Engineering Noble-Metal Nanoparticles for Sensing and Imaging with Surface-Enhanced Raman Scattering

Matthew Rycenga
Washington University in St. Louis

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WASHINGTON UNIVERSITY

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ENGINEERING NOBLE-METAL NANOPARTICLES FOR SENSING AND IMAGING WITH SURFACE-ENHANCED RAMAN SCATTERING

By

Mathew Rycenga

A dissertation presented to the Graduate School of Arts and Sciences of Washington University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

May 2011
Saint Louis, Missouri
ABSTRACT OF THE DISSERTATION

Engineering Noble-Metal Nanoparticles for Sensing and Imaging with Surface-Enhanced Raman Scattering

by
Matthew Rycenga

Doctor of Philosophy in Biomedical Engineering
Washington University in St. Louis, 2011

Professor Younan Xia, Chairperson

This research investigated the surface-enhanced Raman scattering (SERS) from Ag and Au nanoparticles with an aim to better understand the SERS mechanism and to implement this technique for single-molecule detection and imaging. In addition, SERS was used as a sensitive probe to study molecules confined to a nanoparticle’s surface.

The first part of this work focused on measuring the SERS from different Ag and Au nanoparticles and determining how their structural and physical properties affect SERS. The effects of shape, size, and Au-Ag composition on SERS are determined using Ag nanocubes, Ag nanospheres, and Au-based nanocages. I also demonstrate several techniques used to study the SERS of single nanoparticles, one at a time, which has provided significant insight into the SERS effect. I then discuss the development of a new and simple way to create large SERS enhancements by taking advantage of the supporting substrate of a nanoparticle. In this technique, simply depositing a single Ag nanocube on a metal substrate can increase its SERS enhancements to levels capable of single-molecule detection.
In the second part of this work I used SERS as a molecular probe to understand how glucose molecules interact at a nanocube’s surface, and as an optical thermometer to quantify the temperature change at the surface of a Au-based nanocage during the photothermal effect. The nanoparticles were coated with highly ordered self-assembled monolayers (SAMs), and then SERS was used to determine the structural and conformational changes in the SAMs as a result of perturbations from the environment. This allowed me to use SERS to directly probe the molecules on the nanoparticle’s surface.

In the final part of this work, I used nanocubes and nanospheres in SERS imaging. The resolution, sensitivity, and penetration depth are determined for our Raman microprobe system. In addition, phantoms are used to generate SERS images of three-dimensional microstructures.
ACKNOWLEDGEMENTS

I would like to express my appreciation to my advisor Professor Younan Xia for his guidance over these years. His knowledge and broad understanding of science have been a vital resource to me, and I will miss his ability to simplify the complex problems and ideas I often bring to him. I would also like to thank Dr. Dong Qin, who has on many occasions pointed me in the right direction and encouraged me to be a leader. I am also very thankful to Dr. Joseph McLellan who introduced me to surface-enhanced Raman Scattering. I would like to recognize Dr. Pedro Camargo, Dr. Claire Cobley, Xiaohu Xia, Weiyang Li and Christine Moran for all their help and collaborations for which I am truly grateful. I would like to express my acknowledgement and appreciation to all past and current members of the Xia group, in particular Dr. Byungkwon Lim, Majiong Jiang, Young Hwan Lee, Dr. Unyong Jeong, Dr. Yujie Xiong, Dr. Jingyi Chen, Dr. Eric Formo, Dr. Eun Chul Cho, Dr. Jingwei Xie, Dr. Ben Wiley, Xiaoran Li, Dr. Alex Briseno, Maureen McKiernan, Dr. Meng-Yi Bai, Dr. Sung-Wook Choi, and Dr. Hirokazu Kobayashi. I would like to express my gratitude to Professor Frank Yin for his help during my transfer to Washington University and all the staff in the BME department, in particular, Glen Reitz, Lori Williams and Karen Teasdale. I am grateful to Prof. Michael Heinekey and Nectaria Llywelyn from the Chemistry Department at UW for their assistance and help during the beginning of my Ph.D. study.
DEDICATION

To my parents,

Ted and Carole Rycenga
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<tr>
<td>adu</td>
<td>Analog-to-Digital Unit</td>
</tr>
<tr>
<td>AFM</td>
<td>Atomic Force Microscope</td>
</tr>
<tr>
<td>1,4-BDT</td>
<td>1,4-Benzenedithiol</td>
</tr>
<tr>
<td>1-DDT</td>
<td>1-Dodecanethiol</td>
</tr>
<tr>
<td>DDA</td>
<td>Discrete Dipole Approximation</td>
</tr>
<tr>
<td>cw</td>
<td>Continuous Wave</td>
</tr>
<tr>
<td>EDX</td>
<td>Energy Dispersion X-ray</td>
</tr>
<tr>
<td>EF</td>
<td>Enhancement Factor</td>
</tr>
<tr>
<td>E-field</td>
<td>Electromagnetic Field</td>
</tr>
<tr>
<td>EG</td>
<td>Ethylene Glycol</td>
</tr>
<tr>
<td>EM</td>
<td>Electromagnetic Mechanism</td>
</tr>
<tr>
<td>fcc</td>
<td>Face Centered Cubic</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full-Width Half Max</td>
</tr>
<tr>
<td>ICP-MS</td>
<td>Inductively Coupled Plasma Mass Spectroscopy</td>
</tr>
<tr>
<td>$I_{sers}$</td>
<td>The SERS Intensity</td>
</tr>
<tr>
<td>LSP</td>
<td>Localized Surface Plasmon</td>
</tr>
<tr>
<td>LSPR</td>
<td>Localized Surface Plasmon Resonance</td>
</tr>
<tr>
<td>4-MBA</td>
<td>4-Mercapto Benzoic acid</td>
</tr>
<tr>
<td>4-MBT</td>
<td>4-Methyl Benzenethiol</td>
</tr>
<tr>
<td>MD</td>
<td>Molecular Dynamics</td>
</tr>
<tr>
<td>$M_{loc}$</td>
<td>Local E-field Intensity Enhancement</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>$M_{rad}$</td>
<td>Radiation Enhancement</td>
</tr>
<tr>
<td>NC</td>
<td>Nanocube</td>
</tr>
<tr>
<td>NIR</td>
<td>Near-Infrared</td>
</tr>
<tr>
<td>$\mathbf{p}$</td>
<td>Dipole Moment of a Molecule</td>
</tr>
<tr>
<td>PEG</td>
<td>Poly(ethylene glycol)</td>
</tr>
<tr>
<td>$P_{\text{laser}}$</td>
<td>Laser Power</td>
</tr>
<tr>
<td>PSP</td>
<td>Propagating Surface Plasmon</td>
</tr>
<tr>
<td>PT</td>
<td>Photothermal</td>
</tr>
<tr>
<td>PVA</td>
<td>Polyvinyl alcohol</td>
</tr>
<tr>
<td>PVP</td>
<td>Poly(vinyl pyrrolidone)</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolutions Per Minute</td>
</tr>
<tr>
<td>SAM</td>
<td>Self-Assembled Monolayer</td>
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<tr>
<td>SEM</td>
<td>Scanning Electron Microscopy</td>
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<tr>
<td>SERS</td>
<td>Surface Enhanced Raman Scattering</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission Electron Microscopy</td>
</tr>
<tr>
<td>TEOS</td>
<td>Tetraethyl Orthosilicate</td>
</tr>
<tr>
<td>UV-Vis</td>
<td>Ultraviolet-Visible</td>
</tr>
<tr>
<td>$\lambda_{ex}$</td>
<td>Laser Wavelength</td>
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<tr>
<td>$\sigma_{ads}$</td>
<td>Chemical Enhancement</td>
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Chapter 1

Introduction

1.1. Metal Nanostructures

Coinage metals, such as Au, Ag, and Cu, have been important materials throughout history.\(^1\) While in ancient cultures they were admired primarily for their ability to reflect light, their applications have become far more sophisticated with our increased understanding and control of the atomic world. Today, these metals are widely used in electronics, catalysis, and as structural materials, but when they are fashioned into structures with nanometer-sized dimensions, they also become enablers for a completely different set of applications that involve light. These new applications go far beyond merely reflecting light, and have renewed our interest in maneuvering the interactions between metals and light in a field known as plasmonics.\(^2-6\)

In plasmonics, the metal nanostructures can serve as antennas to convert light into localized electromagnetic fields (E-fields) or as waveguides to route light to desired locations with nanometer precision. These applications are made possible through a strong interaction between incident light and free electrons in the nanostructures. With a tight control over the nanostructures in terms of size and shape, light can be effectively manipulated and controlled with unprecedented accuracy.\(^3\) While many new technologies stand to be realized from plasmonics, with notable examples including superlenses,\(^7\) invisible cloaks,\(^8\) and quantum computing,\(^10,11\)
conventional technologies like microprocessors and photovoltaic devices could also be made significantly faster and more efficient with the integration of plasmonic nanostructures.\textsuperscript{[11-14]} Metal nanostructures are also widely regarded as the materials for the next-generation of biomedical technologies, including biomedical imaging, diagnostics, and cancer therapy.\textsuperscript{[3,15]}

1.2. Plasmonics

Plasmonics is related to the localization, guiding, and manipulation of electromagnetic waves beyond the diffraction limit and down to the nanometer length scale.\textsuperscript{[4,6]} The key component of plasmonics is the metal nanostructures, because they support surface plasmon polariton modes, which are electromagnetic waves coupled to the collective oscillations of free electrons in the metal.

While there are a rich variety of plasmonic metallic nanostructures, they can be differentiated based on the plasmonic modes they support: localized surface plasmons (LSPs) or propagating surface plasmons (PSPs).\textsuperscript{[5,16]} In LSPs, the time-varying electric fields associated with the light exerts a force on the gas of negatively charged electrons in the conduction band of the metal and drives them to oscillate collectively. At a certain excitation frequency, this oscillation will be in resonance with the incident light, resulting in a strong oscillation of the surface electrons, commonly known as localized surface plasmon resonance (LSPR).\textsuperscript{[17]} This phenomenon is illustrated in Figure 1.1A. Structures that support LSPRs experience a uniform electric field when excited by light, as their dimensions are much smaller than the wavelength of the light.
In contrast, PSPs are supported by structures that have at least one dimension that approaches the excitation wavelength, as shown in Figure 1.1B. In this case, the excitation field is not uniform across the structure and retardation effects must be considered. In such a structure, like a nanowire for example, surface plasmons propagate back and forth between the ends of the structure generating a standing wave of electron density. Both PSPs and LSPRs can generate intense local electromagnetic fields (E-fields) that can be thousands of times more intense than the incident light.

One of the reasons why so much attention has been paid to metal nanostructures is because their strongly localized and enhanced E-fields can profoundly alter the light-emission properties of nearby molecules in interesting ways. For fluorescent molecules, nanostructures can increase the optical absorption rate by $pE^2$, where $p$ is the dipole moment of the molecule and $E$ is the magnitude of the enhanced local field of a metal nanostructure. These nanostructures can also affect the relaxation of excited molecules back to their ground states by introducing new electromagnetic decay pathways and thus increasing the decay rate. Similarly, for Raman scattering the signals can be enhanced by a factor of $E^4$. While there are numerous applications of LSPRs and their enhanced E-fields, this work is focused on one of the most prominent applications: surface-enhanced Raman scattering (SERS).

### 1.3. Surface-Enhanced Raman Scattering (SERS)

SERS is a fascinating process by which normally weak Raman signals can be amplified by many orders of magnitude. This impressive enhancement is mainly
caused by the enhanced, light-induced E-fields on the surface of a metal nanoparticle caused by excitation of the LSPR.\cite{21, 23, 24} Measured enhancement factors (EFs) range from $10^4$ to $10^{15}$ and even single molecules have been detected with SERS for a dimer of nanoparticles and larger aggregates.\cite{25-28} In addition, SERS provides the unique vibrational spectrum of a molecule, a Raman fingerprint, and does not require labels or other markers. It also does not just infer the presence of a molecule through spectral shifts, but can be used to identify its structure based on the spectroscopic fingerprint. For these reasons SERS is a direct and sensitive technique, and its use in biomedical sensing and imaging has been actively explored over the past decades.\cite{29, 30, 31}

The enormous enhancement in SERS can be attributed the mechanisms shown in Figure 1.2. The first is the electromagnetic enhancement that arises due to the LSPR modes which can focus light into nanosized volumes drastically increasing the local E-field intensity relative to the incident E-field.\cite{32, 33} This is known as the local E-field enhancement ($M_{loc}$). The second is the result of the molecules interacting with the LSPR, which enhances the emission process, and is called the radiation enhancement factor ($M_{rad}$). Together, both these enhancement mechanisms are known as the electromagnetic mechanism (EM). In practice these two mechanisms are typically assumed to be equal which results in the $E^4$ approximation, as shown in Figure 1.2.

The other mechanism is the chemical enhancement ($\sigma_{ads}$), which arises from interactions between the molecule and the nanoparticle that can alter the molecule’s electronic states. This leads to an enhancement from charge transfers between the
molecule and the nanoparticle.\textsuperscript{[34,35]} The EM is typically thought to contribute most of the enhancement ($10^5 - 10^8$) and the chemical enhancement contributes much less ($10 - 10^2$), however, this remains an active area of research.\textsuperscript{[36,37]} The larger contribution of the EM has made this a favorite handle for engineering SERS, and many synthetic methods now exist to create plasmonic nanostructures than focus light into tiny volumes for giant electromagnetic enhancements called “hot spots”. Dimers of nanoparticles and sharp features on nanostructures are excellent examples of the engineering strategies used to form hot spots for SERS applications.

\subsection*{1.4. Applications of SERS}

From an engineering standpoint, SERS is typically implemented using two different strategies. In the first strategy, scientists attempt to capture and identify exogenous molecules at a metal nanoparticle’s surface for ultra-sensitive detection. For this approach many review articles have been written, with a focus on sensitivity and single-molecule detection.\textsuperscript{[30,38]} The second strategy relies on the use of predetermined, endogenous molecules to create SERS tags.\textsuperscript{[39]} These molecules have unique SERS spectra and, due to the narrow widths of Raman bands, are ideal for identifying and imaging many different tags in the same SERS spectrum, a technique commonly known as multiplexing.\textsuperscript{[40,41]} These two strategies have resulted in the use of SERS in a variety of applications. These applications can be organized into three main groups, all of which are discussed in this work, and are shown in Figure 1.3. In the first group SERS is used as a probe to infer the molecular structure and conformation of molecules on metal nanoparticles. In the second group, SERS is used
for the trace-detection of molecules. In the third group, SERS is used as an imaging technique with metal nanoparticles serving as contrast agents. By far, a majority of SERS applications focus on trace-detection. In contrast, the development of SERS as an imaging technique is still in its infancy.

While there are many studies extolling potential application of SERS, there are even more studies concerned with understanding and harnessing the SERS effect. Due to the sensitivity of SERS to small fluctuations in the nanostructure, and the extreme localization of the enhanced E-fields, much of the work with SERS has focused on the fabrication of nanoparticles with reproducible and controllable enhancements.\textsuperscript{[29,42]} Correlating the physical and structural parameters of a nanoparticle with its SERS has advanced both the understanding of SERS and also the techniques used to probe single nanoparticles.\textsuperscript{[43,44]} Characterization of single nanoparticles and dimers has shown that shape, size, composition (Ag vs. Au), and the excitation polarization all affect SERS enhancements.\textsuperscript{[45-47]} Studies have also attempted to probe the hot spot (the region with the highest E-field enhancement) on single nanoparticles and dimers in an attempt to detect single molecules and determine the relative contribution of the hot spot to the SERS enhancement factor.\textsuperscript{[48,49]}

1.5. Scope of this Work

In this dissertation, SERS is implemented as: \textit{i}) a tool to probe molecules, \textit{ii}) a single-molecule detection technique, and \textit{iii}) an imaging technique. Concomitantly, this work aims to better understand the relationship between the properties of metal
nanoparticles and the creation of large SERS EFs. A better understanding of this relationship will ultimately lead to strategies for the design and fabrication of metal nanoparticles capable of strong and reproducible SERS enhancements.

