing artifacts in the
image. Both surfaces displayed approximately spherical nanoparticle morphologies with an estimated average diameter of ~75 nm, but with different size distributions. NPS-H displays a uniform, random distribution in the nanoparticle size regardless of the sampled location, with a typical image shown in Figure 30. In contrast, NPS-C only displays a uniform distribution of sizes in the central regions of the large silver islands, like the one shown in the center of Figure 29. On the edges of these island and in the space between them, a large variety of particle sizes were measured that changed drastically in their distribution depending on the sampled location. An example of a poorly distributed area is shown in Figure 31. The significant increase in the uniformity of the silver coverage and nanoparticle morphology confirm that the direct decomposition of the Ag complex eliminates the internal coffee stain effects that resulted in the flower pattern seen in Figure 27.

All attempts to spin coat AgNWs failed to produce adequate quality surfaces. Neither the concentration of AgNWs, the solvent used, nor the rpms used affected the results obtained. In every experiment, the wires would be rolled up and deposited in rope-like structures, shown in Figure 32. The rope-like structure was formed because of the assembly of AgNWs at the air-liquid interface. As the meniscus moved outward and evaporated, the wires were rolled into the rope-like bundles. The internal section of these structures exhibited a small portion of isolated wires or occasionally small clusters. The only influencing factor was whether dynamic or static spin casting was used. In dynamic spincasting, the solution is deposited on the surface while it is already spinning at the designated rpm. In static spincasting, the solution is deposited on the surface while it is stationary, and the surface is then accelerated to the designated rpm.
Figure 30. A typical AFM image of the middle region of NPS-H. The image was acquired using tapping mode AFM.
Figure 31. An AFM image of the middle region of NPS-C that displays poor nanoparticle coverage. The image was acquired using tapping mode AFM.
Figure 32. A microscope image of the typical rope-like structure deposited during AgNW spincasting experiments.
In the case of static spincasting, a small portion of wires persisted in the area of initial deposition, while in dynamic spin casting, they did not.

In contrast, the #2.5 and #10 Mayer rods were able to produce uniform coatings of AgNWs with relative ease. All microscope and AFM images presented so far were taken of surfaces produced using the #2.5 rod. The surfaces were used primarily with diluted AgNW solutions to produce isolated wires or small wire clusters for the qualitative analysis of synthetic conditions. The #2.5 rod deposits a significantly smaller volume of liquid due to the smaller diameter of the wire winding. The smaller volume results in a thinner coat with a smaller number of wires and therefore a lower number of wire intersections. The intersection points are critical to produce SERS hotspots, so the #10 rod was used to produce all NWS used for SERS experiments. A microscope image of a typical NWS that was produced by Mayer rod coating with the #10 rod is shown in Figure 33. The wires prefer to align with directly adjacent wires, forming swirling patterns on the surface. This is a result of a tendency for the AgNWs to align in the direction of flow for the surrounding fluid. Presumably, the swirling pattern represents eddy currents present during the deposition of the AgNWs. The linear portions contain a larger density of wire intersections which is necessary to generate SERS active hotspots. However, the areas of the swirls with tight curvature contain significantly less wire intersections, making the linear regions the desired structure.

The NWS were produced with variable qualities. In the industrial setting, the variability in coat quality would be significantly lower due to automation. The industrial use of Mayer rod coating is extremely prevalent in the form of roll-to-roll, or R2R, printing. Substrates to be coated are attached to a belt that is driven around a roller
Figure 33. A microscope image of a typical NWS produced by Mayer rod coating with a #10 rod.
into a vat of the coating solution. The belt then brings the wet substrate out of the solution and is passed over a second roller that is itself a Mayer rod.

The excess solution that is pushed off falls back into vat of coating solution, resulting in little to no waste. The rate the wet substrate passes the Mayer rod, the contact pressure of the rod and surface, and other factors such as a rotational rate of the Mayer rod are all tightly controlled in R2R printing to produce extremely uniform surface coatings. These factors are all highly variable when performed by hand. In addition, the dragging of wire aggregates often produced defects like the one shown in Figure 34. Small diameter wires that are flexible in solution are more prone to aggregation, however even mechanically rigid wires form aggregates with age. Therefore, either the removal of wire aggregates or a methodology to address their presence would be necessary for industrial processing.

For the AgNW surfaces to be uniform in SERS response, the number of hotspots inside the ~60 μm laser spot must be consistent and large enough in magnitude to eliminate their natural deviation. The individual enhancement factors of the hotspots vary due to differences in junction gap sizes, the number of analyte molecules in the gaps, the orientation of the excitation laser, and the orientation of the detector. However, the same factors that make the AgNWs desirable for processing limit the amount of intersections, and therefore SERS hotspots that can exist within the laser spot.

The ~100 nm diameter of the wires allow them to be mechanically rigid which lowers the rate of aggregate formation and increases the uniformity of the coating. However, the rigidity also limits any two individual wires to one intersection with each
Figure 34. The microscope image of ridges produced by the dragging of wire aggregates on a NWS.
other along their length; or at the very most two for the extreme case of the highly aligned wires with curvature in opposite directions. The ~20 μm length of the wires also makes it highly improbable that any one wire is able to lie flat in the Z plane, where the XY plane contains the plane of the surface. The result is a large number of wire junctions exist as one wire “stacked” on top of another wire.\textsuperscript{55} As discussed in the introduction, the maximum SERS enhancement in nanostructure junctions occurs when the incident light is perpendicular to a plane containing the analyte molecule between two plasmonic structures. The junctions stacked in the Z direction would therefore experience the greatest enhancement from incident light from the XY plane. Since the SERS surface is sampled from either the Z or –Z direction, 90° from the ideal excitation angle, these stacked junctions will produce the minimum enhancement factor possible.

The limitation of basically one intersection between any two wires, the comparable lengths of the wires to the laser spot size, and the prevalence of stacked junctions that restrict the number and efficacy of SERS hotspots that can be produced by AgNWs alone. In addition, the natural variability in the quality of the surfaces produced by hand, the areas with a low density of intersections, and the presence of defects all present additional spatial variations in the number of SERS hotspots relative to the spot size of the laser. However, it has been shown that:

- The AgNWs themselves have surface layer of PVP
- PVP can be used to increase the coating characteristics of the Ag complex
- The AgNWs easily produce uniform coats on scales larger than the spot size of the laser
Therefore, the easily produced AgNW coating can be utilized as a substrate to produce a uniform AgNP surface through the decomposition of the aqueous ink of the Ag complex without the need of any additives. The AgNPs will be produced in the interstitial space between and on the AgNWs, creating a dual-nanostructured surface. On NPS-H, the ~75 nm, spherical AgNPs were shown to form with a tight packing and uniform distribution. Therefore, a large number of SERS hotspots will be formed relative to the spot size of the laser. The dual-nanostructured surface effectively circumvents the processability problem of the Ag complex ink alone, will increase the magnitude of the SERS response by increase the number of hotspots, and decrease the variability of the signal obtained from the AgNWs alone.

DUAL-NANOSTRUCTURE FABRICATION

To prove that AgNW substrates can be used to improve the processability of the Ag complex without the aid of surfactants, a dual-nanostructured surface (DNS) was produced using two Mayer rod coatings. The first coat was done using 25 μl of the AgNW stock solution with a #2.5 rod and allowed to dry for 30 minutes. The second coat was done using 25 μl of an 11mg/ml aqueous ink of only the Ag complex and a #10 rod. Immediately after the second coating, the slide was placed on a hotplate preheated to 125 °C and the complex was allowed to decompose for 5 minutes. Figure 35 shows a microscope image of the surface, with a lower magnification image of the surface in the inset.