In Chapter 2, I focus on how the shape and Ag/Au composition of metal nanoparticles affects their SERS. Nanoparticles with a cubic shape are compared to nanoparticles with a spherical shape, and theoretical simulations are used to understand the properties of their LSPRs and SERS EFs. In addition, the size of the cubic and spherical nanoparticles is also varied, while their shape is maintained, and the effects of size are discussed. Finally, metal nanoparticles with varying Ag/Au compositions are probed and the effect of increasing the Au content on the SERS intensities is shown.

Chapter 3 and 4 focus on the study of single nanoparticles, one at a time, which ultimately leads to a new technique for generating hot spots. By dispersing single nanoparticles onto a substrate they can be located with microprobe techniques and their SERS recorded. This allows for a close comparison between the structural properties of a single nanoparticle and its SERS EF.

Chapter 5 demonstrates the use of SERS as a molecular probe. A SERS spectrum contains information about the structure and conformation of a molecule. This allows us to probe the conformation of self-assembled monolayers (SAMs) on the surface of Ag nanocubes and Au-based nanocages. The SAMs’ conformation is sensitive to the environment and provides rich information about the surroundings of a metal nanoparticle. We use SERS to monitor the SAMs’ conformation on nanocubes in the presence of aqueous glucose and also to determine the temperature change near Au-
based nanocages.

Chapter 6 covers the characterization and implementation of our Raman microprobe system for use in SERS imaging. The sensitivity, resolution, and penetration depth are determined for aqueous dispersions of nanoparticles and single nanoparticles on a solid support. The importance of nanoparticle aggregation to SERS imaging is demonstrated, and the ability of our system to image a three-dimension phantom is also shown.
Figure 1.1 A schematic illustration of a metal nanosphere excited by the electric field ($E_0$) of incident light with wavevector (k). (A) For nanoparticles smaller than the wavelength of light, their free electrons can be displaced from the lattice of positive ions (consisting of nuclei and core electrons) and collectively oscillate in resonance with the light. In (B) the nanowire has one dimension much larger than the wavelength of light. In this case light coupled to the nanowire will excite the free electrons to create a propagating evanescent wave that can travel along the surface of the nanowire. Note that the wavelength of light is usually much larger compared to a metal nanoparticle, but is drawn here for clarity, and is not strictly to scale.
Figure 1.2 A schematic of the SERS process and the enhancement mechanisms responsible for the high intensities ($I_{\text{sers}}$) of SERS. The SERS intensities are proportional to the number of adsorbed molecules on the metal nanoparticle ($N_{\text{ads}}$), the power of the incident light ($I_i$), the local E-field intensity enhancement ($M_{\text{loc}}$), the radiation enhancement ($M_{\text{rad}}$), and finally the chemical enhancement ($\sigma_{\text{ads}}$). In practice $M_{\text{loc}} = M_{\text{rad}}$ so the SERS intensity is expected to be proportional to the local E-field enhancement raised to the power of four.
Figure 1.3 The three broad areas of application for SERS: for probing the surface or interface of metal nanoparticles, for single-molecule detection, and for imaging and mapping of nanoparticle distributions.
1.6. Notes to Chapter 1


Chapter 2

Effects of Nanoparticle Shape and Composition to SERS

2.1. Introduction

Understanding the optical properties of metal nanoparticles remains one of the most important and fascinating subjects in nanoscience and beyond. One of the well-known properties is the LSPR, which is responsible for the strong absorption and scattering of light by metal nanoparticles.[1] The LSPR is the origin of the bright and unique colors of nanoparticles that can be seen with the naked eye, and has been widely studied resulting in numerous methods for tuning the LSPR wavelength.[2] While less obvious, the strong E-fields generated by the LSPR are also important, but have only recently been studied in an effort to control their distributions and magnitudes. As a result, a nanoparticle’s shape, size, and Au-Ag composition have been primarily used as handles to control the LSPR wavelength. However, these properties hold great promise for the control of local E-fields. In this chapter I explore how these properties affect the distributions and magnitudes of the local E-fields of a nanoparticle and their SERS.

The enhancement of a SERS signal is directly proportional to the E-field enhancements of a metal nanoparticle.[3] It follows that control over the properties of metal nanoparticles that generate large E-fields is vital to the design and implementation of SERS.[4] Theoretical studies have suggested that shape and size can
play important roles in creating large SERS enhancements.\textsuperscript{[5]} In addition, nanoparticles composed of multiple metals (like Au and Ag) are becoming much more common due to their unique properties, but the effects of varying the Au-Ag content on the local E-field intensities is unknown.\textsuperscript{[6]} By carefully comparing the SERS of nanoparticles with these properties, I hope to elucidate their relationship to SERS and their ability to form hot spots.

In the first section, I use suspensions of similarly sized Ag nanocubes and nanospheres to determine the effect of sharp features on SERS. I also calculate the EF from nanocubes and nanospheres for various sizes from 30 to 150 nm. In the second section, I compare the SERS of Au-based nanocages with different Au-Ag compositions. I then discuss how increasing the Au content can result in the attenuation of SERS. In this Chapter, each section explains in detail the synthesis and optical properties of the nanoparticles. This will not be repeated in subsequent Chapters in order to avoid redundancies as these particles are used throughout this work.

### 2.2. Silver Nanocubes and Nanospheres

In this work the nanocubes and nanospheres have a size in the range of 20 to 200 nm. Both nanocubes and nanospheres are single crystalline, meaning that the crystal lattice of Ag atoms in the entire particle is continuous and unbroken to the edges. Figure 2.1 shows typical scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images of the nanocubes and nanospheres used in this experiment.
2.2.1. Synthesis of Silver Nanocubes and Nanospheres

Both Ag nanocubes and nanospheres are synthesized using the polyol process, in which a polyol (a molecule containing multiple hydroxyl groups), such as ethylene glycol (EG), serves as both a solvent and source of reducing agent.\textsuperscript{[7,8]} In a typical procedure, a capping agent, poly(vinyl pyrrolidone) (PVP), and a Ag precursor are injected into pre-heated EG, and the reduction of Ag\textsuperscript{+} ions results in the nucleation and growth of Ag clusters known as seeds. These seeds then grow into Ag nanoparticles. A seed will form one of three predominant structures each with different crystallinity: single crystalline, single twinned, and multiply twinned. The structure the seed takes at this early stage will ultimately determine the shape of the nanoparticle. When multiply twinned seeds are formed, the growth will occur more rapidly at the twin defects of the seed, resulting in the formation of wires with a pentagonal cross section.\textsuperscript{[9]} If single twin seeds are formed, the growth will ultimately lead to right bipyramids.\textsuperscript{[10]} The single crystalline seeds will initially grow into cuboctahedrons with a spherical profile. As additional Ag atoms are added, the corners start to sharpen, resulting in the formation of nanocubes enclosed by (100) side faces.\textsuperscript{[11]}

Reducing AgNO\textsubscript{3} in a polyol reaction in the presence of PVP will result in a mixture of the morphologies described above: pentagonal wires, bipyramids, and cubes. All these nanoparticles are capped primarily by \{100\} facets. This facet selectivity can be attributed to the preferential binding of PVP to the Ag(100) surface.\textsuperscript{[12]} To produce only nanocubes, the twinned seeds that form wires and
bipyramids must be eliminated, leaving only single crystalline seeds in the solution. This is achieved by controlling the type and amount of an oxidative etchant added into the reaction system. In general, single twinned and multiply twinned seeds are more susceptible to oxidative etching due to the presence of defects on their surfaces. The amount of oxidative etchant in a system is typically controlled through the introduction of trace ions like Cl\(^-\). The Cl\(^-\)/O\(_2\) pair will dissolve both multiply twinned and singly twinned seeds, leading to a final product of nanocubes.\(^{[12]}\) Additionally, sulfide (S\(^2^-\)) or hydrosulfide (HS\(^-\)) has been shown to dramatically increase the reduction rate of AgNO\(_3\) making large-scale production of Ag nanocubes considerably easier.\(^{[13]}\) Due to the importance of nanocubes, our group continues to improve the quality and yield of the final product, and currently uniform samples of Ag nanocubes with edge lengths from 30-250 nm can be produced on a scale approaching 0.2 grams per batch.\(^{[14,15]}\)

In contrast to nanocubes, single crystalline Ag nanospheres with good uniformity were not available until only recently.\(^{[16]}\) The synthesis is based on the etching of Ag nanocubes with a wet etchant based on ferric nitrate (FeNO\(_3\)) or a ferricyanide-based etching solution (see Experimental) to truncate the sharp corners and edges of the cubes. Additional etching results in nanospheres without sharp features and with a diameter similar to the edge length of the original cubes. This preferential elimination of sharp edges and corners could be due to their higher surface energy when compared to flat faces. Furthermore, PVP has been shown to cap Ag(100) side faces of cubes more strongly than other facets located at corners and edges. For nanocubes ~50 nm in edge length FeNO\(_3\) can be used to transform them into nanospheres.
However, for larger nanocubes (145 nm in edge length), a more powerful etching solution based on ferricyanide was needed to produce nanospheres. This method is capable of producing nanospheres from 25 to 140 nm in diameter, allowing for accurate optical measurements of Ag nanospheres for the first time.

2.2.2. Optical Properties of Silver Nanocubes and Nanospheres

As discussed in Section 1.2, the optical properties of metal nanoparticles are largely dependent on their LSPR. In Figure 2.2 the extinction spectra of Ag nanocubes (45 and 150 nm in edge length) and nanospheres (44 and 144 nm in diameter) in water are shown. For nanocubes, the LSPR wavelength is typically red-shifted (or is shifted to longer wavelengths) compared to nanospheres of a similar size.\[17,18\] This is shown in Figure 2.2 for both small (~45 nm) and large (~150 nm) nanocubes. In addition, the spectra of the nanocubes contain more peaks compared to the nanospheres. For the smaller cubes, shown in Figure 2.2A, there is a peak at 350 nm along with a strong dipole peak near 437 nm. The spectrum of the spheres only shows one resonance peak at 410 nm.\[17,19\] The additional peaks arise because the lower symmetry of the cube relative to the sphere, making it possible to polarize the electrons in more than one way.\[20\] Figure 2.2 also shows that increasing the size of the nanocubes or nanospheres will result in a red-shift of its main dipolar LSPR.

These spectral shifts can be explained by examining the properties of the LSPR with respect to each nanoparticle’s unique shape and size. For the nanocube, the surface electrons accumulate at the sharp corners increasing charge separation and reducing the restoring force for electron oscillation.\[21-23\] The corners effectively
concentrate charge density, which can dramatically alter the near-fields close to the surface of the nanocube.\textsuperscript{[24-27]} Theoretical calculations suggest this effect could increase the local E-field intensity in these regions by factors up to 2,000.\textsuperscript{[26,28]} This is shown in Figure 2.3, where the E-field enhancements for both nanocubes and nanospheres are plotted using the discrete dipole approximation (DDA) method.\textsuperscript{[29]} The simulation confirms that the E-fields are confined to the corners of the nanocube which greatly increases their intensity relative to the nanospheres.

As the size of the nanoparticle is increased, the charge separation on the nanoparticle increases, leading to a lower frequency (or higher wavelength) for the collective oscillation of electrons. This can explain the red-shift from 410 to 620 nm for the dipole resonance peak of nanospheres, and 437 to 740 nm for the nanocubes as they increase in size, as shown in Figure 2.2.\textsuperscript{[16]} For nanocubes, the relationship between the LSPR peak position (in terms of wavelength) and the edge length is linear, allowing one to conveniently monitor and control the size of Ag nanocubes during a synthesis.\textsuperscript{[30]} In addition, the size also determines the types of LSPRs that can be excited on a nanoparticle. In small particles, typically there is only a dipole LSPR, whereas both dipole and quadrapole LSPRs are possible in larger particles.\textsuperscript{[31]} For the 44 nm nanosphere, there is only one strong, dipole mode at 410 nm whereas the 144 nm sphere shows a quadrapole mode at 433 nm in addition to the dipole mode at 620 nm.

\textbf{2.2.3. A Comparative Study of the SERS for Silver Nanocubes and Nanospheres}
In this section, I determine the SERS EFs of Ag nanocubes and nanospheres. This work aims to corroborate theoretical results that compare smooth nanoparticles to nanoparticles with regions of high surface curvature like the corners of a nanocube. The main goal is to provide experimental evidence to support the argument that creating sharp features on a nanoparticle is a good engineering strategy for increasing the SERS enhancements from a metal nanoparticle.

2.2.3.1. Experimental Setup

Aqueous suspensions of nanocube and nanosphere solutions with known concentrations (particles/mL) were functionalized with 1,4-benzenedithiol (1,4-BDT) and 4-methyl benzenethiol (4-MBT). These particles were then sampled with a Raman microscope while in the solution phase and the SERS spectra from 1,4-BDT and 4-MBT were recorded and analyzed to determine the SERS EF for each type of particle. In SERS experiments, the empirical indicator of the prominence of a specific SERS system is the EF. The EF explicitly denotes the magnitude of the enhancement in a SERS measurement. It is extremely important for applied applications of SERS devices and also for comparison with theory. While there are many ways to define the EF, throughout this chapter it is defined as below:

\[
EF = \frac{I_{sers} \times N_{\text{normal}}}{I_{\text{normal}} \times N_{sers}},
\]

where \( N_{\text{normal}} \) is the number of molecules in the scattering volume for the Raman measurement and \( N_{sers} \) is the number of adsorbed molecules in the scattering volume for SERS. \( I_{sers} \) is the peak area of a band from the SERS measurement and \( I_{\text{normal}} \) is the peak area of the same band from the Raman measurement. A major problem in
measuring the EF is determining $N_{sers}$ or the number of molecules on the nanoparticle’s surface.\cite{31} Without knowing this parameter, interpreting the measured EF can be difficult. To attenuate this problem 1,4-BDT and 4-MBT are used to determine the EF as these molecules bind to metal surfaces in a known way and tend to form a monolayer.\cite{32-35} This was confirmed throughout the SERS measurements by periodically checking for S-H stretching bands (~2550 cm\(^{-1}\)) and S-S stretching bands (~530 cm\(^{-1}\)) as these bands would develop in a multilayer.

The SERS EF that was measured in this study was the average EF experienced by the molecule on a specific nanoparticle. The EFs presented herein are averages for three reasons: \(i\) the nanoparticles have no specific orientation to the polarization of the excitation source; \(ii\) the SERS data originates from the entire surface of the nanoparticle; and \(iii\) the measurement involves many hundreds of nanoparticles. In contrast, in Chapters 3 and 4 single nanoparticles on a support are probed, and the measurements are considered to be more accurate estimates of the EFs.

### 2.2.3.2. Comparison of Shape

The SERS spectra and the average EFs measured for the nanocubes 45 nm in edge length and nanospheres 44 nm in diameter are shown in Figure 2.4 for several Raman bands. Figure 2.4 also shows the expected orientation of the molecules on the nanoparticle’s surface. 1,4-BDT has been shown to form a monolayer with both sulfur groups forming a thiolate bonds on the Ag surface.\cite{36} The benzene ring of the molecule is parallel to the surface of the nanoparticle. In contrast, 4-MBT forms only one thiolate bond is perpendicular to the metal surface.\cite{32} Figure 2.4C shows that the
average EFs are consistently higher for the nanocubes compared with the nanospheres for both molecules by a factor of nearly \( \sim 500 \). This agrees with our previous work comparing nanocubes and truncated nanocubes (cubes with the corners attenuated by etching) and smaller nanocubes and nanospheres.\(^{[18,37]}\) The average EF measured for 1,4-BDT and 4-MBT were similar for the nanocubes and nanospheres, with small differences due to the proximity of the benzene ring to the metal surface.\(^{[38,39]}\) The data show the EFs for 1,4-BDT and 4-MBT on nanocubes and nanospheres had standard deviations less than 20%.

The larger EFs measured for the nanocubes compared with the nanospheres can be understood in terms of particle shape and E-field properties. The DDA calculations of the E-field enhancement distributions of a nanocube and nanosphere shown in Figure 2.3 agree with the measured EFs and provides insight into how shape affects the SERS. The calculation predicts that the SERS enhancements from the nanocube should be \( \sim 1,000 \) times larger compared with the nanosphere. This is due to the sharp corners of the nanocube, which create hot spots that are responsible for the large SERS EFs. In contrast no hot spots are formed for the nanospheres. The difference between the EF measurements from nanocubes and nanospheres in Figure 2.4 are not as large as predicted in the DDA calculation due to the fact the data are averaged over the entire nanoparticle’s surface. This work shows the large impact shape can have on a metal nanoparticle’s SERS enhancements. Sharp corners and edges are therefore important design parameters for engineering hot spots for SERS.

### 2.2.3.3. Comparison of Size
We have also investigated how size plays a role in the SERS of a nanoparticle. Figure 2.5 plots the average EFs measured from nanospheres and nanocubes with different diameters and edge lengths, respectively. Increasing the size of a nanoparticle will result in a red-shift of its LSPR wavelength, the formation of higher-order plasmon modes (like a quadrapole mode), and an increase in scattering from the nanoparticle. Our data suggests these factors can affect the SERS of the nanoparticle.

For the nanospheres, the average EFs increase as the diameter increases from 27 nm to 155 nm by nearly a factor of ~80. The largest nanospheres have an EF of $1.1 \times 10^4$. For the nanocubes the EFs also increase but only by a factor of 7. The strong effect of size on the SERS of nanospheres, in contrast to nanocubes, can be explained by the formation of higher-order LSPR resonances.

Higher-order plasmon modes, like the quadrapole, can generate enhanced E-fields with distributions that cover a much larger area over a nanoparticle’s surface compared with the dipolar E-field distributions. When molecules are uniformly distributed on the nanoparticle’s surface, there are SERS enhancements over a larger area resulting in a stronger SERS signal for the quadrapole excitation.$^{40,41}$ This is especially true for nanoparticles with a small variation in the E-field enhancements, like nanospheres. For example, in Figure 2.3 the E-field enhancements for the nanosphere varies from 35 to 0. In contrast, the nanocube has a much larger variation in enhancements that ranges from 1,020 to 0. E-fields not localized near the corners are ~1,000 times less enhanced, and the SERS originating from these areas will be negligible. These reasons largely support the data that show the EFs of the nanocubes are relatively static and dominated by the E-field enhancements from its sharp
corners, regardless of size.

For both nanocubes and nanospheres, the increase in size results in a red-shift of their LSPR. A better overlap of the excitation (514 nm in these experiments) with the LSPR wavelength is expected to increase SERS\cite{42}. The increased scattering of the larger nanoparticles affects the strength of the dipolar plasmon mode and can also affect the SERS. The LSPR band has a broader linewidth, which can be seen in Figure 2.2B for the peaks at 740 and 620 nm for the nanocubes and nanospheres, respectively. However, the higher-order LSPR modes (the bands at 536 and 433 nm in Figure 2.2B) have small linewidths and are not broadened by energy loss through scattering. This can explain the increase in the EFs for the nanospheres, as the quadrapole LSPR is expected to generate larger SERS and is closer to the 514 nm excitation. In addition, this mode is expected to become more prevalent with larger sphere sizes, and is not broadened like the dipolar mode\cite{40}. Size can therefore play a significant role in SERS, but our data suggest shape control is a more meaningful parameter to optimize SERS enhancements.

2.3. Gold-Based Nanocages

Gold-based nanocages are nanoparticles with a cubic shape, a hollow interior, and porous walls. They are an alloy of Au and Ag and they have sizes ranging from 30 to 150 nm.

2.3.1. Synthesis of Gold-Based Nanocages

Gold-based nanocages are formed from Ag nanocubes via the galvanic
replacement reaction.[43] In which Au is deposited epitaxially on the surface of the Ag nanocube while the interior of the Ag nanocube is oxidized and removed upon the reduction of the metal salt AuHCl₄.[44] In this mechanism, AuCl₄⁻ oxidizes the sacrificial Ag nanocube to AgCl, which is highly soluble at the boiling temperature of water used in this reaction, shown below:

\[
3\text{Ag} (s) + \text{AuCl}_4^-(aq) \rightarrow \text{Au}(s) + 3\text{Ag}^+(aq) + 4\text{Cl}^-(aq) \quad (2)
\]

The main driving force behind this reaction originates from the difference in the standard reduction potential for the AuCl₄⁻/Au pair (0.99 V) against the AgCl/Ag pair (0.22 V). The electrons generated in the oxidation process migrate to the surface of the Ag nanocube and reduce AuCl₄⁻ to Au atoms. Gold atoms are able to epitaxially nucleate and grow on the surface of the Ag nanocube because Au and Ag have the same face-centered cubic (fcc) structure with lattice constants of 4.0786 Å for Au and 4.0862 Å for Ag. This close match means these metals can easily form an alloy. The evolution of this replacement reaction can be controlled so that the nanocage porosity, composition, and morphology can be steered for specific applications.[45]

For example, as the molar ratio of Au to Ag increases during the replacement process, Au-Ag alloy nanoboxes form and a red-shift from 440 to 700 nm occurs for the major LSPR peak. When additional HAuCl₄ is added, the AuCl₄⁻ ions start to remove Ag atoms from the nanoboxes to generate Au-based nanocages.[30,46] As a result, the mole ratio of Au to Ag continues to increase and the LSPR peak red-shifts to 900 nm and beyond.
2.3.2. Optical Properties of Gold-Based Nanocages

Au-based nanocages have unique optical properties due to their LSPR tunability and large absorption cross section. As mentioned above, the LSPR of a nanocage can be tuned from 500 to 1200 nm. This allows for the nanocages to be optimized for maximum absorption at the wavelength needed for a specific application. Their broad tunability, particularly into the near-infrared (NIR) range (800 to 900 nm) is ideal for biological applications where soft tissue and blood are optically transparent.

The size and wall thickness of a Au-based nanocage can have a strong effect on the absorption and scattering cross sections, in addition to the LSPR wavelength. As discussed above for the Ag nanocube and nanosphere, the scattering from the nanoparticle will increase as the size of a nanoparticle increases, and can dominate the optical properties. Therefore, smaller nanocages have larger absorption coefficient relative their scattering coefficient. For a nanocage the typical wall thickness can change from 2 to 10 nm and as the wall thickness decreases from 5 to 3 nm, for example, the LSPR shifts from 710 to 820 nm. A thinner wall thickness can therefore result in a significant red-shift for the LSPR. In contrast, the effect of wall thickness on the absorption cross section is thought to be relatively small.[30] While size and wall thickness are important, the hollow nature of the nanocage and its Au-Ag composition largely determine its optical properties.

Figure 2.6 shows the TEM images and LSPR wavelength of the Au-based nanocages used in this study. The size and wall thickness were relatively constant, but the Au-Ag composition was varied dramatically. What is immediately noticeable is
the shift in the LSPR wavelength as the Au content in the nanocages increases. There is also a large increase in the linewidth of the LSPR for the nanocages compared to the nanocubes. This is a result of plasmon damping, which has been investigated by our group for nanocages.\textsuperscript{[46,47]} This broadening of the LSPR is also expected to affect the SERS of the nanocages. Broad linewidths imply a short dephasing time ($T_2$) or damping of the LSPR, which can attenuate local E-field enhancements.\textsuperscript{[48]} Consequently, we expect that the nanocages will have lower SERS enhancements compared to the nanocubes, because LSPRs are responsible for the enhanced E-fields which are essential for SERS.\textsuperscript{[49]}

2.3.3. SERS of Gold-Based Nanocages with Different Compositions

2.3.3.1. Experimental Setup

The experiment closely followed the protocol detailed in Section 2.2.3.1. Nanocages with LSPR wavelengths of 525, 585, 625 and 760 nm were synthesized \textit{via} the galvanic replacement reaction using Ag nanocubes with LSPR at 450 nm. The Ag nanocubes and nanocages were functionalized with 1,4-BDT. These particles were then sampled with a Raman microscope and the SERS spectra from 1,4-BDT was recorded and analyzed. The SERS spectra were taken with 514 and 785 nm excitation for each sample. The metal composition of each nanocage sample was determined to be 85% Ag and 15% Au with LSPR at 525 nm, 73% Ag and 27% Au for LSPR at 585 nm, 45% Ag and 55% Au for LSPR at 685 nm, and 9% Ag and 91% Au for LSPR at 790 nm. In addition, the nanocage concentration was also measured.
for each sample and used to normalize the SERS spectra.

### 2.3.3.2. The Effect of Composition

Figure 2.7A shows SERS spectra of the Ag nanocubes and Au-based nanocages taken with 514 nm excitation. The SERS intensities diminished as the nanocage’s LSPR shifted to longer wavelengths. No signals were generated for the nanocages with LSPRs beyond 700 nm. In Figure 2.7B, the intensity of the 1561 cm\(^{-1}\) SERS band from 1,4-BDT is plotted for both 514 and 785 nm excitation. The plots reveal a significant difference between the excitation wavelength and SERS intensity. For the 514 nm excitation, the SERS intensities diminish rapidly to undetectable levels with increasing LSPR wavelength, however with 785 nm excitation the intensities diminish but remain detectable. For both excitations, the Ag nanocubes reported a considerably stronger SERS compared with the nanocages, as expected from the LSPR linewidths.

The sensitivity of the SERS signal to the excitation wavelength is surprising. As mentioned above, the nanocages change composition considerably as they are tuned to the NIR, becoming more inundated with Au. Increasing the Au content may increase plasmon damping, or plasmon dephasing, a cause of weak SERS intensities. While the processes that induce damping are not clear, damping of the plasmon is generally thought to have two different pathways: i) the plasmon decay occurs via transformation of surface plasmons to photons (radiative damping),\(^{[50,51]}\) and ii) via non-radiative decay into electron-hole excitations. The latter process can involve either intraband excitations (within the conduction band) or interband excitations due
to transitions between bands like the d-orbitals of a noble metal and the conduction band. Because the particles in this study have a similar morphology and small-size, the contribution from radiative decay will be similar. However, the changing composition can introduce new dependencies to the intraband excitations as a route for plasmon damping.

For Au, the interband transitions have an edge at 2.5 eV (500 nm) compared to Ag with an edge at 3.8 eV (330 nm). Excitation at interband transitions can lead to plasmon damping, but this has not been observed in alloy nanoparticles. The data in Figure 2.7 shows SERS intensities are attenuated when the excitation wavelength is near 514 nm but not 785 nm for nanocages with a high Au content. As the Au content of the nanocages increase, the SERS intensities derived from 514 nm excitation decrease to a point where bands cannot be distinguished from the background. The fact that the LSPR of the particles is tuned far away from these intraband transitions does not matter. The relationship between the Au content, excitation wavelength, and SERS intensities suggests that damping of the plasmon is occurring non-radiatively, through intraband excitations. For SERS, our data indicates that not only is it advantageous to engineer a nanoparticle’s LSPR away from intraband transmission wavelengths, but the excitation wavelength should also be away from these transitions.

2.4. Summary

In this Chapter, I showed that nanocubes could generate greater SERS enhancements compared to similarly sized nanospheres due to the formation of hot
spots localized at nanocube’s corners. This study confirms DDA calculations, and experimentally shows that sharp features can increase the EFs of a Ag nanoparticle by a factor of 500. I have also shown that size can affect the SERS of both Ag nanocubes and nanospheres. The effect of size is more pronounced for nanospheres, primarily due to the beneficial excitation of higher-order plasmon modes which generated larger SERS intensities when compared with the dipolar plasmon mode. The SERS of nanocubes is less sensitive to changes in size because the E-field enhancements localized at the corners of the nanocube do not change dramatically with higher-order plasmon modes. Our data shows that shape is a more powerful handle compared to size for optimizing SERS enhancements.

I have also experimentally demonstrated the relationship between SERS and Au-Ag composition in a metal alloy nanoparticle. I found that the wavelength of excitation, independent of the LSPR, could dramatically affect the SERS enhancements of a Au-based nanocage. The relationship between the Au content and excitation wavelength is clear: the more Au in the particle, the weaker the SERS intensity with 514 nm excitation, regardless of the LSPR. This trend was not observed for 785 nm excitation and strongly suggest the involvement of Au interband transitions as a damping mechanism of the LSPR which in turn attenuates the SERS intensities.

2.5. Experimental Section

Synthesis of Ag Nanocubes. The Ag nanocubes were prepared using the sulfide-mediated polyol process. In a typical synthesis, 6 mL of EG was preheated to 155
°C for 1 h under magnetic stirring. EG solutions containing 3 mM Na$_2$S, 0.18 M PVP (calculated in terms of the repeating unit, Mw 55,000) were prepared. A 80 µL aliquot of the Na$_2$S solution was injected into the hot EG, followed by 1.5 mL of PVP solution and finally 0.5 mL of the AgNO$_3$ solution. The reaction underwent color changes from yellow to reddish brown to opaque green with plating on the vial walls. The reaction was completed in 20 min. The reaction solution was diluted with acetone, and the product was isolated by centrifugation. The product was washed twice with deionized water and then collected by centrifugation at 13,000 rpm for 5 min and re-dispersed by brief sonication in 4 mL of deionized water.

**Synthesis of Ag nanospheres.** In a typical process, a small aliquot of Ag nanocubes in water (10 µL of small nanocubes or 50 µL of large nanocubes) was added to a small centrifuge tube containing a PVP solution (1 mg/mL in water). The exact volume of PVP solution was adjusted slightly for each reaction so that the total volume was 0.5 mL to allow for straightforward comparisons of UV-vis spectra. Different amounts of etching solution were then added, and the centrifuge tube was immediately capped and transferred to a vortex mixer for 15 s. The products were allowed to equilibrate for 10 min, at which point a UV-vis spectrum was taken and the particles were quickly washed via centrifugation and re-dispersed in ethanol a minimum of 3 times before being re-dispersed in water for imaging. The particles were typically collected by spinning at 13,200 rpm for 4-7 min. It was critical that all etching solutions were made fresh daily. The 45 nm Ag nanocubes were etched with a ferric nitrate solution, typically 0.5-5 mM depending on the concentration of the cube suspension. The 144 nm Ag nanocubes were etched with a light-sensitive
ferricyanide-based solution that contained 100 mM K₂S₃O₃, 10 mM K₃Fe(CN)₆, and 1 mM K₄Fe(CN)₆.

**Synthesis of Au-Based Nanocages.** The Au-based nanocages were prepared using the galvanic replacement reaction between Ag nanocubes and AuCl₄⁻. In a typical synthesis, 50 µL of a 3.5 nM Ag nanocube solution was dispersed in 5 mL of deionized water containing 1 mg/mL of PVP in a 50 mL flask under magnetic stirring and then heated to boil for 10 min. Simultaneously, a 0.2 mM AuCl₄⁻ aqueous solution was prepared. An aliquot of the AuCl₄⁻ solution was added to the reaction flask via a syringe pump at a rate of 45 mL/h under magnetic stirring. The solution was heated for another 10 min until the color of the reaction was stable. Once cooled to room temperature, the sample was washed with NaCl saturated solution to remove AgCl and the with deionized water several more times to eliminate PVP and NaCl. The product was collected by centrifugation and re-dispersed in water.

**Electron Microscopy and Spectroscopic Characterization.** The nanoparticles were characterized by both TEM and SEM. An FEI TEM (Tenai G2 Spirit Twin, Hillsboro, OR) operated at 120 kV was used to take images of the nanocubes and nanocages. An FEI field-emission SEM (Nova NanoSEM 230, Hillsboro, OR) with an accelerating voltage of 15 kV was used to image the particles as well as to take energy-dispersive X-ray spectroscopy (EDX) measurements on the Au-based nanocages. Nanoparticle dimensions were obtained from the TEM and SEM images using ImageJ (Wayne Rasband, NIH) software. The LSPR spectra of the nanoparticles were recorded with a Varian Cary 50 UV-vis spectrophotometer equipped with a tungsten lamp. For each sample, the nanoparticle concentration was
estimated by determining the Ag$^+$ and/or Au$^+$ concentration with an inductively coupled plasma spectrometer (ICP-MS, Agilent 7500ce) and using this knowledge with the nanoparticle dimensions from TEM/SEM imaging and element compositions from EDX analysis. For this study, nanoparticle concentrations were determined in order to remove the effect of concentration on SERS, as more concentrated samples would report higher SERS intensities.