As predicted, the AgNW substrate allowed the ink to be evenly coated down the entire length of the slide. The AgNW surface itself was made with a #2.5 Mayer rod to
Figure 35. The microscope image of the DNS produced by Mayer rod coating the Ag Complex ink over a AgNW coated substrate. The inset is a lower magnification of the same surface.
allow a second pass with a #10 rod to do minimal damage. Since this was done by hand, there was minimal control over the pressure between the Mayer rod and the surface. Even with the precaution, the physical abrasion of the Mayer rod resulted in a visible resuspension of some AgNWs during the second coat. In addition, the glass surface could not be held at 125 °C during the process because a homemade aluminum brace without heating capabilities was used to hold the glass slide stationary. The resuspension of some wires and the lack of a preheated surface during the second coat caused the coffee stain effect to result in the formation of the wire clusters shown in the inset of Figure 35.

The higher magnification image in Figure 35 clearly shows that the majority of silver mass from the decomposition of the ink is in the vicinity of the AgNWs. This localized coffee stain effect shows that the ink experienced a separation between the drying and decomposition events without the preheating of the substrate. Compared to the random distribution of silver islands seen on NPS-C, the AgNW substrate provided a guiding template for the deposition of the Ag complex as the solvent evaporated. The nanostructure of the deposited silver was examined using AFM. Multiple locations were imaged, with a typical image shown in Figure 36. The most striking feature is the appearance of a two-lobed structure in the morphology of the nanoparticles. In addition, the size of one lobe is consistently larger than the other and the larger lobe appears preferentially on one side. The morphology was originally interpreted as an artifact of the tapping mode AFM, however the trace and retrace showed identical features and the morphology was consistent across all areas sampled. While the directionality changed orientation with the sampled area, the event was observed in every image
Figure 36. An AFM image of the DNS produced by Mayer rod coating the Ag complex directly onto a AgNW substrate. The image was acquired using tapping mode AFM.
Figure 37. A higher magnification AFM image of the central region of the DNS shown in Figure 36. The image was acquired using tapping mode AFM.
taken, suggesting that the morphology is not an artifact of the AFM. Figure 37 is a higher magnification of the AgNW free region in the middle of Figure 36. The lobed structure can clearly be seen even in the smaller particles that were beyond the resolution of the lower magnification image. To fully characterize the morphology, a higher resolution technique such as FEB-SEM is required.

The average size of the particles is also larger, with an estimated average diameter of ~125 nm. In addition, the particles exhibited a much wider range of sizes, with particle as small as 25 nm and as large as 250 nm. However, just like with the dropcasting system discussed earlier, the uniformity of the diameter suffered from the existence of a separation between the drying and decomposition of the Ag complex. A coating performed at the decomposition temperature would restore the uniformity in the nanoparticle morphology.

The first method of DNS fabrication serves as a proof of concept that a AgNW substrate can be used for the direct deposition of the Ag complex using a Mayer rod system. The ability to utilize R2R printing in both coating steps will allow a single production line to produce the dual-nanostructured device. The optimization of parameters that are outside of the control of hand coating, such as the pressure between the rod and the substrate, will allow the dual-nanostructured surface to be fabricated with R2R processing on an industrial scale.

To examine the SERS activity, a second method of producing a DNS from the combination of Mayer coating and dropcasting was used. In this method, a #10 rod was used to produce a AgNW substrate on a glass slide using 25 μl of the AgNW stock solution. After drying at room temperature for 30 minutes, the glass slide was placed on
Figure 38. The microscope image of the DNS produced by dropcasting the Ag complex ink onto a AgNW coated substrate. The inset is a lower magnification of the same surface.
a hotplate and preheated to 125 °C. Once the slide reached the decomposition
temperature, 25 μl of the 11 mg/ml Ag complex ink was dropcasted onto the center of
the lower half of the slide and allowed to decompose for 5 minutes. The microscope
image of the central region of a typical dual-nanostructured area is shown in Figure 38
while the inset of Figure 38 depicts the surface at a lower magnification.

Just as with the previous dropcast system, the outer edge of the deposited ink
spot experienced a concentration of silver deposition due to the coffee stain effect.
Contrary to the first dual-nanostructure fabrication method, the dropcasted ink only
resuspended a small amount of AgNW clusters (~1-3) at the edge of the ink spot. This
suggests that the presence of mechanical forces unique to the air-liquid interface of the
meniscus drove the phenomenon\textsuperscript{56} and it can be assumed that it has a negligible effect
on the internal area. The mechanical displacement of the clusters through the bubbling
of gaseous decomposition products was considered unlikely because the displacement
events did not continue after the initial deposition.

The inset of Figure 38 shows the swirling pattern of the original AgNW substrate
is still present in the DNS. As with the previous dropcast system, craters due to the
elimination of the gaseous decomposition products are seen in Figure 38. The shapes
are asymmetric compared to the primarily round or rod-shaped craters seen previously
(Figure 28 and Figure 29). This is the result of the asymmetrical distribution of the
interstitial space between the wires. The surface tension of a bubble likely resulted in
them becoming trapped in the gaps between wires. As they grew, they merged with
other spatially close, trapped pockets of gas, resulting in the odd shapes.
The morphology of the DNS was examined using AFM, with a typical image shown in Figure 39. The extreme variation in the Z height of the AgNWs prevented the imaging of the bottom layer of the glass surface. However, clusters of AgNPs at wire intersections were able to be imaged. The particles exhibit extremely small sizes and exist on a large range of Z heights. The existence of discrete particles is supported by the defined ridges visible in the image, however the tapping mode AFM is limited to the highest feature in the Z direction. Therefore, particles that are not discretely separated in the XY plane cannot be differentiated.

While the two methods used to produce the dual-nanostructured surfaces resulted in significantly different surface characteristics, together they show the feasibility of the large-scale device production. The low-quality surface produced using two Mayer rod coats proved that a AgNW substrate could be produced in a R2R process and then used as a substrate to Mayer rod coat an aqueous solution of the Ag complex. The limitations of the hand coating process are easily overcome in the industrial implementation of the R2R process. In order to mimic the quality of the surface that would be achieved, a simplified method using a Mayer rod coat of AgNWs and a dropcast deposition of the aqueous Ag complex was used.
Figure 39. An AFM image of the central region of the DNS produced by dropcasting the Ag complex directly onto a AgNW substrate. The image was acquired using tapping mode AFM.
SINGLE-NANOSTRUCTURE SERS

Thiophenol-based molecule are a powerful reporter for the SERS activity of a device. The thiol moiety has a high affinity for the surface and chemisorbs very rapidly. This results in a strong CE factor, increasing the SERS enhancement that the class of molecules receive. In addition, surface localization increases the EM the molecule receives. The phenyl ring gives rise to a set of strong, characteristic vibrations that occur in a relatively unique region of the vibrational spectrum. The first occurs at ~1000 cm⁻¹ and the second is ~1600 cm⁻¹. They are associated with symmetrical vibrations of the ring system, which inherently has a large Raman cross section due to the large changes in polarizability associated with the vibrations. In addition, the covalent bond between the sulfur orients the phenyl ring away from the metal surface. The relatively perpendicular geometry of the system results in strong enhancement factors for the ring system.