**Particle Preparation and Functionalization.** The nanoparticles were functionalized with a 1 mM ethanol solution of 1,4-BDT or 4-MBT over a period of 6 h. Ethanol was used to wash the samples several times before re-suspension in water to achieve concentration of 3-6 nM (particles per liter).

**Raman Spectroscopy Measurements.** Raman spectroscopy was done with solutions of 10 mM of benzenethiol in basic NaOH (~6 M) where the molecule is in anion form and much more soluble. This was verified by the absence of any S-H stretching bands for solutions containing 1,4-BDT and 4-MBT. For these samples $\lambda_{ex} = 514$ nm, $P_{laser} = 4$ mW, $t = 30$ s.

**Surface-Enhanced Raman Spectroscopy Measurements.** The SERS spectra were recorded from a solution phase using a Renishaw inVia confocal Raman spectrophotometer coupled to a Leica microscope with a 50× objective (N.A.=0.90) in backscattering configuration. The 514 nm wavelength was generated from an argon continuous wave (cw) laser and used with a holographic notch filter based on a grating of 1,200 lines per millimeter. The 785 nm excitation was from a semiconductor cw diode laser and used with a holographic notch filter with a grating of 1,200 lines per millimeter. The backscattered Raman signals were collected on a
thermoelectrically cooled (-60 °C) CCD detector. Sample cells were constructed by attaching the cap of a microcentrifuge tube to a glass slide. The cap acted as a vessel for the liquid sample, and a glass cover slip (0.17-0.13 mm) was carefully placed on top to eliminate solvent evaporation and to act as a reference point from which the focal volume was lowered to a depth of 200 µm into the sample. SERS data was collected with $\lambda_{ex} = 514$ nm, $P_{laser} = 3.1$ mW, and $t = 30$ s and $\lambda_{ex} = 785$ nm, $P_{laser} = 5.1$ mW, and $t = 30$ s, or as indicated otherwise.

Processing of the Raman spectra and all data analysis was done with IGOR Pro software (Portland, OR). All data was baseline corrected before normalization. For the baseline correction a fourth order polynomial was fitted to the raw Raman spectrum and subtracted. Vector normalization was done by calculating the sum of the squared intensity values of the spectrum and using the squared root of this sum as the normalization constant. The spectra were then corrected for differences in nanoparticle concentration.
Figure 2.1 SEM and TEM (inset) images of Ag nanocubes (A, C) and Ag nanospheres (B, D). The average edge length of the nanocubes and average diameter of the nanospheres, along with standard deviation, were: (A) 45±6 nm, (B) 44±7 nm, (C) 150±10 nm, and (D) 144±13 nm. The scale bars correspond to 500 nm.
**Figure 2.2** UV-vis-NIR extinction spectra of (A) 45-nm Ag nanocubes and 44-nm Ag nanospheres; and (B) 150-nm Ag nanocubes and 144-nm Ag nanospheres suspended in water. Major LSPR peaks are labeled. For each spectrum the peak with the longest wavelength represents the dipole LSPR mode, and other peaks labeled in the spectrum are higher-order plasmon modes.
Figure 2.3 E-field enhancement ($|E|^2$) contour plots for a 45-nm Ag nanocube (A) and a 44-nm Ag nanosphere (B) when irradiated at a wavelength of 514 nm with water as the suspension medium. The cartoon at the bottom of each contour plot shows the plane of the nanoparticle represented by the calculated contour plot. For (A) the incident light is along the z-axis and the E-field is along the [110] direction (red line in cartoon) and for (B) the incident light is along the z-axis and E-field is along the x-axis.
Figure 2.4 SERS spectra taken from the 45 nm nanocubes (top) and 44 nm nanospheres (middle) and the normal Raman scattering spectra (bottom), for two different molecules: (A) 1,4-benzenedithiol (1,4-BDT), and (B) 4-methyl benzenethiol (4-MBT). The numbers above each spectrum represents the scale in adu mW$^{-1}$ s$^{-1}$. (C) The average EFs calculated for the 8a vibrational mode (1561 cm$^{-1}$) and the 9a vibrational mode (1183 cm$^{-1}$) for 1,4-BDT; the 8a vibrational mode (1593 cm$^{-1}$) and the 7a vibrational mode (1072 cm$^{-1}$) for 4-MBT. All experiments used a 514 nm excitation laser and the particles were suspended in water. Bands are listed in wavenumber (cm$^{-1}$). Note that in the molecular cartoon white is hydrogen, black is carbon, yellow is sulfur and the plane is a metal surface.
Figure 2.5 The average EF calculated from the 1561 cm\(^{-1}\) band of 1,4-BDT chemisorbed on Ag nanocubes and nanospheres suspended in water. The nanoparticle size was determined from SEM measurements from the edge length of a nanocube or the diameter of a nanosphere. \(\lambda_{ex} = 514\) nm, \(P_{laser} = 5\) mW, \(t = 30\) s.
Figure 2.6 TEM images of Au-based nanocages. The scale bars are 100 nm. (A) nanocages with an LSPR of 525 nm and a composition of 85% Ag and 15% Au; (B) LSPR of 585 nm and 73% Ag and 27% Au; (C) LSPR of 685 nm and 45% Ag and 55% Au; (D) LSPR of 760 nm and 9.0% Ag and 91% Au. (E) UV-vis-NIR extinction spectra recorded from aqueous suspensions of the Ag nanocubes (LSPR at 450 nm) and the Au-based nanocages used in this study. As the Au/Ag ratio increased, the LSPR band of the nanocages red-shifted.
Figure 2.7 (A) The SERS spectra of 1,4-BDT taken from Ag nanocubes (black), nanocages with an LSPR at 525 nm (red), 585 nm (blue), 685 nm (green), and 760 nm (violet) with 514 nm excitation. (B) The SERS intensity measured from the 1561 cm\(^{-1}\) peak of 1,4-BDT on nanocages from 514 (black circle) and 785 nm (red square) excitation as a function of the LSPR of the nanocage. The number near the scale bar corresponds to adu mW\(^{-1}\) s\(^{-1}\).
2.6. Notes to Chapter 2


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Chapter 3

Correlating the SERS of a Single Nanoparticle with its Structural Parameters

3.1. Introduction

There has been a resurgence of SERS studies, catalyzed by the demonstration of single-molecule detection,\cite{1,2} which have focused on understanding the mechanisms of SERS and also how to implement this technique as a reliable method for trace detection.\cite{3,4} These inquiries have resulted in a revolution of SERS experiments, characterized by a high level of scrutiny and control at the nanometer level.\cite{5-7} SERS is, after all, a nanoscale phenomenon and to fully understand it experiments must take into account the subtle variables that have troubled SERS studies from their very beginning. It is with this in mind that correlated-SERS studies have become prominent. Correlated-SERS studies feature excellent characterization at the nanometer level of the actual nanostructure(s) that the SERS measurements were taken from.\cite{8-13} This allows for investigation into which specific attributes of the nanostructure control the SERS enhancements. These studies record SERS signals from a single nanoparticle, dimer, or nanostructure with the goal of quantifying the SERS EF and determining what physical variables of the nanostructure (i.e., size, shape, and distance to neighboring particles) prominently affect the SERS. This is in
contrast to studies where the specific nanostructure probed by SERS is not investigated and requires assumptions about the nanostructure’s morphology.

In this Chapter, I discuss our work correlating the SERS of Ag nanoparticles with their structural parameters. We have developed several methods to carefully study single nanoparticles and their dimers using microprobe techniques. Our well-defined nanoparticle samples allow us to collect a large amount of data on the same type of nanoparticle, and has proven critical to meaningful correlation with their SERS. Our studies have focused on the effects of nanoparticle dimerization, excitation polarization, the supporting substrate, and also hot spot isolation. Together these correlated-SERS studies have provided a wealth of information about the mechanisms of SERS and also how to optimize the Ag nanoparticles for large SERS enhancements.

3.2. Procedure for Studying Single Nanoparticles with Correlated-SERS

Early correlated-SERS studies often simply determined whether or not the nanostructure sampled under a Raman microprobe was an aggregate or single particle, which at the time was significant. More recent correlated-SERS studies have emerged with developments in the fabrication and synthesis of Au and Ag nanostructures of controlled size, composition, and shape. These studies use TEM, SEM, and atomic force microscopy (AFM) characterization to quantitatively determine the structural variables such as size, shape, and particle-to-particle distances. These studies also require that the particles have a fixed, knowable
geometry with respect to the laser polarization, and that the particles can be located with both a Raman system and an imaging system like SEM.

Figure 3.1 shows a simple procedure we have developed for correlated-SERS studies. A small aliquot of an aqueous solution of nanoparticles is drop-cast onto a clean Si substrate patterned with registration marks. The nanoparticles are then allowed to disperse and dry to fixed locations. The substrate is washed briefly and the locations of individual nanoparticles are determined from their Rayleigh scattering with dark-field microscopy.\[11] It is currently difficult to use the Rayleigh scattering for anything except to determine a nanoparticle’s location.\[15] After the nanoparticles are probed with SERS, they will be imaged nanoparticles are imaged with SEM to define their structural parameters.

The parameters measured with the SEM allow for a better estimate of the SERS EF compared to solution measurements. This is due to the dependence of the EF on the number of molecules in a SERS acquisition (\(N_{\text{sers}}\)), which can be estimated from the surface area of a nanoparticle measured by SEM. Our group uses 4-MBT and 1,4-BDT as the SERS probe molecules because they tend to form monolayers on Ag surfaces and have known molecular “footprints”. These attributes are integral to estimating the total number of molecules being probed. Additionally, these molecules are expected to penetrate into regions between nanostructures owing to their small size and strong interaction with Ag.

3.3. The Effect of Laser Polarization on SERS

One of the most important contributions of correlated-SERS measurements is the
clear dependence of the excitation polarization on the SERS EFs. Such studies were first done with large aggregates,\textsuperscript{[16,17]} but have been extended to single nanoparticles as their optical properties have become more complex with advances in synthetic techniques. As a result, the SERS from a single nanoparticle can be greatly influenced by laser polarization. Figure 3.2 shows the effect of laser polarization on the SERS of a Ag nanosphere, nanocube, and nanowire. For a nanosphere no polarization effects are expected due to its isotropic shape because the electrons will be polarized similarly in all directions. The SERS is therefore similar regardless of the laser polarization, shown in Figure 3.2D. This is not the case for the nanocube. Figure 4E shows the SERS spectra from 1,4-BDT on a nanocube (edge length of 115 nm) with two different laser polarizations. The difference in the intensity of the 1562 cm\textsuperscript{-1} band is 2-3 times greater when the laser polarization is along an axis that connects diagonal corners of the nanocube. This strong dependence on laser polarization can be attributed to the anisotropic shape of the nanocube, which results in unique local E-field distributions and enhancements due to the different excitation polarizations.\textsuperscript{[18]} In general, our data has shown that the strongest SERS intensity were generated when the polarization was along an axis connecting the sharp features of a nanostructure.

Nanowires of Ag and Au have also been the subject of correlated-SERS studies.\textsuperscript{[5,9,10,12,19]} Silver nanowires are relatively easy to synthesize,\textsuperscript{[20,21]} and they can be identified under an optical microscope.\textsuperscript{[10]} One drawback of them is their large size, as they often have one dimension in micrometers. This can have an attenuating effect on SERS.\textsuperscript{[9,10,12]} Figure 3.2C shows the SEM of a Ag nanowire 5 \(\mu\)m long and 95 nm in diameter. The long axis of the nanowire causes polarization dependences to
the SERS. This is because the excitation sources used in SERS cannot couple with the longitudinal plasmon mode of the nanowire. As a result no SERS could be detected when the excitation polarization was along the longitudinal axis, as seen in Figure 3.2F.\textsuperscript{[9]} However, when the polarization was along the transverse mode, which has dimensions in nanometers, the characteristic 1,4-BDT peaks at 1069, 1181, and 1562 cm\textsuperscript{-1} are present in the SERS spectrum. For an Ag nanowire with a diameter between 50 to 100 nm, the transverse plasmon resonance is approximately 400 nm and can be excited with our 514 nm excitation. For these studies, the laser was focused at the center of the nanowire (relative to its longitudinal axis) in all measurements. This ensures a maximum number of probe molecules are in the SERS measurement and eliminates any fluctuations arising from the tips.

3.4. The Effect of Nanoparticle Dimerization on SERS

While studying the SERS of single nanoparticles is a necessary step in the design of structures with large SERS EFs, it is widely expected that a single nanoparticle will not have single-molecule sensitivity. However, combining two or more nanoparticles can lead to extremely large enhancements due to the formation of hot spots. Early theoretical studies predicted hot spots\textsuperscript{[22]} and early correlated-SERS experiments suggested that these hot spots, which allow for single-molecule detection, were a consequence of nanoparticle aggregation.\textsuperscript{[14]} However, studying the random assembly of undefined nanoparticles cannot reveal a rational design strategy for hot spot formation. Simple aggregates must be studied, and the simplest type of aggregate is a dimer. A dimer can be used detect single molecules,\textsuperscript{[6]} and its
simplicity allows for an easy correlation of its structural properties with SERS.\textsuperscript{[17,19,23]} The effect of dimerization on SERS can be seen in Figure 3.3 where single nanoparticles are compared to their dimers. In Figure 3.3B the SERS spectrum from 1,4-BDT is plotted for an individual nanosphere and a dimer of nanospheres. The EF for a single nanosphere 80 nm diameter is on the order of 7.0\times10^5 and for a dimer the EF is 1.7\times10^8, an increase of approximately two orders of magnitude.\textsuperscript{[24]} This dramatic effect is also seen for nanocubes. The nanocube dimer in Figure 3.3C has a face-to-face configuration, meaning that their faces were in proximity, as opposed to their edges. The SERS intensity of the nanocube dimer is increased by a factor of 96 compared to a single nanocube, as seen in Figure 3.3C.\textsuperscript{[25,26]} The EF measured for the cube dimer was 7.0\times10^7 and the individual cube had an EF of 7.3\times10^5. For both the nanocube and nanosphere dimer, the hot-spot region is located between the nanoparticles and is expected to give much greater EFs as compared to the entire dimer.

In Figure 3.3E, the nanowire-nanocube dimer had a face-to-face configuration in which the hot-spot region was between a nanocube’s face and a nanowire’s side face. The nanowire was 93 nm in diameter and 4.1 \mu m in length, while the nanocube had an edge length of 142 nm. The EF for the dimer was measured as 1.4\times10^7. This corresponds to an increase of the EFs by 22 and 24 times with respect to the individual nanowire and nanocube.\textsuperscript{[9]} The spectra in Figure 3.3F show the SERS of the nanowire-nanocube dimer, as well as an individual nanocube for comparison. All together the data shows that dimerization increases the SERS EF by a factor of 22 to 250 and is sensitive to the shape of the nanoparticles in the dimer.
Much like single nanoparticles, a dimer requires that the polarization of the laser matches the geometry of the structure so that the largest local E-fields will be generated. As a consequence, a dimer needs the polarization of the laser along the axis connecting the two particles, with the key feature being the interparticle gap.\cite{27,28} For molecules located in the gap with light polarized along the interparticle axis, the proximity of the molecules to the local E-fields is extremely small resulting in large SERS enhancements. In addition, due to the coupling of the plasmons from each nanoparticle a hot spot is formed in the gap region.\cite{22,28} When light is polarized perpendicular to the interparticle axis, it is expected that the local E-fields will not localize in the gap region and no hot spot will be formed. Figure 3.4 shows that a dimer of nanospheres or nanocubes both have larger SERS EFs when the laser is polarized parallel to the interparticle axis. The EFs for the two polarizations differ by a factor of 11 for the nanospheres, and 77 for the nanocubes. Similarly, for the nanowire-nanocube dimer in Figure 3.4C, the EFs were $1.9 \times 10^5$ and $1.4 \times 10^7$ for polarization perpendicular and parallel to the interparticle axis. For all the different dimers, when the polarization is perpendicular to the interparticle axis, the EFs are similar to those for a single nanoparticle, supporting the conclusion that a hot spot is not formed. These differences demonstrate that polarization is indeed a key parameter for probing SERS hot spots.