Generally, a para-substituted derivative of thiophenol is used. These molecules are attractive because they retain all the benefits of the thiophenol moiety while simultaneously offering a functional group for further chemical modifications. The para position points predominately away from the silver surface, open to the bulk solution for derivatization. With care and proper implementation, selectively for an analyte can be introduced into device for the implementation to a specific application. The thiophenol-based molecules have therefore become widespread for the characterization of the enhancement factor, or EF, of a SERS device. The EF is calculated by Equation 4:

\[
EF = \frac{I_{\text{SERNS\text{bulk}}}}{I_{\text{bulk\text{SERS}}}} \quad \text{Eq. 4}
\]
where $I_{\text{SERS}}$ and $I_{\text{Bulk}}$ are the experimental intensities from a peak resulting from identical vibrations in the SERS spectrum and the standard Raman spectrum of an analyte acquired under the same instrumental parameters. $N_{\text{Bulk}}$ and $N_{\text{SERS}}$ represent the number of molecules that give rise to the acquired signal.

While no doubt effective, the use of thiophenol-based reporter molecules must be interpreted cautiously. Many SERS methods reported in literature\textsuperscript{28, 57-59} utilize a sampling method that requires a long-term exposure, $>1$ hour, to the solution of the analyte. This sampling method allows for a preconcentration effect to occur due to the chemisorption of the thiophenol-based molecules to the silver surface.\textsuperscript{13} Without the rigorous characterization of the Langmuir isotherm of the system, the actual $N_{\text{SERS}}$ cannot be estimated realistically and may superficially enhance the EF reported. In order to avoid this, the following Raman experiments were performed by depositing 5 or 10 μl of a solution of the analyte in ethanol directly to the center of a surface. The drop was allowed to dry, and the diameter of the resulting spot was measure with a micrometer. The avoid alterations due to the coffee stain effect, all sampling was done at least 5 mm from the edge of the drop.

The silver surface produced by Mayer rod coating the PVP-ink was screened for SERS activity by using a 5 μl aliquot of 46 mM MPAA. The spot was measured to be 21 mm in diameter and all SERS spectra were acquired at least 1 mm from the edges. The spectrum in Figure 40 is an average of the 3 most intense spectra acquired. Each spectrum was taken with a laser power of 100 mW, a 5 second integration time, and a 4-scan averaging. A background correction was performed using a custom written LabView program. The two major peaks at 1079 cm$^{-1}$ and 1584 cm$^{-1}$ are attributed to
Figure 40. The SERS spectrum of MPAA on the silver surface produced by Mayer rod coating the PVP-ink. The spectrum is an average of 3 spectra and the error bars represent the STD in the peak intensity.

Figure 41. The Raman spectrum of 240 mM ATP in ethanol.
vibrations associated with the aromatic ring of MPAA. The error bars represent the standard deviation (STD) of the peak intensities. A low number of samples was taken due to the lack of appropriate sampling equipment. The slide was positioned manually above the spectrometer during the acquisition.

Assuming a uniform deposition, a concentration of $6.6 \times 10^{-8}$ mole/cm$^2$ was detected. The solution spectrum of the 46 mM MPAA was under the detection limit of the instrument. In addition, the deposition of 100 μl aliquots were deposited, allowed to dry, and the acquisition of a Raman spectrum was attempted. The process was continued until a visible amount of residual still failed to produce a detectable signal. While no quantitative information was afforded by the analysis, the presence of a SERS response suggested that the PVP-ink surface was composed of nanostructured surface.

All following surfaces were examined for SERS activity by using ATP. Figure 41 shows a Raman spectrum of a 240 mM solution of ATP in ethanol. The spectrum was taken with a laser power of 5 mW, a 1 second integration time, and a 4-scan averaging. A background correction was performed using a custom written LabView program. The two major peaks at 1095 cm$^{-1}$ and 1604 cm$^{-1}$ are attributed to SERS-relevant vibrations associated with the aromatic ring of ATP. The Wasatch Photonics 638 Raman system has a spot size of ~60 μm. Assuming that a sphere at the focal point of the laser is generating the signal, the $N_{\text{Bulk}}$ is equal to $1.16 \times 10^{14}$ molecules of ATP. The value of $I_{\text{Bulk}}$ is dependent on which vibrational band is used at the time, with $I_{1096}$ and $I_{1606}$ referring to the intensities of 3239.8 and 2074.2 for the respective wavenumber of the subscript.

Following the confirmation of the presence of a nanoparticle morphology, the SERS properties of NPS-H and NPS-C were explored. Prior to the use of an analyte,
the background spectra were recorded and are presented in Figure 42, normalized to the highest intensity peak. A broad background signal begins rising rapidly at ~1000 cm⁻¹ and falls rapidly at 1600 till ~1750 cm⁻¹ where is slows the decline. Both surfaces exhibit a peak at 1340 cm⁻¹, which is attributed to a remaining portion of incompletely decomposed silver oxalate. If present, the residual oxalate ion would persist as a surface cap on the AgNP, resulting a SERS enhancement of even trace amount. The peak at 930 cm⁻¹ in the spectrum of NPS-H could also be due to residual oxalate. A weak stretch in this region is characteristic of carboxylic acid dimers. The presence of the water solvent during the decomposition process of NPS-H would facilitate the protonation required for dimerization. This is also supported by the presence of the notably reduced intensity of the peak in the background spectrum of NPS-C, which was dried prior to decomposition.

The wide peak at 2021 cm⁻¹ is possibly the result of a small portion of isocyanide formation during the decomposition of the ethylene diamine. Thermal cleavage of the C-C bond and rapid oxidation could presumably result in a surface bound isocyanide group, since the nitrogen of the ethylene diamine is coordinated to the silver. The SERS spectrum of cyanide from KCN treated silver electrodes has been reported to have a spectral band at this location and it was shown to broaden upon aqueous exposure. This behavior is seen in Figure 42, where the peak at 2021 cm⁻¹ is sharper for NPS-C compared to NPS-H. NPS-C was dried prior to decomposition and therefore the surface bound isocyanide experience no water exposure while it did for NPS-H. The peak at 2140 cm⁻¹ was attributed to surface bound carbon monoxide, again presumed to the a result of thermal decomposition.
Figure 42. The background spectrum acquired prior to the introduction of the analyte solution for NPS-H and NPS-C. The spectra are normalized to the highest intensity peak.

Figure 43. The most intense SERS spectrum acquired for NPS-H and the absence of a SERS spectrum for NPS-C. The spectra are normalized to the intensities at 1340 cm\(^{-1}\).
To test for SERS activity, 10 μl of a 1 μM solution of ATP in ethanol was dropped in the center of the ink spot for both NPS-H and NPS-C. A homemade, spring-loaded glass slide holder was used to manipulate the surface with rough repositioning while fine position control was done by hand. The surfaces were sampled with a laser power of 5 mW, an integration time of 500 ms, and with no scan averages. No SERS signal was acquired, so the sampling was repeated with laser powers of 10, 20, 30, and 50 mW. The power was varied instead of the integration time because the samples were being positioned by hand, which is highly variable. The short integration time is therefore necessary to maintain a real-time signal and adjust the position of the surface as needed. At a laser power of 50 mW, a SERS spectrum was found on NPS-H, shown in Figure 43. The spectrum of NPS-C taken with identical parameters is included for comparison and the spectra are normalized to the common background peak at 1335 cm\(^{-1}\). The presence of a small peak at 1084 cm\(^{-1}\) is barely visible. The shift of the vibrational band to a lower frequency is the result of the chemisorption to the silver surface and is a common behavior of the thiophenol based molecules during a SERS analysis.\(^{13}\)

The other characteristic ring vibration around 1600 cm\(^{-1}\) is qualitatively visible in the spectrum by comparison of background shape between NPS-H and NPS-C. However, it is inadequately resolved for quantitative application. While absent in the solution spectrum of ATP, the appearance of peaks at 1150 and 1438 cm\(^{-1}\) also confirms the presence of an ATP derived SERS signal, however their origin will be addressed further. The 1084 cm\(^{-1}\) peak intensity was corrected for the background signal by fitting a third-degree polynomial to points in the valleys between adjacent
peaks. The regression used is shown in Figure 70 and the corrected peak is shown in Figure 44. With an estimated noise of ~30 counts over the corrected region, the 1084 cm\(^{-1}\) peak has an estimated signal-to-noise ratio (SNR) of ~6, so while above the detection limit (a SNR above 3:1), the value is under the quantitation limit (a SNR above 10:1).