3.5. Isolating Hot Spots on Nanoparticles with Plasma Etching

A new strategy for fabricating ultra-sensitive SERS substrates is to isolate the hot spot on a nanoparticle so that only molecules are present in this hot-spot region. Hot
spots are areas of intense local E-fields which are responsible for 25% of the SERS signal but represent only a small fraction of the molecules (<1%). Additionally, the ability to probe only the hot spot region could provide significant insight into the mechanism of SERS. We have developed a new method to probe the hot spot which involves plasma etching. Plasma etching can remove molecules on a nanoparticle’s surface. Additionally, this technique can help change the probe molecules adsorbed on the surface of a nanoparticle or dimer. This concept is illustrated in Figure 3.5A. In the first step, a nanocube is functionalized with 4-MBT. Then, the 4-MBT molecules are removed by briefly subjecting the sample to plasma etching. Finally, the sample is immersed in a solution containing 1,4-BDT. Figure 3.5B shows the SERS spectra recorded from a sample going through these steps. The initial spectrum (top trace) has the characteristic peaks from 4-MBT at 1073 and 1583 cm\(^{-1}\), which completely disappeared after plasma etching (middle trace). After immersion in a 1,4-BDT solution, the characteristic peaks from 1,4-BDT at 1182 and 1561 cm\(^{-1}\) appeared in the SERS spectrum as a result of the adsorption of 1,4-BDT onto the nanocube. We observed a shift in the benzene ring stretching band (8a mode) from 1582 cm\(^{-1}\) to 1562 cm\(^{-1}\) indicating the presence of 1,4-BDT. This clearly shows our ability to change the probe molecules on a single nanoparticle.

To determine if the plasma etching would lead to any physical or chemical changes to the Ag nanocube, including its SERS capability, we subjected a single nanocube to numerous cycles of plasma etching. We found that plasma etching and refunctionalization with 4-MBT could be repeated up to three times without observing a major deterioration of the SERS spectrum. However, after the fourth
cycle, no 4-MBT peaks could be detected. It is possible that surface oxidation after extended exposure to the oxygen-based plasma hampered the adsorption of 4-MBT onto the surface.[25]

To determine the ability of the plasma etching technique to isolate a hot spot region we used a dimer of nanocubes. Figure 3.6B shows the SERS spectra from a nanocube dimer that was functionalized with 4-MBT (top trace), with subsequent plasma etching for 2 min (middle trace), and then immersion in a 1,4-BDT solution (bottom trace). For a nanocube dimer the hot spot region comprised the narrow gap between two nanocubes. Therefore, in addition to being isolated from the oxygen plasma, the 4-MBT molecules in the gap region form a multilayer resist relative to the plasma. This can explain why plasma etching can serve as an effective method for isolating the hot spot, because it will require a longer time to remove molecules in the gap region between two nanocubes compared to their other surfaces. After plasma etching (Figure 3.6B, middle trace), a slight decrease in the intensity was observed from the 4-MBT bands at 1072 and 1582 cm\(^{-1}\). This slight reduction was due to the removal of 4-MBT molecules from the region outside the hot spot and indicates that the molecules in the hot spot were the major contributors to the SERS signals of the dimer. Assuming that only the 4-MBT molecules adsorbed in the hot spot region were present in the nanocube dimer after plasma etching, the measured EF of the dimer increased by a factor of ~5 to 1.0\(\times\)10\(^8\).

The sample was then immersed in a 1,4-BDT solution, and all the peaks arising from 4-MBT were replaced by the characteristic peaks from 1,4-BDT (Figure 3.6B, bottom trace). This indicated that, in addition to being adsorbed onto the faces of the
nanocubes outside the hot spot, 1,4-BDT replaced the 4-MBT molecules in the hot-spot region. The stronger interaction between 1,4-BDT and Ag compared to 4-MBT may be the driving force for this process.[13,15,17] After complete replacement of 4-MBT by 1,4-BDT, the EF for the dimer was 1.9×10^7, which is close to the initial EF obtained with 4-MBT, indicating that no significant changes to the nanocube dimer occurred as a result of plasma etching.

Other techniques to isolate hot spots rely on photoresist to block areas of low enhancement, exposing only the hot spot regions to exogenous molecules.[30] Isolation of hot spots is an active area of research because it can eliminate the attenuating effects of the broad distribution of SERS EFs over a nanostructure, essentially allowing molecules to absorb only in the regions of highest SERS enhancements. However, when a single molecule is in a hot spot its orientation to the nanostructure and the incident laser field can have an enormous impact on their SERS, and the spectrum can become transient and ephemeral.[23,31] Single-molecule SERS remains an active area of research, particularly in terms of its reproducibility.

### 3.6. Summary

We have studied single nanocubes, nanospheres, nanowires, and their dimers. We have shown that as a nanoparticle becomes more anisotropic (from sphere, to cube, to wire) the larger the effect laser polarization had on the SERS. Similarly, our work supports the idea that if the laser is not polarized along the interparticle axis of a dimer no hot spot is formed. The hot spots formed in a dimer could increase the SERS EF by several orders of magnitude and be isolated with plasma etching.
Isolation of the hot spot further increased the EF by a factor of 5 and provided experimental evidence to the existence of hot spots, and demonstrated a new way to potentially improve the sensitivity of SERS.

The studies discussed in this Chapter focus on just one hot spot at a time, allowing for a high level of characterization that is largely responsible for confirming what had been previously predicted theoretically, like polarization dependencies,\cite{19,17,32} and the hot spot nature of a nanoparticle dimer\cite{8,6,17,33} and sharp nanoscale features.\cite{11,34} Such characterization has also allowed for unprecedented connection of experiments and simulations,\cite{5,6} and perhaps most importantly has provided new engineering pathways for the creation of simple and reliable SERS of substrates.\cite{10,35}

3.7. Experimental Section

Raman Instrumentation and Correlated SERS/SEM measurements. The SERS spectra were recorded from single nanoparticles using a Renishaw inVia confocal Raman spectrophotometer coupled to a Leica microscope with a 50× objective (N.A.=0.90) in backscattering configuration. The 514 nm wavelength was generated from an argon cw laser and used with a holographic notch filter based on a grating of 1,200 lines per millimeter. The backscattered Raman signals were collected on a thermoelectrically cooled (-60 °C) CCD detector. The laser power was measured before each experiment and adjusted to a value of 0.5 mW.

Samples for correlated SERS/SEM experiments were prepared by drop casting an aqueous suspension of the functionalized nanoparticles onto a substrate that had been patterned with registration marks via lithography, or by scoring the substrate with a
diamond pen. The substrate was briefly rinsed with ethanol to remove any dust that might interfere with locating the nanoparticles under a dark-field microscope. The nanoparticles were allowed to dry under ambient conditions and the locations of several nanoparticles (typically 20 to 50) were identified by their Rayleigh scattering and their positions on the substrate were noted for correlation with SEM. After the nanoparticles had been probed with SERS, the sample was immediately imaged by SEM to determine the sizes, shapes, and orientations of the nanoparticles.

Preprocessing of the Raman spectra and all data analysis was done with IGOR Pro software (Portland, OR). All data was baseline corrected before normalization. For the baseline correction a fourth order polynomial was fitted to the raw Raman spectrum and subtracted. The spectra were normalized by using the Si peak centered at 950 cm\(^{-1}\). Peak areas were used, not intensities, to determine the EF. Lorentzian fits for the Raman modes were found with IgorPro, MultiPeakFit software.

**Determination of the SERS Enhancement Factor.** We used the peaks at 1582 cm\(^{-1}\) (for 4-MBT) and 1561 cm\(^{-1}\) (for 1,4-BDT) to estimate the EF through the following equation:

\[
EF = \frac{I_{\text{sers}} \times N_{\text{normal}}}{I_{\text{normal}} \times N_{\text{sers}}} \tag{1}
\]

where \(I_{\text{sers}}\) and \(I_{\text{normal}}\) are bands from SERS and normal Raman spectroscopy, and \(N_{\text{normal}}\) and \(N_{\text{sers}}\) are the number of molecules probed in each experiment. The areas of the bands at 1562 cm\(^{-1}\) and 1582 cm\(^{-1}\) were used for \(I_{\text{sers}}\) and \(I_{\text{normal}}\). \(N_{\text{normal}}\) was determined based on the Raman spectrum of a 0.1 M solution of the probe molecule in 12 M NaOH and the focal volume of our Raman system (which was determined to be 1.48 pL). \(N_{\text{sers}}\) was obtained by assuming that the thiolate molecules form a
complete monolayer with a molecule footprint of 0.19 and 0.54 nm$^2$ for 4-MBT and 1,4-BDT respectively. These assumptions represent the theoretically maximum number of molecules and are an overestimate. Therefore, the EFs reported herein are likely an underestimate. The thiolate probe molecules do not have any absorption bands that overlap with the wavelength of laser used in this study, eliminating the resonance Raman effect.

**Plasma Etching.** Nanocubes were deposited onto a clean Si substrate and subsequently functionalized with 4-MBT. The substrate was then rinsed with ethanol and dried with a stream of air. After deposition, the nanoparticles were located via their Rayleigh scattering for SERS measurements. The sample was then plasma etched, which removed all exposed organic molecules on the surface of nanocubes. The plasma etching was performed in a plasma cleaner/sterilizer (Harrick Scientific Corp., PDC-001) operated at ~60 Hz and 0.2 Torr air, with power being set to high. Plasma etching of the sample was performed by placing the Si substrate containing the Ag particles on a petri dish in the plasma cleaner chamber and exposing it to the oxygen plasma for 2-4 min. All samples were used immediately for SERS measurements after preparation. After plasma etching, the SERS spectra for individual nanocubes were subsequently re-recorded and the sample was then imaged with SEM for correlation.
Figure 3.1 The major steps involved in a typical procedure for a correlated-SERS measurement. Silver nanoparticles are deposited on a substrate and then rinsed with water. The nanoparticles are then located by their Raleigh scattering using a dark-field microscope. A Raman microprobe is then used to collect SERS data from the individual nanoparticles. Finally, the substrate is imaged with SEM to reveal the size, structure, and morphology of the individual nanoparticles. The scale bar is 5 µm in large (top) SEM image, and 100 nm in the inset.
Figure 3.2 SEM images of a Ag nanosphere 110 nm in diameter (A), nanocube 115 nm in edge length (B), and nanowire 5 µm long and 95 nm in diameter (C). The SERS spectra of 1,4-BDT from the nanoparticles are plotted below each image. The colored arrows in the images indicate the laser polarization direction relative to the nanoparticle. For the nanosphere, no significant variation was observed for the spectra taken at different polarization directions. For the nanocube, the SERS signals were more enhanced when the laser was polarized along the side diagonal direction. For the nanowire, no SERS signal was collected when the polarization was along the long axis of the wire. The scale bars correspond to 10 adu mW⁻¹ s⁻¹.
Figure 3.3 The effect of dimerization on the SERS of Ag nanoparticles. (A) SEM images of a nanosphere 80 nm in diameter and a sphere-sphere dimer. The corresponding SERS spectra from 1,4-BDT of the nanosphere and the dimer are plotted in (B). (C) SEM images of a nanocube 91-nm in edge length and a cube-cube dimer, and their SERS spectra plotted in (D). (E) SEM image of a nanowire-nanocube dimer. The nanowire was 4.1 µm long and 93 nm in diameter. The nanocube was 142 nm in edge length. (F) The SERS spectra of the dimer in (E) and an individual nanocube. For all SERS spectra, the laser polarization was along the arrow at the bottom of (E). The scale bars in the SERS spectra correspond to 10 adu mW⁻¹ s⁻¹.
Figure 3.4 Summary of the SERS EFs measured for a dimer of nanocubes, nanospheres, and a nanowire-nanocube dimer. The arrows indicate the laser polarization direction. The EFs for two laser polarizations are shown for each dimer, one parallel to the axis connecting the two particles and the other perpendicular to it. The scale bars represent 100 nm. The samples were functionalized with 4-MBT.
Figure 3.5 (A) Schematic of how plasma etching works. Plasma etching with oxygen can remove the exposed molecules on a nanocube’s surface. After immersion in 4-MBT, the nanocube on the substrate is plasma etched, which eliminates all the molecules on the surface. Re-immersion with 1,4-BDT re-functionalizes the nanocube. (B) The corresponding SERS spectra for the steps in (A) showing the SERS spectrum of 4-MBT, its complete removal after plasma etching, and the SERS spectra of 1,4-BDT. The broad peak centered at 950 cm$^{-1}$ was from the Si substrate.
Figure 3.6 Probing the hot spot in a dimer of Ag nanocubes. (A) Schematic of the approach employed for selectively probing the hot spot region formed between a pair of nanocubes. The dimer was functionalized with 4-MBT and then exposed to oxygen plasma to remove the adsorbed 4-MBT molecules. In this case, only the 4-MBT molecules outside the hot spot region (i.e., outside the two touching faces) were removed during the plasma etching. The nanocube dimer was then immersed in a 1,4-BDT solution, resulting in the complete replacement of 4-MBT by 1,4-BDT over its entire surface. (B) The corresponding SERS spectra, where the middle spectrum represents the SERS signals from molecules in the hot spot region only. The scale bar in the SEM represents 100 nm.
3.8. Notes to Chapter 3


Chapter 4


4.1. Introduction

SERS relies on the enhanced local E-fields associated with the LSPR modes of metal nanostructures. While it has been demonstrated that hot spots with highly localized and strongly enhanced E-fields can amplify Raman signals by as much as $10^{10}$ for single-molecule detection, the structures used for generating hot spots are typically difficult to fabricate and/or troubled by unpredictable performance. Two such methods to harness the surface plasmons of nanostructures for SERS are the lightening rod effect and the formation of closely spaced nanoparticles with small gaps between neighboring particles. In both cases hot spots are formed in known areas (i.e., at the tip of the nanostructure or in the gap between nanoparticles two nanoparticles, respectively) and SERS enhancements up to $10^8$ are calculated for the sharp tips of nanostructures and $10^{10}$ for the gap between two nanoparticles. Dimers and aggregated nanoparticles are the only structures that have reported large SERS enhancements (reportedly up to $10^{14}$) and are used exclusively in single-molecule SERS studies. Significant efforts have been made to create nanostructures with well-defined and consistent gap lengths to address the problem of reproducibility in SERS, while maintaining large enhancements. However, such structures, prepared by assembly techniques or fabricated by lithography, remain difficult to create and
are often prohibitively complex for large-scale manufacturing and implementation as sensors.

In this Chapter, I report a novel approach to the fabrication of hot spots with strong and reproducible SERS enhancements that simply involves the deposition of Ag nanocubes on a metal substrate.\textsuperscript{[8-12]} Our measurement and simulation results indicate that hot spots are created at the corners of a Ag nanocube in contact with the substrate, where the E-fields are strong enough for SERS detection with single-molecule sensitivity. Our approach, while mechanistically similar to the creation of a gap between two metal nanoparticles\textsuperscript{[13,14]} depends solely on the junction between a Ag nanocube and a metal substrate. It requires minimum fabrication steps and offers great simplicity for the formation of accessible and robust hot spots, providing a new approach to hot spot formation and an effective SERS platform for single-molecule.