The inability to locate acquire a SERS spectrum from NPS-C was attributed to the low concentration of analyte used, the heterogeneous structure of NPS-C, and the manual sampling technique used. NPS-C was composed of sporadic islands of silver nanostructures that contained a large variety and distribution of the AgNPs. Conversely, NPS-H was extremely uniform in surface coverage and distribution of AgNP sizes. Therefore, finding and maintaining an appropriate Z distance to focus the laser on the SERS active surface was much easier.

The uniformity of the SERS response generated from the direct decomposition was tested by preparing another NPS-H. Afterwards, 10 μl of a 1 mM solution of ATP in ethanol was dropped in the center of the ink spot and allowed to dry. The spectrum shown in Figure 45 is the average of ten randomly sampled locations and the error bars indicate the STD of the intensity of the peaks. Each spectrum was acquired using a laser power of 25 mW, an integration time of 100 ms, and no averaging.

The 1084 cm\(^{-1}\) peak is clearly evident, and the other characteristic ring vibration is seen at 1584 cm\(^{-1}\). Again, the shift to a lower frequency is characteristic of thiophenol compounds when chemisorbed to a metal surface. A new prominent peak at 1400 cm\(^{-1}\) appears in addition to the 1150 and 1438 cm\(^{-1}\) peaks briefly mentioned earlier. The STD of the SERS of all the peaks are large, however the three peaks that only appear in the
Figure 44. The background corrected intensity of the 1084 cm\(^{-1}\) in the SERS spectrum from NPS-H.

Figure 45. The SERS spectrum of 1 mM ATP on NPS-H. The spectrum is the average of 10 spectra. The equation of the regression used to correct the intensity of the 1084 cm\(^{-1}\) is shown.
SERS spectrum have larger STD values. The 1150, 1400, and 1438 cm\(^{-1}\) peaks have
STDs of 2254, 2665, and 2129 counts respectively. The 1085 and 1584 cm\(^{-1}\) peaks
have STDs of 1740 and 1850 counts. Due to the larger intensity and lower STD, the
1085 cm\(^{-1}\) peak was corrected using the linear regression shown in Figure 45. The
corrected, average intensity of the peak was 8469 counts with a relative STD (RSD) of
0.364. The extremely high RSD of the peak with the lowest STD was originally
attributed to the continuation of the manual sampling, however the use of ATP as a
SERS indicator was found to be a major contributing factor.

The new peaks at 1150, 1400, and 1444 have been largely attributed to selective
elevation of the \(b_2\) vibrational modes of ATP during a SERS analysis.\(^{57-58}\) While the
migration, appearance/disappearance, or change in relative intensities of normal
Raman spectral bands can happen during SERS analysis, the new peaks are actually
formed through a dimerization of ATP to 4,4\(^{\prime}\)-dimercaptoazobenzene (DMAB).\(^{33}\) Ren et.
Al showed that the \(a_g\) vibrational modes of DMAB not only give rise to the new peaks,
but also produce bands that are almost identical in position to the \(a_l\) bands of ATP at
1084 and 1600 cm\(^{-1}\).\(^{33}\) They proved the dimerization occurs with an impressive array of
evidence:

- Calculated Raman spectrums from computational modeling of \(a_g\) modes of
  DMAB match exactly and fail to produce the \(b_2\) modes of ATP
- Observation of the predicted Raman spectrum of ATP without the DMAB
  bands by using extremely low power densities
- Realtime conversion of ATP to the predicted DMAB Raman spectrum by
  increasing the powder density of the laser
• Electrolytic reduction of the DMAB back to ATP with simultaneous Raman acquisitions and the disappearance of DMAB modes

• Laser desorption ESI-MS identification of the [H-DMAB]$^+$ parent ion exclusively after laser exposing silver surface bound ATP

• Full synthesis and characterization of DMAB, followed by acquiring a direct DMAB SERS spectrum that matched the predicted values

Despite the overwhelming body of evidence for dimer formation, ATP is still a prevalent reporter molecule for calculating the EF of SERS-active devices.\textsuperscript{13-14, 25, 58} The DMAB formation happens at even low laser powers and severely complicates the quantitative applicability of ATP because the Raman cross section of DMAB is inherently larger than ATP.\textsuperscript{33}

Regardless of the complication of the SPR catalyzed dimerization of ATP, the molecule is still commonly used for quantifying the EF of both AgNW and AgNP based SERS devices. Therefore, the molecule was continued to be used as the reporter molecule for the characterization of the AgNW and dual-nanostructured surfaces. To reduce the influence of the dimerization on the analysis, a laser power of 5 mW and an integration time of 1 second was used for the remainder of the acquisitions. In addition, the XYZ stage controller with micrometer control was implemented to remove manual sampling variation from influencing the dataset.

To determine the effects of the dimer formation in the system, a 5 μl aliquot of the 423.5 μM ATP standard was deposited on a NWS. First, the slide was mounted to the stage and the NWS was brought into focus. The laser spot was then rapidly moved to a new location that had no previous laser expose and 2000 spectra were collected.
Figure 46. The intensity of the peaks at 1084, 1150, 1190, 1400, and 1438 cm$^{-1}$ as a function of laser exposure time.

Figure 47. The intensity of the peaks at 1584 and 1438 cm$^{-1}$ as a function of laser exposure time.
consecutively. The intensity of relevant peaks as a function of time are shown in Figure 46 and Figure 47. The 1084 cm\(^{-1}\) peak is a combination of the overlapping spectral bands of ATP and DMAB due to a vibration of their phenyl rings. The intensity of the 1084 cm\(^{-1}\) peak therefore represents a combination of ATP and DMAB contributions. The 1150, 1190, 1400, and 1438 cm\(^{-1}\) peaks are associated with spectral bands that are unique to DMAB vibrations.\(^{33}\) The second phenyl ring vibration of ATP appears at 1600 cm\(^{-1}\) but is found at 1584 cm\(^{-1}\) for DMAB. The comparison of these two intensities can be used to partially differentiate their individual contribution. However, the differentiation is far from quantitative since in the SERS spectra they overlap each other and typically appear as one broad peak.