4.2. Preparation of Different Substrates

To reveal the effect a substrate has on the SERS of individual nanoparticles, we performed a series of SERS measurements on different substrates. In a typical experiment, we functionalized the Ag nanocubes and nanospheres with 1,4-BDT and then deposited them on a substrate. We recorded the SERS spectra for individual particles under 514 nm laser excitation. After SERS measurements, we used SEM to characterize the orientation of each nanoparticle on the substrate and then correlated it with the corresponding SERS spectrum. Figure 4.1 shows SEM images of nanocubes and nanospheres on thermally evaporated (~100 nm thick), macroscopically smooth (5 nm in root-mean-square roughness) films of gold (Au) and silver (Ag), a polished
silicon wafer (Si), and a glass coverslip (glass). These substrates were chosen due to their availability, different electrical properties, and ease of use with our correlated-SERS measurements.[15]

4.3. Comparison of the SERS from Single Nanoparticles on Different Substrates

When a single nanoparticle is deposited on a substrate, a substrate-nanoparticle interface is formed, similar to a dialectic gap between two neighboring nanoparticles.[16] To determine if this interface could form hot spots, we measured the SERS EFs for individual Ag nanocubes deposited on different types of substrates. Figure 4.1A shows typical SERS spectra taken from Ag nanocubes that were supported, respectively, on Au, Ag, Si, and glass. The lowest SERS signals were observed from the glass substrate and the highest for the Au substrate, with a factor of ~2,000 separating the obtained EFs. This trend supports past work comparing SERS on semiconductor and metal substrates,[11] however, for the nanocubes the large increase in the SERS EFs to $10^8$ are unique.

Figure 4.1B shows representative SERS spectra of the nanospheres on the same substrates as the nanocubes in Figure 4.1A. For the nanospheres the substrate-induced effects are less pronounced compared to the nanocubes. In general, nanospheres are predicted to have lower SERS EFs compared with nanocubes. The SERS EFs measured for both Ag nanocubes and nanospheres on different substrates are summarized in Figure 4.1C. For nanocubes, the EF increased by a factor of 2,500, from $9.4 \times 10^4$ to $2.3 \times 10^8$ when the substrate was merely switched from glass to Au or
Ag. The extraordinarily strong enhancement suggests the formation of hot spots when a Ag nanocube was brought into proximal contact with a metal surface. In contrast, the EF was only increased by a factor of 120 for the Ag nanospheres as the substrate was changed from glass to Au or Ag. The EFs in Figure 4.1C suggest that the formation of hot spots between a Ag nanoparticle and its supporting substrate is very sensitive to both the shape of the particle and the electrical property of the substrate. In other words, hot spots are only generated when a Ag nanoparticle with sharp corners is brought into contact with a metal surface.

To gain a better understanding of the hot spots formed between a Ag nanocube and its supporting substrate, we used the discrete dipole approximation (DDA) method to calculate the E-field enhancement distributions (Figure 4.2). We performed simulations for both nanocubes and nanospheres supported on Au and Si substrates, and suspended in air. The E-field enhancement contour plots in Figure 4.2, are consistent with the trend of the SERS EFs measured for the nanoparticles supported on the different types of substrates (Figure 4.1C). The largest E-field enhancement occurs for the nanocube on Au and is highly localized at the corner sites as seen in Figure 4.2C. For a nanosphere, while its E-fields are enhanced on Au compared with Si and air, hot spots are not formed. The experimental and DDA simulations clearly show that the substrate can influence the E-fields of metal nanoparticles and cause further increase to their SERS EFs. While the interaction between a nanoparticle and substrate has been investigated in terms of LSPR properties,\[17-19] there has been little investigation into how the substrate affects SERS, and the exact mechanism behind particle-substrate interactions remains unknown. For non-metal substrates, a
nanoparticle can induce image charges in the substrate, which then interact with the nanoparticle’s plasmon modes.\cite{19,20} In contrast, a metal substrate can support propagating surface plasmon (PSP) modes, which can hybridize with the nanoparticle’s LSPR.\cite{21,22} This plasmon coupling could generate huge local E-field enhancements.\cite{11,23,24} The large increase in SERS EF for a nanocube on a metal substrate is evidence of this strong interaction. Unlike glass or Si, the metal substrate can couple with the LSPR of the nanocube and significantly enhance its local E-fields.

4.4. Isolation of the Hot Spot between a Nanocube and Substrate

The dependency of the SERS intensity on the substrate composition and nanoparticle shape point to particle-substrate interactions that form hot spots. These interactions should be strongest at the nanoparticle-substrate interface, creating hot spots at the corners of the nanocube in contact with the substrate, as shown in Figure 4.2C. To determine if hot spots are formed at the nanoparticle-substrate interface, we isolated molecules between the nanocube and its supporting substrate by plasma etching. We then measured their SERS spectra and compared the intensities to nanocubes that were completely functionalized. If hot spots are formed at the nanocube-substrate interface, eliminating all the molecules outside this region should still result in strong SERS intensities from the nanocubes as 25% of the SERS signal is produced from less than 1% of the molecules in the hot spots.\cite{25}

As discussed in Chapter 3, plasma etching can remove molecules from the surfaces of a nanoparticle while leaving the particles and substrate intact. However,
molecules between two adjacent nanocubes (e.g., in a dimer of nanocubes) or a nanocube and substrate are protected from plasma etching.\textsuperscript{[26]} For nanocubes functionalized in solution and then deposited on a substrate, molecules located between the nanocube and substrate, as schematically shown in Figure 4.3A, are preserved during plasma etching. By comparing the SERS spectra from nanocubes before and after plasma etching, we can determine if the molecules at the nanocube-substrate interface contribute to the large SERS enhancements. Plasma etching attenuated the SERS peak of 4-MBT at 1582 cm\(^{-1}\) by 96\%, 76\%, and 43\% when the Ag nanocubes were supported on glass, Si, and Au substrates, respectively. The data in Figure 4.3B indicates that the molecules at the nanocube-glass interface did not contribute significantly to the SERS signals, implying the absence of hot spots in this system. For nanocubes on Si, Figure 4.3C shows that ~24\% of the SERS signals remained after plasma etching. This data also indicates the absence of intense hot spots capable of single-molecule SERS because the reduction in SERS intensity was proportional to the reduction in number of molecules on the surface. For the Au substrate, however, ~57\% of the SERS intensity remained after plasma etching as shown in Figure 4.2D. In this case, approximately 80\% of the 4-MBT molecules should have been removed during plasma etching. As a result, the remaining strong SERS peaks provide clear evidence to support our claim that a relatively small number of molecules on the nanocube were positioned in the hot spots at the nanocube-substrate interface. This data, along with the large SERS EFs derived for Ag nanocubes on Au or Ag substrates (Figure 4.1C), clearly demonstrates the formation of hot spots at the nanocube-metal interface with exceptionally strong
enhancements.

4.5. The Effect of Varying the Separation between a Nanocube and Substrate

We also examined how the hot spots between a Ag nanocube and its supporting substrate change as the gap distance between them is varied. As shown in Figure 4.4A, we can easily tune the gap distance \( (d) \) between a Ag nanocube and its supporting substrate by coating the nanocube with a dielectric shell of SiO\(_2\). In this case, the surfaces of Ag nanocubes were derivatized with 4-mercaptobenzoic acid (4-MBA, the SERS probe) and then coated with SiO\(_2\) shells of different thicknesses. The plot in Figure 4C shows that, for both the Au and Si substrate, the SERS peak from 4-MBA at 1583 cm\(^{-1}\) decreased in intensity as \( d \) was increased from 0 to 45 nm, while there were very little changes in SERS intensity for the samples supported on glass. In comparison to the EF values in Figure 4.1C, the system with the largest EF was most sensitive to \( d \). For both dielectric and metal substrates, the interaction of the plasmon modes of the nanoparticle with the substrate will decrease as the gap distance between the nanoparticle and the substrate increases.\(^{[11,23]}\) This data shows that the near-fields of the Ag nanocube were affected by its supporting substrate, resulting in an additional enhancement in its SERS and formation of hot spots.

Prior studies suggest that electronic interactions between a nanoparticle and a substrate are sensitive to the gap distance.\(^{[6,11,23]}\) For a metal substrate, plasmon coupling will occur when the gap distance between nanoparticle and substrate is extremely small.\(^{[16,27,28]}\) The sensitivity of the SERS EFs of the nanocubes to \( d \) is a
strong indication that plasmon coupling between the metal substrate and Ag nanocube is occurring. In contrast, the low sensitivity to $d$ for glass and Si suggest that coupling is not occurring for these substrates.

4.6. Single-Molecule Detection with Nanocubes on Metal Substrates

Since the SERS EFs measured for single nanocubes on a metal substrate were on the order of $10^8$ at 514 nm excitation, their hot spots should allow for the detection of single molecules under resonant conditions.$^{[31]}$ The use of a single nanoparticle for single-molecule detection has not received much attention, and when it has, often through simulations, a contacting substrate was never taken into consideration.$^{[6]}$ Single-molecule detection with SERS has benefited greatly from the demonstration of a bi-analyte technique that uses two different molecules in equal concentrations as the SERS probes.$^{[3,32]}$ In this technique a majority of the SERS spectra should contain both types of molecules unless a hot spot is involved, then the spectrum will be dominated by only one type of molecules at the hot spot. In a typical experiment, both rhodamine 6G (R6G) and crystal violet (CV) dyes at a concentration of 100 nM were incubated with the Ag nanocubes (at a ratio of approximately 500 molecules per nanocube) that were subsequently deposited onto a thermally evaporated film of Ag for SERS measurements. Figure 4.5A shows a dark-field optical micrograph with a color-map overlay indicating spectra unique to R6G (red) and CV (green), recorded with 514 nm excitation and an acquisition time of 1 s. The two spectra unique to R6G and CV are also shown, together with SEM images of the two corresponding Ag nanocubes. The histogram in Figure 4.5B shows a distribution of the spectra recorded
from single nanocubes and the percentage of spectrum was characterized as R6G ($P_{R6G}$). At $P_{R6G}=100\%$ the spectrum would be representative of only R6G, and at $P_{R6G}=0\%$ the spectrum would be only CV. Because each nanocube had approximately 500 molecules of both dyes on it, the spectrum recorded from a nanocube should be a combination of both molecules and the data should be concentrated at $P_{R6G}\approx50\%$ in Figure 4.5B. This was not the case, however, and some nanocubes showed spectra that were dominated by R6G or CV, indicating that these spectra originated from only a few molecules positioned in the areas of the highest enhancements or the hot spots. With a higher concentration of R6G and CV, the single molecule spectra should become less evident, as more molecules would decrease the probability of positioning only a few molecules at the hot spots. This is exactly what we observed when the concentration was increased to 500 nM and the spectra from individual nanocubes were representative of both types of molecules. The existence of two hot spots between the nanocube and its supporting substrate, and our limited data set (50 nanocubes were probed) make it difficult to conclude that the spectrum originated from one molecule only (two molecules might have contributed to the signal, for example). To our knowledge, though, this is the first demonstration of SERS detection on the single-molecule level with a single Ag nanoparticle, and many parameters stand to be optimized including the excitation wavelength, as well as the thickness and roughness of the metal substrate.

4.7. Summary

This method for increasing the SERS EF of a nanoparticle is dependent on the
substrate effect whereby the supporting substrate affects its plasmonic properties resulting in a dramatic increase SERS. By varying the shape of the nanoparticle, the composition of the substrate, the distance between the substrate and the nanoparticle, and the spatial location of the molecules on the nanoparticle, we provide experimental evidence to support the formation of hot spots between a nanocube and a metal substrate.

This simple and reproducible approach has great potential to produce SERS substrates with accessible hot spots for excellent performance in both the enhancement of local E-fields and amplification of SERS intensities for the following reasons: *i*) Ag nanocubes can be synthesized with good uniformity in terms of shape and size distributions by various methods,[33] and their sharp corners and relatively large dimensions ensure stronger SERS signals relative to smaller or rounded nanoparticles; *ii*) the gap distance between a Ag nanocube and the substrate should approach proximity, and is automatically formed during the deposition; *iii*) the nanocube-substrate interface creates hot spots, leading to tremendous enhancement of the local E-fields; and *iv*) unlike a dimer of nanoparticles that needs probe molecules to be localized at a single point between the two particles, hot spots are formed at the corners of a nanocube in contact with the substrate and molecules near these regions are in ideal locations for sensitive SERS detection.

### 4.8. Experimental Section

**Fabrication of Metal Substrates.** The Au and Ag thin film substrates were prepared by thermally evaporating (BOC Edwards Auto 306 Thermo evaporator) ~30
nm of Cr onto polished Si wafers, followed by ~100 nm of Au or Ag (99.9999%, Alfa Aesar). A Veeco Nanoman V atomic force microscope was used to determine the surface roughness of the evaporated films. For Au and Ag films the root mean squared surface roughness was determined to be 5 nm.

**Raman Instrumentation and Correlated-SERS Measurements.** The SERS spectra were recorded from single nanoparticles using a Renishaw inVia confocal Raman spectrophotometer coupled to a Leica microscope with a 50× objective (N.A.=0.90) in backscattering configuration. The 514 nm wavelength was generated from an argon cw laser and used with a holographic notch filter based on a grating of 1,200 lines per millimeter. The backscattered Raman signals were collected on a thermoelectrically cooled (-60 °C) CCD detector. The laser power was measured before each experiment and adjusted to a value of 0.5 mW.

Samples for correlated SERS/SEM experiments were prepared by drop casting an ethanol suspension of the functionalized nanoparticles onto a substrate that had been patterned with registration marks via lithography or by scoring the substrate with a diamond pen. The substrates (a polished Si wafer, a thermally evaporated film of Au or Ag, and a glass cover slip) were briefly rinsed with ethanol to remove any dust particles that might interfere with locating the nanoparticles under a dark-field microscope. The nanoparticles were allowed to dry under ambient conditions and the locations of several nanoparticles (typically 20 to 50) were identified by their Rayleigh scattering and their positions noted for correlation with SEM. After the nanoparticles had been probed with SERS, the sample was immediately imaged by SEM to determine the sizes, shapes, and orientations of the nanoparticles. The
method benefits greatly from uniformity of the nanoparticles as this ensures the majority of the SERS data were taken from nanoparticles with essentially the same parameters. Dimers or undesirable nanoparticles were identified with SEM and their SERS data eliminated from analysis.

Preprocessing of the Raman spectra and all data analysis was done with IgorPro software (Portland, OR). All data was baseline corrected before normalization. For the baseline correction a fourth order polynomial was fitted to the raw Raman spectrum and subtracted. Vector normalization was done by calculating the sum of the squared intensity values of the spectrum and using the squared root of this sum as the normalization constant. Peak areas were used, not intensities, to determine the EF. Lorentzian fits for the Raman modes were found with IgorPro MultiPeakFit software. A cubic polynomial baseline defined by the fit program was used. Four variables were fit including the area, peak amplitude, width, and the center of frequency. Goodness of fit was gauged by comparing the standard deviation ($\sigma$) of the fit parameters with being less than 10%.

**Determination of the SERS Enhancement Factor.** We used the peaks at 1582 cm$^{-1}$ (for 4-MBT) and 1561 cm$^{-1}$ (for 1,4-BDT) to estimate the EF through the following equation:

$$EF = \frac{(I_{\text{sers}} \times N_{\text{normal}})}{(I_{\text{normal}} \times N_{\text{sers}})}$$

where $I_{\text{sers}}$ and $I_{\text{normal}}$ are bands from SERS and normal Raman spectroscopy, and $N_{\text{normal}}$ and $N_{\text{sers}}$ are the number of molecules probed in each experiment. The areas of the bands at 1562 cm$^{-1}$ and 1582 cm$^{-1}$ were used for $I_{\text{sers}}$ and $I_{\text{normal}}$. $N_{\text{normal}}$ was determined based on the Raman spectrum of a 0.1 M solution of the probe molecule.
in 12 M NaOH and the focal volume of our Raman system (which was determined to be 1.48 pL). \( N_{\text{sers}} \) was calculated by assuming the thiolate molecules form a complete monolayer with a molecule footprint of 0.19 and 0.54 \( \text{nm}^2 \) for 4-MBT and 1,4-BDT respectively. These assumptions represent the theoretically maximum number of molecules and are an overestimate. Therefore, the EFs reported herein are likely an underestimate. The thiolate probe molecules do not have any absorption bands that overlap with the wavelength of laser used in this study, eliminating the resonance Raman effect.