In the initial spectrum, t=0, the 1084 and 1600 cm\(^{-1}\) peaks are the most intense, suggesting that the SERS signal is predominantly generated by ATP. However, the presence of the other DMAB associated peaks at t=0 suggests that the dimerization occurs faster than the 1 second acquisition time; even at a laser power of 5 mW. The spectral bands unique to DMAB (1150, 1190, 1400, and 1438 cm\(^{-1}\)) rapidly rise in intensity and the 1438 cm\(^{-1}\) peak becomes the most intense peak in just under 500 seconds. Interestingly, the growth of the peak at 1190 cm\(^{-1}\) occurs much slower than the other bands unique to DMAB. The intensity at 1084 cm\(^{-1}\) also increases much slower and parallels the growth pattern of the 1190 cm\(^{-1}\) peak.

Both DMAB and ATP exhibit a phenyl ring vibration at 1084 cm\(^{-1}\), however as the dimerization occurs the number of phenyl rings that produce the signal does not change. Therefore, the increase in the intensity at 1084 cm\(^{-1}\) shows that DMAB has a significantly larger SERS cross section than ATP. Without further investigation, the
reason for the larger SERS cross section can only be speculated. Presumably, the increased length of the conjugated system may redshift the excitation of the $\pi$ electrons, resulting in an increased resonance Raman effect. In addition, DMAB exhibits a bidentate thiol binding motif. With both thiols bound to one nanostructure, this may optimize the orientation of the molecule to surface for SERS enhancement. Alternatively, if the thiols bind two separate nanostructures, the DMAB would bridge a nanometer size gap between the surfaces; an idealized SERS hotspot.

The 1584 cm$^{-1}$ and 1600 cm$^{-1}$ spectral bands of DMAB and ATP respectively actually appear as one peak that broadened as the time series progressed. At $t=0$, the peak exhibited a maximum at 1600 cm$^{-1}$ and it continually shifted to a lower frequency until it reached 1584 cm$^{-1}$ at 120 seconds. Past $t=120$, the peak maximum stayed at 1584 cm$^{-1}$, indicating that the broadening and shifting maximum was due to the dimerization of ATP to DMAB. This was also supported by the growth of the 1584 cm$^{-1}$ peak, which followed the pattern of the other peaks unique to DMAB. The 1600 cm$^{-1}$ peak still experienced growth because of the contributions of the overlap with 1584 cm$^{-1}$ peak and DMAB’s large SERS cross section. Overall, the time series exemplifies the complications that occur as ATP dimerizes to DMAB.

DUAL-NANOSTRUCTURE SERS

The number of hotspots in the DNS should be significantly higher than the NWS, due to the incorporation of AgNPs that are much smaller in relation to the spot size of the laser. Therefore, the DNS should give a more uniform SERS response in relation to spatial variations in sampling. To demonstrate this, SERS spectra were taken every 500
μm along a 1 cm linear path on a NWS and a DNS. The distance ensures that the sampled areas are sufficiently far enough away from each other to avoid preemptive laser exposure. This minimizes the influence of the dimer formation between measurements. A 10 μl aliquot of a 0.5 μM solution of ATP was used and resulted in a spot size of 25.1 mm. Figure 48 and Figure 49 depicts the SERS spectra with the z-axis divisions normalized to keep the height of the images in scale relative to one another.

The SERS response of the NWS is highly dependent on the position that is sampled. In each spectrum, the most intense peak was consistently the 1440 cm\(^{-1}\) band associated with DMAB. As seen from Figure 48, the intensity of the spectra fluctuates drastically from location to location. As the distance was sampled, some areas did not exhibit any visible SERS activity. This behavior is most evident in the first 7 spectra, traveling from 0.00 to 0.30 cm. Following the 1600 cm\(^{-1}\) peak, the intensity decreases until the disappearance of the signal at 0.15 cm. The intensity then increases again to a local maximum at 0.35 cm. As discussed previously, the inconsistent spatial response of the NWS is due to the length of the AgNWs. Their length is comparable to the spot size of the laser, limits the number of SERS hotspots, and inherently prevents the ideal orientation of the hotspots.

As seen from Figure 49, the SERS response of the DNS retains spatial fluctuations in the intensity of the acquired spectra. This is expected because the variation due to the underlying AgNW layer should remain present in the DNS. However, the consistent presence of a SERS response in seen in every sampled location. Unlike the NWS, the 1084 cm\(^{-1}\) peak was the most intense. This feature suggests that the either the rate of dimerization is slower for the AgNPs or that the...
Figure 48. The SERS spectra acquired on the NWS over a spatial distance of 1 cm.

Figure 49. The SERS spectra acquired on the DNS over a spatial distance of 1 cm.
significantly higher surface area of available silver resulted in an increased likelihood of isolated ATP molecules.

As seen from Figure 49, the SERS response of the DNS retains spatial fluctuations in the intensity of the acquired spectra. This is expected because the variation due to the underlying AgNW layer should remain present in the DNS. However, the consistent presence of a SERS response in seen in every sampled location. Unlike the NWS, the 1084 cm$^{-1}$ peak was the most intense. This feature suggests that the either the rate of dimerization is slower for the AgNPs or that the significantly higher surface area of available silver resulted in an increased likelihood of isolated ATP molecules.

Figure 50 compares the RSD of each set of spectra. In the spectral region where relevant vibrational bands are present, the NWS has a value close to 1 in every location except near the 1084 cm$^{-1}$ band. The DNS maintains a RSD under 0.5, showing that the DNS produces a significantly lower variation in the SERS signal. As discussed previously, the DNS should exhibit is a consistent signal with an overlaid variable signal, due to the relative sizes of the AgNPs and AgNWs to the laser spot respectively. This is supported by the behavior of the 1084 cm$^{-1}$ band, however the conclusion is only tentative due to the complication of the dimerization of ATP. For the 1084 cm$^{-1}$ peak, the average intensity was $801 \pm 501$ and $4587 \pm 1343$ counts for the NWS and DNS respectively and the error in the values represents the STD. Interestingly, the STD of the DNS is comparable in magnitude to the intensity of the average intensity of the NWS.
Table 2. The analyte spot diameter for each standard on the NWS and DNS.

<table>
<thead>
<tr>
<th>Surface</th>
<th>Standard [ATP]/μM</th>
<th>Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>847</td>
<td>423.5</td>
</tr>
<tr>
<td>NWS</td>
<td>20.4 mm</td>
<td>17.2 mm</td>
</tr>
<tr>
<td></td>
<td>84.7</td>
<td>42.35</td>
</tr>
<tr>
<td></td>
<td>19.8 mm</td>
<td>18.6 mm</td>
</tr>
<tr>
<td></td>
<td>18.7 mm</td>
<td>19.7 mm</td>
</tr>
<tr>
<td></td>
<td>19.0 mm</td>
<td>18.7 mm</td>
</tr>
<tr>
<td>DNS</td>
<td>18.7 mm</td>
<td>18.0 mm</td>
</tr>
<tr>
<td></td>
<td>8.47</td>
<td>4.235</td>
</tr>
<tr>
<td></td>
<td>18.2 mm</td>
<td>17.9 mm</td>
</tr>
<tr>
<td></td>
<td>18.2 mm</td>
<td>14.3 mm</td>
</tr>
<tr>
<td></td>
<td>0.847</td>
<td>19.1 mm</td>
</tr>
</tbody>
</table>

Figure 50. The RSD of the two sets of spectra from the NWS and the DNS acquired over a distance of 1 cm.
The surfaces were tested for a concentration-dependent SERS response to ATP by using standards of various ATP concentrations. Standards of 847, 423.5, 84.7, 42.35, 8.47, 4.235, and 0.847 μM ATP in ethanol were prepared. The SERS spectra were acquired for each concentration using a NWS and a DNS, both fabricated on the same slide. This was done to reduce the effect of inter-slide variation. For each standard, 5 μl of the analyte was dropped onto the surface and the diameter of the resulting spot was measured. The analyte spot sizes of the series are shown in Table 2.