**Plasma Etching.** Nanocubes functionalized with 4-MBT were deposited onto a Si, glass, or Au. For all the substrates, after deposition, the nanoparticles were located \textit{via} their Rayleigh scattering and for recording of SERS spectra from individual particles. The sample was then plasma etched, which removed all exposed organic molecules on the surface of nanocubes. The plasma etching was performed in a plasma cleaner/sterilizer (Harrick Scientific Corp., PDC-001) operated at \(~60\) Hz and 0.2 Torr air, with power being set to high. Plasma etching of the sample was performed by placing the Si substrate containing the Ag particles in the plasma cleaner chamber and exposing it to the oxygen plasma for 4 min. All samples were used immediately for SERS measurements after preparation. After plasma etching, the SERS spectra for individual nanocubes were subsequently re-recorded and the sample was then imaged with SEM for correlation.

**Coating of Silver Nanocubes with Silica Shells.** The nanocubes were coated with SiO\(_2\) using a modified Stöber process. In a typical procedure, 5 \( \mu\)L of Ag nanocubes (5 nM in water) was dispersed in 2 mL ethanol and then mixed with 10 \( \mu\)L
of 1 mM 4-MBA ethanol solution. After stirring at room temperature for 1 h, 500 µL of 1 mg/mL PVP solution in ethanol was added, followed by continuous stirring for 10 min. Then, the Ag nanocubes functionalized with 4-MBA were coated with SiO₂ by stepwise adding 300 µL of deionized water, 60 µL of ammonium hydroxide (28% NH₃), and 3 µL (TEOS) under magnetic stirring. After reaction at room temperature for 3 h, the product was collected by centrifugation and washed three times with ethanol and re-suspended in 1 mL ethanol for use. The thicknesses of SiO₂ coating was controlled by adjusting the volume of TEOS. The thickness was determined from TEM measurements. The silica-coated nanocubes were then dispersed onto different substrates for correlated SERS-SEM measurements.

**Single-Molecule Detection by SERS.** For the single-molecule study, a 1 nM nanocube sample was incubated with a solution containing both CV and R6G at a concentration of 100 nM or 500 nM, with the dyes being at equal concentrations, for 2-3 hours in ethanol and then deposited onto a Ag or Au substrate. The maximum number of dye molecules on each nanocube was estimated to be 500 and 2,500 for the 100 and 500 nM concentration, respectively. The substrate was allowed to dry at ambient conditions and then briefly rinsed with ethanol, dried and used immediately. The nanoparticles were identified by their Rayleigh scattering and were subsequently sampled with the Raman system. Data was acquired at 0.5 mW for 1 s with 514 nm excitation. Sampling of the individual nanocubes occurred manually or automatically via WiRE acquisition software. In the latter case, the SERS data could be analyzed quickly using the WiRE software. However, a more thorough analysis of the data was done on IgorPro software, which was used to generate the histogram in Figure 4.5B.
For data analysis, control samples were prepared with only R6G or CV on the Ag nanocubes. Approximately 50 nanocubes were probed from each control sample and these were then averaged to create spectra used as metric for the analysis.

We found that the SERS spectra from R6G and CV had similar intensities and the band positions and shapes were similar to previous studies. The low concentration of dye molecules ensures they do not interact with each other, and therefore we can combine the average spectra of CV and R6G. We assume this spectrum corresponds to 1:1 dye ratio, which was used to ensure the weighted fits are effectively normalized for different cross sections of the dyes. The weighted fits to the bi-analyte experiments were then used to determine the total signal percentage in each spectrum originating from R6G ($P_{R6G}$) to be determined as the average CV and R6G spectrum was fixed at $P_{R6G} = 50\%$. The average R6G spectrum was then fit to the SERS data from the bi-analyte experiment using several fit parameters (peak positions and shapes) which were ultimately combined into a single percentage indicating the total signal percentage originating from R6G. The percentages were binned into 7 bins with the extreme events $P_{R6G} > 90\%$ or $P_{R6G} < 10\%$.

**Theoretical Calculations.** The discrete-dipole approximation (DDA) method was used to calculate the near-field distributions at an excitation wavelength of 514 nm for both the Ag nanocubes and nanospheres. Two different substrates were used: Au and Si. For comparison, simulations were also performed without a substrate, with the nanoparticles suspended in air. The nanoparticles were position 2 nm above the substrate. We used 64,000 dipoles to approximate the nanocube, with the incident light polarized along the [110] direction. For the Ag nanosphere, Mie theory was
employed to calculate the near-field distribution as the sphere is irradiated at a wavelength of 514 nm.
Figure 4.1 The SERS spectra of 1,4-BDT taken from individual Ag nanocubes (A) and nanospheres (B) supported on a Au film, Ag film, Si wafer, and glass cover slip, respectively. The insets show their corresponding SEM images. The nanocubes on metal substrates had much higher SERS intensities compared with those supported on Si and glass. The scale bar for the SERS spectra is 10 adu mW$^{-1}$ s$^{-1}$. (C) The EFs for single Ag nanocubes and nanospheres, respectively, supported on different substrates. Each value reported in this table represents an average of the data from 40 particles. Unlike the nanospheres, the EFs for the nanocubes increased dramatically when the substrate was switched from a dielectric to a metal. The cartoon shows propagation and polarization directions of the laser used in this study and simplified distribution of dipolar charges on each type of particle.
Figure 4.2 Schematic of the coordination systems for calculating the E-field enhancement distribution of a nanocube (A) and nanosphere (B), respectively, positioned 2 nm above a substrate. The grey region represents the plane plotted in the simulations and was 1 nm above the underlying substrate. For nanocube, the polarization was along the green line. The E-field enhancement distributions calculated using the DDA method for nanocubes (C) on Au, (E) on Si, and (G) in air; as well as for nanospheres (D) on Au, (F) on Si, and (H) in air.
Figure 4.3 (A) Schematic of the isolation of hot spots between a Ag nanocube and its supporting substrate showing the selective removal of 4-MBT molecules (red) from the surface of a Ag nanocube supported on a substrate. Plasma etching with O$_2$ can remove the exposed molecules on the particle’s surface, only leaving behind molecules at the nanocube-substrate interface. B-D, SERS spectra taken from Ag nanocubes functionalized with 4-MBT (with a peak at 1582 cm$^{-1}$) and then deposited on glass (B), on Si (C), and on Au microplate substrates (D), respectively. The SERS spectra were recorded from the same nanocube before and after plasma etching.
Figure 4.4 Controlling the gap distance between a Ag nanocube and its supporting substrate for SERS characterization. (A) Schematic showing how the gap distance \( d \) between a Ag nanocube and its underlying substrate is controlled by the thickness of the SiO\(_2\) shell (blue). The nanocube was functionalized with SERS-active 4-MBA (green) prior to coating with SiO\(_2\). (B) TEM images of Ag nanocubes coated with SiO\(_2\) shells of 0, 5, 10, and 45 nm, respectively, in thickness. (C) Plots of SERS peak intensity as a function of thickness for the SiO\(_2\) shell. The peak intensities at 1583 cm\(^{-1}\) were recorded for the 4-MBA molecules on single nanocubes supported on Au film, Si wafer, and glass substrates, respectively.
Figure 4.5 (A) Dark-field optical micrograph of Ag nanocubes on a Ag film, with a SERS color map overlay to mark the SERS spectra uniquely from CV (red) or R6G (green). The other colors indicate that the spectra were a combination of both dyes. The bright white spots are dimers of Ag nanocubes. Because there were on average 500 molecules on each nanocube, the SERS spectrum recorded from each nanocube should be a combination of both dye molecules; however, this was not the case, suggesting that the SERS signal was dominated by a few molecules in the hot spots. The scale bars are 10 µm and 8 adu mW$^{-1}$ s$^{-1}$, respectively, for the dark-field image and SERS spectra. The inset show SEM images (at a tilt angle of 45°) of the nanocubes from which the two spectra were recorded. (B) Histogram of the percentage the SERS spectrum from a nanocube was characterized as R6G (P$_{R6G}$) at concentrations of 100 nM and 500 nM. The SERS spectra were acquired with 514 nm excitation, 1 s acquisition, and 0.5 mW laser power.
4.9. Notes to Chapter 4


[31] Camden, J. P.; Dieringer, J. A.; Wang, Y.; Masiello, D. J.; Marks, L. D.; Schatz,


Chapter 5

Probing the Surfaces of Silver Nanocubes and Gold-Based Nanocages with SERS

5.1. Introduction

The high sensitivity of SERS has shaped the mainstream view of this technique as one primarily to be implemented for trace detection.\textsuperscript{[1,2]} Yet, SERS is a vibrational spectroscopy technique and can also provide rich spectral information from molecules. In addition, due to the enhancement mechanism, a SERS spectrum is representative of the molecules localized only at a nanoparticle’s surface. SERS is therefore primed to reveal the structure and conformation of molecules on a nanoparticle’s surface. These surfaces continue to gain importance as nanoparticle synthesis and surface functionalization become evermore sophisticated to meet the demands of new, innovative applications of nanoparticles.\textsuperscript{[3-5]}

In this Chapter, I use SERS to probe a molecular retention layer for aqueous glucose on Ag nanocubes, and to examine the temperature gradients near the surfaces of Au-based nanocages. In both systems, I use alkanethiolate self-assembled monolayers (SAMs) as they form readily on metal nanoparticles and can change their conformation in the presence of environmental perturbations, which can be monitored with SERS.
5.2. Preparation of Silver Nanocubes and Gold-Based Nanocages

Covered with Self-Assembled Monolayers (SAMs) of Alkanethiolates

Many molecules that contain a thiol group can spontaneously form ordered SAMs on metal surfaces.[6,7] The formation of SAMs on extended metal surfaces and metal nanoparticles has been investigated extensively.[8] Together, these studies have indicated that SAMs exhibit a well-defined geometry, composition, packing density and other physical properties on metal surfaces. SAMs can be used to effectively change the surface chemistry of a metal surface, imparting to the surface a desired functionality, like hydrophobicity. SAMs of alkanethiolates are particularly well known due to the highly ordered structures they form on metal surfaces.[3]

The formation of alkanethiolate SAMs on Ag nanocubes and Au-based nanocages involves simply dispersing the nanoparticles in a ~1 mM solution of the thiol molecule for approximately 12 h. The weak interaction between the capping agent PVP and the nanocubes or nanocages is easily displaced by the covalent thiolate bond formed between the sulfur and metal surface. The extent of the SAMs coverage and its conformation on the nanoparticles can be determined by characterization with SERS. SAMs on extended metal surfaces (surfaces with μm² to cm² areas) of Au and Ag have been extensively characterized with SERS, so their SERS bands are well known.[9-12] A comparison of these band positions and shapes suggests that the alkanethiol SAMs form well-ordered structures on Ag nanocubes and Au-based nanocages.[13,14]

5.3. Probing the Interaction between Alkanethiolate SAMs and
Aqueous Glucose with SERS

Molecular retention with alkanethiolate SAMs can be used to concentrate molecules for detection applications like SERS.\textsuperscript{[15-17]} However, the interaction between SAMs and small molecules remains largely unexplored and is an active area of research. Of particular interest is the interaction of glucose with the alkanethiol SAMs. Several studies have used alkanethiolate SAMs to detect glucose with SERS, however it is not known how glucose interacts with these SAMs.\textsuperscript{[15-17]} One idea is that the glucose penetrates into the SAMs.\textsuperscript{[16,17]} If penetration does occur the SAMs are expected to become more disordered. In this study, we aim to determine if glucose penetrates into the SAMs or superficially adsorbs onto the SAMs. I use uniform Ag nanocubes covered with alkanethiolate SAMs to investigate the structural relationship of the SAMs to the retention of glucose with SERS. SERS is gaining a strong presence in the analytical sciences for detection, but this technique is also primed to study how molecules and SAMs interact on a nanoparticle’s surface. A better understanding of the interactions between small molecules and SAMs is vital in controlling the retention of molecules like glucose to SAMs on metal nanostructures.

5.3.1. Characterization of Silver Nanocubes Covered with Alkanethiolate SAMs

The Ag nanocubes used in this study are shown in Figure 5.1. The particles have flat square faces that are smooth.\textsuperscript{[18,19]} The LSPR of the Ag nanocubes is shown in Figure 1B. The dipole peak at 510 nm and the quadropole peak at 450 nm are red-shifted 22 nm after functionalization with the alkanethiol 1-dodecanethiol (1-DDT)
due to a change of refractive index caused by thiol adsorption to the Ag nanocube surface.\textsuperscript{[20]}

The binding of 1-DDT to the Ag surface proceeds by chemisorption of the sulfur head group to Ag through a loss of its hydrogen to form a thiolate.\textsuperscript{[7]} The 1-DDT molecules then interact with one another to minimize their free energy by forming a densely packed, highly ordered structure. This can be observed in the SERS spectrum of as-prepared SAMs on Ag nanocubes shown in Figure 5.2. The vibrational contributions of the gauche (G) and trans (T) intensities in the $\nu$(C-S) region are indicative of the extent of order and crystallinity in the SAM.\textsuperscript{[9,21]} The area of the T band (730 cm$^{-1}$) to the G band (636 cm$^{-1}$) of the 1-DDT SAM formed on Ag nanocubes differs by a factor of $\sim$50, indicating that the monolayer is highly ordered which in turn signifies extensive SAM coverage on the Ag nanocubes. The 1125 cm$^{-1}$ $\nu$(C-C)$_T$ band is also nearly equal in intensity with the 1081 cm$^{-1}$ $\nu$(C-C)$_G$ band which is a characteristic of 1-DDT SAMs. The SERS data from the 1-DDT SAMs on the Ag nanocubes agree well with bands reported for extended Ag surfaces shown in Table 5.1, indicating the formation of an ordered monolayer on the nanocubes.

5.3.2. Interaction between Glucose and Alkanethiolate SAMs

Figure 5.3F shows the SERS spectrum of a saturated ($\sim$5 M) solution of glucose. The Raman bands at 1465 cm$^{-1}$ $\nu$(CH), 1365 and 1267 cm$^{-1}$ $\delta$(C-C-H), 1125 cm$^{-1}$ $\nu$(C-C), 1065 cm$^{-1}$ $\nu$(C-H), 915 and 847 cm$^{-1}$ $\nu$(C-O), and 519 cm$^{-1}$ $\delta$(C2-C1-O1) correspond to aqueous glucose.\textsuperscript{[22]} The spectra in Figures 5.3G-J are from the subtraction of the 1-DDT nanocubes with glucose from pure 1-DDT-covered
nanocubes, and clearly demonstrate the presence of glucose. It is important to note that the nanocubes shown Figure 5.1 were not able to detect glucose without functionalization with 1-DDT, even at high glucose concentrations.

Figure 5.4 compares the SERS bands of the C-H stretching region and the C-C stretching region from 1-DDT as these bands changed in the presence of aqueous glucose. From the C-C spectral region in Figure 5.4B, the structural integrity of the broad feature centered at 1440 cm$^{-1}$, composed of the CH$_2$ scissor deformation at 1433 cm$^{-1}$ and the CH$_3$ symmetric deformation band at 1454 cm$^{-1}$, suggest that no gauche bonds were formed in the presence of glucose. An increase in gauche bond formation would result in the split-peak pattern to degrade towards a single peak, which was not observed.$^{[23,24]}$ Furthermore, while both the $\nu$(S-C)$_T$ and $\nu$(C-C)$_G$ bands were attenuated in the presence of glucose the ratio between the peaks, $I[\nu$(S-C)$_T]/I[\nu$(C-C)$_G]$, was constant, indicating that the gauche bond population was static in the inner monolayer near the nanocube’s surface.