The sampling method of each surface type was slightly different due to the qualities of the respective surface respectively. Since the SERS response of the NWS is spatially variable and sometimes nonexistent, the SERS spectra were sampled by moving the laser spot along either the X or Y axis until a SERS-active spot was encountered. The position along the axis was optimized to maximize the intensity and then the other axis was optimized. This was done to separate the effects of spatial variability from concentration dependence as much as possible. However, the process is slow compared to the rapid rate of dimer formation so the time that any given sampled location was exposed to the laser could not be controlled. Therefore, each spectrum was collected once per location as soon as the optimal position was reached. To prevent sampling a location more than once and simultaneously avoid sampling the edges, this process was done 5 times along the X axis, 5 times along the Y axis, and then 5 times along the X axis again.

The stable existence of a SERS response eliminated the necessity to optimize the location with the DNS. This allowed each location to be sampled as soon as an arbitrary distance was moved, affording control over the amount of exposure to laser
radiation for the acquired spectra. One spectrum was taken as soon as the laser moved to the sampling area and a second spectrum was acquired 30 seconds later. The time point was chosen as an estimate for the amount of time the spatial optimization took for the NWS sampling, however the large variability in the latter excludes their direct comparison. The instantaneously acquired spectrum and the spectrum acquired after 30 seconds, referred to as DNS-1 and DNS-30 respectively, were treated separately. The surface was sampled in 5 locations along an X axis of movement and then 5 location along the Y axis of movement.

The 847 μM standard was not sampled properly for the DNS because the large influence of the dimer formation was unknown at the time of the experiment. The standard was the first slide sampled, and the large effect of the dimer formation was observed on the second sampled slide. The time series experiment presented previously was performed on the second slide, where an experimental anomaly resulted in the observation. Unknown at the time, a bubble formed during the deposition of the standard, resulting in a significantly smaller diameter spot. The spot was 11.5 mm in diameter while the average diameter was 18.5 mm for all other trials. In addition, the bubble resulted in a secondary coffee stain effect in the internal region, preventing an appropriate sampling region. Together, this resulted in a much more intense signal. The increased intensity allowed the variation in peak growth rates to be visually noticeable during the optimization of the position. The slide was then used to perform the time course experiment and the only extra slide prepared under identical conditions was used to repeat the 423 μM standard. The 847 μM standard slide could not be resampled since the position of sampling points was only known relative to the original position.
Therefore, the laser exposed areas from the first experiment were unknown and could potentially bias its resampling.

The 15 NWS, the 10 DNS-1, and DNS-30 spectra were averaged separately, background corrected using a custom LabVIEW program, and the resulting spectra are shown in Figures 51, 52, and 53 respectively. The NWS spectra in Figure 51 was effectively corrected, however the DNS spectra in Figure 52 and 53 still display a significant background. The DNS-30 spectra display the most intense SERS response, which is expected due to the formation of DMAB. Together, the spectra exhibit a range of spectral features that show the presence of ATP and DMAB.

Figure 51 shows that the 1084 cm\(^{-1}\) peak is the most intense peak for the NWS SERS spectra. The 847 \(\mu\)M standard clearly shows two distinct peaks with almost equal intensity for ATP and DMAB at 1584 and 1600 cm\(^{-1}\). The 1600 cm\(^{-1}\) peak of the 423.5 \(\mu\)M standard was significantly more intense than the 1584 cm\(^{-1}\) peak. For the 84.7 \(\mu\)M standard, the dominance of the 1600 cm\(^{-1}\) peak continued but is not as intense. At all lower concentrations of ATP, the region appears as a broad, single peak and the maximum lowers in frequency towards 1584 cm\(^{-1}\).

Unlike the NWS, the DNS-1 spectra in Figure 52 exhibit 1440 cm\(^{-1}\) as their most intense peak. In addition, even at high concentrations only one broad peak with a maximum at 1584 cm\(^{-1}\) is present in the ~1600 cm\(^{-1}\) region. However, by comparing to DNS-30 in Figure 53, DNS-1 spectra do exhibit a significant shoulder at 1600 cm\(^{-1}\). This suggests that the DNS may display a larger portion of DMAB signal due to the increased number of hotspots instead of the faster dimerization of ATP.
Figure 51. The SERS spectra of ATP at various concentrations for the NWS. Each spectrum is the average of 15 spectra sampled at different positions.

Figure 52. The SERS spectra of ATP at various concentrations for the DNS-1. Each spectrum is the average of 10 spectra sampled at different positions.
Figure 53. The SERS spectra of ATP at various concentrations for the DNS-30. Each spectrum is the average of 10 spectra sampled at different positions.
The effects of the dimerization are also seen through the STD and RSD of each set of spectra. Figure 54 and 55 show the STD and RSD respectively of the 847 μM standard on the NWS and the DNS. The 847 μM standard was not included in Figure 52 or 53 because it was not sampled appropriately and therefore could not be compared to either set. At 1084 cm\(^{-1}\) the STD of the NWS is higher than the DNS. At 1584 cm\(^{-1}\), they have almost identical STDs while they differ heavily at 1600 cm\(^{-1}\). The NWS displays another local maximum in its STD while the STD of the DNS is rapidly dropping. Together, this indicates that the amount of dimer formation contributing to the SERS signal is much more variable for the NWS compared to the DNS. This is supported by the RSDs of each surface, shown in Figure 55. The RSD of the DNS is consistently lower than the NWS and has local minima at 1084 and 1600 cm\(^{-1}\). This indicates that the large STD values of the DNS are primarily from the poor background correction and that the DNS has less variation in the SERS signal from dimerization events.

The STD and RSD of the 423.5 μM standard spectra are shown in Figure 56 and 57 respectively. The STD of the DNS-1 and DNS-30 spectra are larger than the NWS, which is expected due to their much larger intensities. However, each sample set displays different peak shapes in the ~1600 region of Figure 56. The STD at 1584 and 1600 cm\(^{-1}\) is almost equal for the NWS while both DNS-1 and DNS-30 display a larger STD at 1584 than 1600 cm\(^{-1}\). In addition, the DNS-1 set displays a much more prominent shoulder at 1600 cm\(^{-1}\) compared to DNS-30. Unlike Figure 55, Figure 57 shows similar values for the RSD of the NWS compared to the DNS at 1084 and 1600 cm\(^{-1}\).
Figure 54. The STD of the 2 sets of spectra for the 847 μM standard.

Figure 55. The RSD of the 2 sets of spectra for the 847 μM standard.
Figure 56. The STD of the 3 sets of spectra for the 423.5 μM standard.

Figure 57. The RSD of the 3 sets of spectra for the 423.5 μM standard.
Figure 58. The STD of the 3 sets of spectra for the 84.7 μM standard.

Figure 59. The STD of the 3 sets of spectra for the 42.35 μM standard.
Figure 60. The STD of the 3 sets of spectra for the 8.47 μM standard.

Figure 61. The RSD of the 3 sets of spectra for the 84.7 μM standard.
Figure 62. The RSD of the 3 sets of spectra for the 42.35 μM standard.

Figure 63. The RSD of the 3 sets of spectra for the 8.47 μM standard.
Figure 58, 59, and 60 show the STD of the 84.7, 42.35, and 8.47 μM standards respectively. As the concentration of ATP is lowered, the STD of the 1600 cm\(^{-1}\) peak lowers in comparison to the 1584 cm\(^{-1}\) peak for each data set. Figure 61, 62, and 63 show the RSD of the 84.7, 42.35, and 8.47 μM standards respectively. At 84.7 μM, DNS-1 and DNS-30 display local maxima at 1084 cm\(^{-1}\) but no corresponding event in the ~1600 cm\(^{-1}\) region. At 42.35 μM, the local maxima at 1084 cm\(^{-1}\) also disappears. Unlike DNS-1 and DNS-30, the NWS continues to display a local minimum in the RSD at 1600 cm\(^{-1}\) for the 84.7, 42.35, and 8.47 μM standards. This supports that the DNS has a larger portion of its SERS signal generated from DMAB, regardless of the laser exposure.

Figure 64 shows the SERS response as a function of concentration using the intensity at 1084 cm\(^{-1}\), which is associated with both ATP and DMAB spectral bands. Due to the inability of the custom background correction to eliminate the background from the AgNPs, the 1084 cm\(^{-1}\) peak intensities were manually corrected at each concentration for DNS-1 and DNS-30. Figures 71 and 72 in the appendix show the points and regressions used for the two sets of spectra respectively. The error bars represent the STD of the intensity. The NWS was found to have a linear relationship with the log of the concentration of ATP while the DNS exhibited an exponential relationship. The equations for the intensity as a function of concentration dependence of each surface are shown in Figure 64. The linear relationship of NWS has a R\(^2\) value of 0.9404, while the exponential relationships of the DNS-1 and DNS-30 have R\(^2\) values of 0.9872 and 0.9822 respectively. The lower fit of the NWS regression was unexpected due to the very similar RSD of the NWS to the DNS at this spectral region. Upon the
Figure 64. The quantitative intensity dependence of the SERS signal at 1084 cm\(^{-1}\) on the logarithm of the ATP concentration. The regression equations appear in the same order as the legend.

\[
\begin{align*}
y &= 970.37x - 144.7 \\
R^2 &= 0.9404 \\
y &= 88.131e^{1.5995x} \\
R^2 &= 0.9872 \\
y &= 58.295e^{2.0887x} \\
R^2 &= 0.9822
\end{align*}
\]

Figure 65. The quantitative intensity dependence of the SERS signal at 1084 cm\(^{-1}\) on the logarithm of the ATP concentration after normalization to the area of the spot size. The regression equations appear in the same order as the legend.

\[
\begin{align*}
y &= 337.38x - 46.959 \\
R^2 &= 0.9879 \\
y &= 37.886e^{1.5695x} \\
R^2 &= 0.9892 \\
y &= 25.06e^{2.0587x} \\
R^2 &= 0.9784
\end{align*}
\]
examination of the data from the 847 and 423.5 μM standards, which deviated from the regression the most, they were found to also have the largest deviation from the diameter of the deposited spot. The average of the standard solution spot sizes for the NWS was 19.1 mm. The 847 μM standard had a spot size of 20.4 mm and resulted in a lower intensity than predicted by the regression. The 423.5 μM standard had a spot size of 17.2 mm and resulted in a higher intensity than predicted. To remove the variation of the spot size from the data set, the intensity of the peaks was normalized to the area of the spot size.

The area normalized SERS response as a function of concentration is displayed in Figure 65. The error bars represent the STD of the intensity normalized to the area of the spot size. The equations for the intensity normalized for spot size as a function of concentration for each surface is shown in Figure 65. The corrected R² values are 0.9879, 0.9892, and 0.9784 for the NWS, the DNS-1, and the DNS-30 respectively. The R² value of NWS increased by 0.0475, while the R² value of DNS-1 and DNS-30 increased by 0.0020 and decreased by 0.0018 respectively. The significantly larger improvement of the R² value of the NWS suggests that it is much more susceptible to variations in the spot size during an analysis. After accounting for the spot size, the NWS, DNS-1, and DNS-30 show highly quantitative relationships between the SERS intensity of the 1084 cm⁻¹ peak and the log of the concentration of ATP. The performance of the DNS surface exceeds the performance of the NWS, with both DNS-1 and DNS-30 displaying significantly higher intensities and DNS-1 displaying a higher R² value.
Figure 66 shows the SERS response as a function of concentration using the intensity at 1584 cm\(^{-1}\), which is the peak intensity of the DMAB spectral band. The 1084 cm\(^{-1}\) peak intensities were manually corrected at each concentration for DNS-1 and DNS-30. Figures 73 and 74 in the appendix show the points and regression used for the two sets of spectra respectively. The error bars represent the STD of the intensity and the equations for the intensity as a function of concentration dependence of each surface are shown in Figure 66. The linear relationship of NWS has a R\(^2\) value of 0.8628, while the exponential relationships of the DNS-1 and DNS-30 have R\(^2\) values of 0.8611 and 0.9575 respectively.

The area normalized SERS response as a function of concentration is displayed in Figure 67. The error bars represent the STD of the intensity normalized to the area of the spot size. The equations for the intensity normalized for spot size as a function of concentration for each surface is shown in Figure 67. The corrected R\(^2\) values are 0.9825, 0.9119, and 0.9859 for the NWS, the DNS-1, and the DNS-30 respectively. The R\(^2\) value of NWS increased by 0.1197, while the R\(^2\) value of DNS-1 and DNS-30 increased by 0.0508 and 0.0284 respectively. Again, the increase in R\(^2\) value was larger for the NWS, supporting that it is more susceptible to variations in the spot size during an analysis. However, the spot size correction increased the R\(^2\) value by a much larger amount and increased the R\(^2\) value for both the DNS-1 and DNS-30 significantly. Together, this indicates that the influence of the dimer formation is attenuated significantly through variations in the spot size of the analyte. The larger R\(^2\) value (0.9859 vs 0.9119) and smaller increase in R\(^2\) value (0.0284 vs 0.0508) of the DNS-30 compared to the DNS-1 is due to the significantly lower portion of dimer formation.
Figure 66. The quantitative intensity dependence of the SERS signal at 1584 cm$^{-1}$ on the logarithm of the ATP concentration. The regression equations appear in the same order as the legend.

Figure 67. The quantitative intensity dependence of the SERS signal at 1584 cm$^{-1}$ on the logarithm of the ATP concentration after normalization to the area of the spot size. The regression equations appear in the same order as the legend.
The increased exposure to laser radiation results in more ATP dimerization and therefore less spectral characteristics of DMAB is expected in DNS-1. After accounting for the spot size, the NWS and the DNS-30 show highly quantitative relationships between the SERS intensity of the 1584 cm\(^{-1}\) peak and the log concentration of ATP. The performance of the DNS-30 exceed the performance of the NWS by displaying significantly higher intensities with comparable R\(^2\) values.

Figure 68 shows the SERS response as a function of concentration using the intensity at 1600 cm\(^{-1}\), which is the peak intensity of the ATP spectral band. The 1600 cm\(^{-1}\) peak intensities were manually corrected at each concentration for DNS-1 and DNS-30. Figures 73 and 74 in the appendix show the points and regression used for the two sets of spectra respectively. The error bars represent the STD of the intensity and the equations for the intensity as a function of concentration dependence of each surface are shown in Figure 68. The linear relationship of NWS has a R\(^2\) value of 0.9724, while the exponential relationships of the DNS-1 and DNS-30 have R\(^2\) values of 0.8488 and 0.9688 respectively.

The area normalized SERS response as a function of concentration is displayed in Figure 69. The error bars represent the STD of the intensity normalized to the area of the spot size. The equations for the intensity normalized for spot size as a function of concentration for each surface is shown in Figure 69. The new R\(^2\) values are 0.9586, 0.8803, and 0.9574 for the NWS, the DNS-1, and the DNS-30 respectively. The R\(^2\) value of NWS decreased by 0.0138, while the R\(^2\) value of DNS-1 and DNS-30 increased by 0.0045 and decreased by 0.0114 respectively. Contrary to the two previously examined peaks, the NWS and the DNS-30 both decrease in R\(^2\) value with
Figure 68. The quantitative intensity dependence of the SERS signal at 1600 cm\(^{-1}\) on the logarithm of the ATP concentration. The regression equations appear in the same order as the legend.

\[ y = 589.74x - 168.93 \quad R^2 = 0.9724 \]
\[ y = 90.402e^{1.0844x} \quad R^2 = 0.8848 \]
\[ y = 95.462e^{1.2503x} \quad R^2 = 0.9688 \]

Figure 69. The quantitative intensity dependence of the SERS signal at 1600 cm\(^{-1}\) on the logarithm of the ATP concentration after normalization to the area of the spot size. The regression equations appear in the same order as the legend.

\[ y = 207.25x - 58.923 \quad R^2 = 0.9586 \]
\[ y = 39.351e^{1.0388x} \quad R^2 = 0.8803 \]
\[ y = 41.037e^{1.2203x} \quad R^2 = 0.9574 \]
comparable values. The $R^2$ value of the DNS-1 did increase, however the increase was small and the final $R^2$ value of 0.8803 was still low. Unlike the other peaks discussed, the quantitative relationships between the SERS intensity of the 1600 cm$^{-1}$ peak and the log concentration of ATP suffered from being normalized to the spot size. This suggests that the peak most unique to ATP has a poor quantitative relationship to the analyte. Regardless, the performance of the DNS-30 exceeded the NWS with an increased intensity and comparable $R^2$ values.

Together, the quantitative relationship of the 1084, 1584, and 1600 cm$^{-1}$ peaks and their behavior in response to the area normalization of the analyte spot size demonstrate the complications that arise using ATP as a reporter molecule. Referring to Equation 4, the EF was calculated for each surface at each peak using the 423.5 mM standard. The results are summarized in Table 3. From the EF values, the influence of the dimer formation on the SERS response is again apparent. Since the EF consistently increased when calculated using DNS-30 compared to DNS-1, the formation of the dimer heavily interferes with the accurate determination of an EF. In addition, EFs calculated from DNS-30 were much higher for the 1084 and 1584 cm$^{-1}$ peaks compared to the 1600 cm$^{-1}$ peak. These are associated with an overlapping spectral band of DMAB with ATP and a spectral band of DMAB respectively, and therefore experience an artificial inflation due to the dimerization of ATP. The presence of DMAB spectral features in all the acquired spectra therefore makes the EF unreliable, however the surfaces all exhibit EF on the order of at least $10^5$. 
Table 3. The EF calculated for each surface using various peaks.

<table>
<thead>
<tr>
<th>Peak/cm$^{-1}$</th>
<th>EFx10$^5$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NWS</td>
</tr>
<tr>
<td>1084</td>
<td>2.2</td>
</tr>
<tr>
<td>1584</td>
<td>1.5</td>
</tr>
<tr>
<td>1600</td>
<td>2.2</td>
</tr>
</tbody>
</table>
CHAPTER V
CONCLUSIONS

The dual-nanostructured SERS surface utilizing AgNPs and AgNWs was shown to overcome the shortcomings of the device characteristics of either nanostructure individually. The AgNPs were produced by the decomposition a shelf-stable Ag complex from the literature. The synthetic method for producing AgNWs with lengths of ~10 μm and diameters of ~100 nm in a high purity was optimized for the large-scale production.

The formation of a NPS through the decomposition of the Ag complex was shown to have poor processing characteristics. The direct decomposition upon deposition eliminates the internal coffee stain effects, however they are still present on the outer edges of the surfaces.

The AgNWs were shown to have excellent processing characteristics, as macroscale uniform coats are easily performed using Mayer rod coating. However they exhibit poor SERS characteristics for a quantitative SERS device, due to the large spatial variation in the number of SERS hotspots relative to the spot size of the laser. The AgNWs were shown to be effective substrates to facilitate the Mayer rod coating of an aqueous solution of the Ag complex without the use any additives.

Together, the feasibility of producing the dual-nanostructured surface through R2R was demonstrated as a proof of concept using a two-step Mayer rod coating method. The limitations of performing the process by hand are easily addressed on a large-scale production line, and a two-step Mayer rod coat and dropcasting method was used to produce a small-scale DNS for the characterization of the SERS response. The
DNS exhibits a larger spatial uniformity in the SERS response compared to the NWS alone with a significant increase in intensity.

A quantitative relationship between the intensity per cm$^2$ of the analyte spot size and the Log[ATP] was shown for both the NWS and DNS at 1084, 1584, and 1600 cm$^{-1}$. The NWS exhibits a linear relationship while the DNS exhibits an exponential relationship. The complications associated with the use of ATP as a quantitative model and a SERS EF reporter molecule were demonstrated by comparing the NWS to DNS with two different times of laser exposure. The EF was calculated to be 2.2x10$^5$ and 4.3x10$^5$, however the values are considered unreliable due to the rapid, light catalyzed dimerization of ATP to DMAB.$^{33}$ The use of ATP was continued in order to obtain EFs that are comparable to existing literature.
REFERENCES


Figure 70. The third-order polynomial regression used to correct for the background at the 1084 cm\(^{-1}\) peak in the SERS spectrum from NPS-H.

Figure 71. The points and linear regressions used to correct the intensities of the 1084 cm\(^{-1}\) peaks of DNS-1.
Figure 72. The points and linear regressions used to correct the intensities of the 1084 cm$^{-1}$ peaks of DNS-30.

Figure 73. The points and linear regressions used to correct the intensities of the 1584 and 1600 cm$^{-1}$ peaks of DNS-1.
Figure 74. The points and linear regressions used to correct the intensities of the 1584 and 1600 cm\(^{-1}\) peaks of DNS-30.
VITA

Kory Brian Castro

Department of Chemistry and Biochemistry
Old Dominion University
4541 Hampton Blvd.
Norfolk, VA 23529
Email: KCastro@odu.edu

Education

M.S. in Chemistry
Old Dominion University, Norfolk
Department of Chemistry and Biochemistry
4541 Hampton Blvd.
Norfolk, VA 23529
Graduation: May 2019

B.S. in Chemistry, Biochemistry, and Biology
Old Dominion University, Norfolk
Department of Chemistry and Biochemistry
4541 Hampton Blvd.
Norfolk, VA 23529
Graduation: May 2015

Experience

Graduate Teaching Assistant
August 2016 – May 2019
Old Dominion University, Norfolk
4541 Hampton Blvd.
Norfolk, VA 23529

Instrument Technician
August 2017 – May 2019
Old Dominion University, Norfolk
4541 Hampton Blvd.
Norfolk, VA 23529

The word processor for this thesis was Microsoft Word 2016. All graphs were generated by Microsoft Excel 2016 or Labview 2017. Molecular structures were drawn using ChemDraw 17.0.