The C-H region is more sensitive to conformation changes, however, analysis of this region is more complex due to the bands of both symmetric ($\nu$s) and antisymmetric ($\nu$as) methylene and methyl modes, many of which overlap. This, in part, makes the use of intensity ratios convenient. These ratios have long acted as empirical indicators of the conformational structure of biological membranes,$^{[25]}$ alkanes,$^{[26,27]}$ and, more recently, alkanethiolate SAMs.$^{[28]}$ The $\nu$as(CH$_2$) 2904 cm$^{-1}$, $\nu$s(CH$_2$) 2850 cm$^{-1}$, and the $\nu$s(CH$_3$, FR) 2936 cm$^{-1}$ are the three bands used to determine changes in the conformation or the rotational disorder of the alkanethiolate SAMs. Table 5.2 lists the various empirical ratios used in this study. These bands were all at least $\sim$10 cm$^{-1}$
from glucose band peak centers.

In the presence of glucose, the \( I[\nu_{as(\text{CH}_2)}]/I[\nu_{s(\text{CH}_3, \text{ FR})}] \) ratio increased. The increase of this ratio has been observed in alkanethiolate SAMs that have become more ordered.\(^{[28]}\) These two bands are sensitive to intermolecular interactions and suggest that, in the presence of glucose, there is a decrease in the rotational movement of 1-DDT and an increase in alkane chain-chain coupling in the SAMs.\(^{[26]}\) Similarly, the \( I[\nu_{as(\text{CH}_3, \text{ FR})}]/I[\nu_{s(\text{CH}_2)}] \) ratio is also sensitive to chain-chain coupling and the rotations of the terminal methyl groups.\(^{[27]}\) This ratio decreases when there is an increase in disorder.\(^{[12]}\) In our study this ratio increased in the presence of glucose, supporting the trend toward more ordered SAMs observed above.

The \( I[\nu_{as(\text{CH}_2)}]/I[\nu_{s(\text{CH}_2)}] \) ratio is sensitive to small changes associated with the conformation and the rotation of the 1-DDT SAMs, and is directly related to the T/G ratio of the C-C stretches.\(^{[12,29]}\) In our study this ratio increased in the presence of glucose. This means the order of the 1-DDT SAMs increased.\(^{[27]}\) In this case, the increase in order is attributed to the decrease in rotational freedom for the terminal methyl groups, and an increase of the intermolecular coupling between 1-DDT chains throughout the SAMs. The ratios are all summarized in Table 5.2.

Together, the data supports the conclusion that glucose did not penetrate into the SAMs. Glucose may have adsorbed to the 1-DDT SAM superficially. Adsorption could reduce the high surface energy of the 1-DDT SAM/water interface and encourage the chain-chain coupling of the 1-DDT SAMs on Ag nanocubes. This could explain the increase in the order of the SAMs as measured above.
5.4. Probing the Photothermal Effect of Gold-Based Nanocages

In the photothermal (PT) effect a metal nanoparticle absorbs light and releases it as heat.\textsuperscript{[30]} This heat can affect the molecules on a nanoparticle’s surface and heat up the local environment, both of which are utilized for drug delivery,\textsuperscript{[3]} cancer therapy,\textsuperscript{[31]} and lithography applications.\textsuperscript{[32]} In the PT effect, a nanoparticle’s surface plays a key role in its utilization as molecules are often released from this surface or change as a result of the released heat. Gold-based nanocages have been utilized in several PT effect studies due to their excellent ability to convert light into heat.\textsuperscript{[4,14]} The nanocages used in this study, and the nanocubes they were derived from, are shown in Figure 5.5.

Quantifying temperature changes during the PT effect is pivotal for understanding and engineering heat-induced changes at a nanoparticle’s surface. Many diverse methodologies have been developed to quantify the PT effect over varying timescales including: theoretical computations,\textsuperscript{[33]} ice melting,\textsuperscript{[34]} bubble formation,\textsuperscript{[35]} and ultrafast absorption techniques.\textsuperscript{[36]} These techniques are not sensitive enough to measure temperature changes localized to the nanoparticle surface and rely on indirectly inferring temperature gradients from the PT effect. In this work we show for the first time that SERS can be used to probe the PT effect, resulting in examination of the temperature gradients generated at the nanoparticle surface.

5.4.1. Calibrating the Response of the Alkanethiolate SAMs to Temperature

Since SERS directly measures the chemical structure of molecules on metal
nanoparticles, the temperature near a nanoparticle’s surface can be determined so long as the nanoparticle is covered with molecules that undergo a measurable temperature-dependent structural change. Figure 5.6A shows the SERS spectra of the gauche (G, at 1080 cm\(^{-1}\)) and trans (T, at 1125 cm\(^{-1}\)) \(\nu(C-C)\) bands of a 1-DDT SAM on Ag nanocubes in water.\(^{[13]}\) The temperature of the solution containing SAM-covered Ag nanocubes was increased manually and the SERS spectra acquired at different temperatures. The spectra show a clear change in the relative band intensities of the 1-DDT monolayer associated with the G and T carbon-carbon stretches. These bands are sensitive to the conformation of the SAMs where the \(\nu(C-C)_T\) or T band is a low-energy conformation and indicative of a well-ordered monolayer. The G band is a higher-energy conformation that can arise when the SAM is disordered due to increasing temperature.\(^{[9]}\) As the temperature of the solution was manually increased from 26 to 61 °C, the intensity of the \(\nu(C-C)_G\) band increased and the \(\nu(C-C)_T\) band decreased (see plots in Figure 5.6B).

### 5.4.2. The Effect of Excitation Wavelength to the Alkanethiolate

**SAMs Conformation on Gold-Based Nanocages**

The sensitivity of these SAMs to solution temperature was well resolved with SERS, and we endeavored to translate this approach with nanocages in an attempt to detect changes in the 1-DDT SAM induced by PT heating. Figure 5.7 shows the SERS spectra, with both 514 and 785 nm continuous wave (cw) excitation, of the \(\nu(C-C)_G\) and \(\nu(C-C)_T\) bands from the 1-DDT SAMs on nanocages with different LSPRs. What is immediately evident is the large discrepancy between the \(\nu(C-C)_G\)
and $\nu(C\text{-}C)_T$ bands from the 514 and 785 nm excitations for all nanocage samples. This was not observed for the nanocubes and only a small difference in peak intensities was measured for different excitations, as shown in Figure 5.6C. In Figure 5.7A nanocages with LSPR of 525 nm show significant disorder with 514 nm excitation but not 785 nm excitation. For the nanocages in Figure 5.7B the LSPR was tuned to 620 nm and when excited with the 514 nm laser the $\nu(C\text{-}C)_T/\nu(C\text{-}C)_G$ intensity ratio decreases relative to the 785 nm excitation. Comparing Figure 5.8A and 5.8B, we see a predictable difference between the G and T band intensities where the nanocages tuned to match the excitation source had a larger G band intensity than the corresponding T band.

In Figure 5.7C, the nanocages were tuned to 790 nm, and with the 785 nm excitation the T and G bands are consistent with a disordered monolayer, although not to the extent seen with the nanocages tuned to lower wavelengths and excited with the 514 nm laser. For the 514 nm excitation of the 790 nm LSPR tuned nanocages there is no appreciable SERS spectra. This has been subject of another study (see Chapter 2),\textsuperscript{[37]} suffice to say here that interband transitions effectively dampen the plasmon and attenuate the SERS.

For all the nanocages studied here, the excitation dependent changes seen in the spectra were entirely reversible. Figure 5.7D shows five acquisitions taken in sequence of 1-DDT nanocages tuned to a LSPR of 525 nm. The reversible nature of this process shows that the collective heat generated by the nanocages was limited to specific acquisitions and no increase in the solution temperature, $\Delta T_{\text{global}}$, occurred. Otherwise, we would expect the T/G band intensities in Figure 5.7D to decrease with
the number of acquisitions, even in the spectra with off-resonance excitation. This also suggests that the SAMs are not being irreversibly damaged. This is further supported by Figure 5.7E, which shows the extended SERS spectra of the 1-DDT nanocages (LSPR of 525 nm) at both the excitation wavelengths. What is apparent from these similar spectra is the relatively minor disorder induced via the PT effect on the 1-DDT SAM: no SAM desorption is evident, bands associated with the $\nu$(C-S)$_T$, 706 cm$^{-1}$ and the $\nu$(C-S)$_G$, 632 cm$^{-1}$ remain relatively unchanged, and the band intensity of the CH$_3$ rocking mode associated with T conformations decreases, as expected. With laser powers of 4-5 mW focused to $\sim$4 kW/cm$^2$, the power densities in this experiment are powerful enough to produce significant $\Delta T_{nano}$.[34,38] However, due to the continuous excitation and interparticle distances (estimated to be less than $\sim$10 um) thermal fields of neighboring particles most likely overlap, contributing to the disorder of the SAMs.

5.4.3. The Effect of Gold-Based Nanocage LSPR to the Photothermal Effect

The temperature-controlled experiments with the nanocubes were used to quantify the changes of the 1-DDT SAMs on the nanocages during the PT effect. In addition, a molecular dynamics (MD) simulation was also performed to simulate the conformation of the 1-DDT SAMs at different temperatures. Coupling the experimental and simulation data of the conformation of the 1-DDT SAMs over several temperatures should provide an accurate picture of the temperatures near a nanocage surface.[39,40] Figure 5.8A shows the optimized alkanethiolate chain
configuration of a 1-DDT SAM on a Au surface at different temperatures. At higher temperatures the planar nature and the all-trans configuration is perturbed and gauche ν(C=C) conformations are formed. This is precisely what was monitored in our SERS experiment, and plotting the experimentally and theoretically derived T/G ratios together in Figure 5.8B, confirms the cogency of the experimentally determined T/G-temperature relationship.

Figure 5.8C shows the relationship between the temperature of the 1-DDT SAMs, the LSPR of the nanocage and the excitation wavelength. For clarity the change in temperature during excitation from the ambient temperature is also shown. For off-resonance excitation of the nanocages the change in temperature is considerably smaller, approximately 5 °C, compared with excitation near the LSPR wavelength. For excitation close the LSPR wavelength a significant increase in temperature was measured; 67, 55, and 37 °C for nanocages with LSPRs of 525, 620 and 790 nm respectively. It is interesting to note the decrease in the magnitude of the temperature gradients is consistent with more Au in the nanocages. This supports past reports with Au and Ag nanoparticles that find Ag nanoparticles report larger temperature increases when excited at plasmon frequencies.\[41\]

The temperature gradients reported here are comparable to those generated by cw irradiation of Au nanoshells,\[42\] Au nanorods,\[43\] and, as expected,\[33\] significantly larger than the values from small Au colloids.\[44\] This simple method to glean information about nanoscale interfaces can be extended to almost any nanoparticle, and also provides insight into the excitation dependencies of PT heating which remain an active area of research and development.\[45\]
5.5. Summary

In this chapter I used SERS to monitor the structural changes of 1-DDT SAMs on metal nanoparticles in response to environmental perturbations. The data suggest that in the presence of aqueous glucose the 1-DDT SAMs do not become disordered. This strongly supports the conclusion that glucose adsorbs to the 1-DDT SAMs superficially and does penetrate into the 1-DDT SAM. These results have implications to the design of SAM retention layers. For example, to retain molecules on SAMs for long periods of time a disorder SAM may be better. This is because molecules could penetrate into the SAM due to defects in its structure. Our data suggest small molecules cannot penetrate into ordered SAMs, which makes them ideal for reversible binding and sensing.\[5,17]\]

The conformation changes of 1-DDT SAMs were also monitored to determine the temperature near a nanocage’s surface. The close relationship between SERS and PT effect makes the union of these two phenomena simple and powerful. I have shown that SERS can be used with 1-DDT SAMs as an all-optical molecular thermometer, and as the excitation wavelength approaches the LSPR of the nanocage more heat is generated from the PT effect. SERS is expected to grow continuously in terms of its applicability and synergistic relationships with various plasmon-associated applications. This study provides insight and stimulus for more investigation between SERS and the PT effect for both fundamental understanding and practical use.

5.6. Experimental Section
Synthesis of Ag Nanocubes. The Ag nanocubes were prepared using the sulfide-mediated polyol process. In a typical synthesis, 6 mL of EG was preheated to 155 °C for 1 h under magnetic stirring. EG solutions containing 3 mM Na₂S, 0.18 M PVP (calculated in terms of the repeating unit, Mw 55,000) were prepared. A 80 µL aliquot of the Na₂S solution was injected into the hot EG, followed by 1.5 mL of PVP solution and finally 0.5 mL of the AgNO₃ solution. The reaction underwent color changes from yellow to reddish brown to opaque green with plating on the vial walls. The reaction was completed in 20 min. The reaction solution was diluted with acetone, and the product was isolated by centrifugation. The product was washed twice with deionized water and then collected by centrifugation at 13,000 rpm for 5 min and re-dispersed by brief sonication in 4 mL of deionized water.

Synthesis of Ag nanospheres. In a typical process, a small aliquot of Ag nanocubes in water (10 µL of small nanocubes or 50 µL of large nanocubes) was added to a small centrifuge tube containing PVP solution (1 mg/mL in water). The exact volume of PVP solution was adjusted slightly for each reaction so that the total volume was 0.5 mL to allow for straightforward comparisons of UV-vis spectra. Different amounts of etching solution were then added, and the centrifuge tube was immediately capped and transferred to a vortex mixer for 15 s. The products were allowed to equilibrate for 10 min, at which point a UV-vis spectrum was taken and the particles were quickly washed via centrifugation and re-dispersion in ethanol a minimum of 3 times before being re-dispersed in water for imaging. The particles were typically collected by spinning at 13,200 rpm for 4-7 min. It was critical that all etching solutions were made fresh daily. The 45-nm Ag nanocubes were etched with
a ferric nitrate solution, typically 0.5-5 mM depending on the concentration of the
cube suspension. The 144-nm Ag nanocubes were etched with a light-sensitive
ferricyanide-based solution that contained 100 mM K$_2$S$_3$O$_3$, 10 mM K$_3$Fe(CN)$_6$, and
1 mM K$_4$Fe(CN)$_6$.

**Synthesis of Au-Based Nanocages.** The Au-based nanocages were prepared using
the galvanic replacement reaction between Ag nanocubes and AuCl$_4^-$. In a typical
synthesis, 50 µL of a 3.5 nM Ag nanocube solution was dispersed in 5 mL of
deonized water containing 1 mg/mL of PVP in a 50 mL flask under magnetic stirring
and then heated to boil for 10 min. Simultaneously, a 0.2 mM AuCl$_4^-$ aqueous
solution was prepared. An aliquot of the AuCl$_4^-$ solution was added to the reaction
flask via a syringe pump at a rate of 45 mL/h under magnetic stirring. The solution
was heated for another 10 min until the color of the reaction was stable. Once cooled
to room temperature, the sample was washed with NaCl saturated solution to remove
AgCl and the with deionized water several more times to eliminate PVP and NaCl.
The product was collected by centrifugation and re-dispered in water.

**Electron Microscopy and Spectroscopic Characterization.** The nanoparticles
were characterized by both TEM and SEM. An FEI TEM (Tenai G2 Spirit Twin,
Hillsboro, OR) operated at 120 kV was used to take images of the nanocubes and
nanocages. An FEI field-emission SEM (Nova NanoSEM 230, Hillsboro, OR) with
an accelerating voltage of 15 kV was used to image the particles as well as to take
energy-dispersive X-ray spectroscopy (EDX) measurements on the Au-Ag
nanocages. Nanoparticle dimensions were obtained from the TEM and SEM images
using ImageJ (Wayne Rasband, NIH) software. The LSPR spectra of the
nanoparticles were recorded with a Varian Cary 50 UV-vis spectrophotometer equipped with a tungsten lamp. For each sample, the nanoparticle concentration was estimated by determining the Ag\(^+\) and/or Au\(^+\) concentration with an inductively coupled plasma spectrometer (ICP-MS, Agilent 7500ce) and using this knowledge with the nanoparticle dimensions from TEM/SEM imaging and element compositions from EDX analysis. For this study, nanoparticle concentrations were determined in order to remove the effect of concentration on SERS, as more concentrated samples would report higher intensities.

**Particle Preparation and Functionalization.** The nanoparticles were functionalized with a 1 mM ethanol solution of 1,4-BDT or 4-MBT over a period of 6 h. Ethanol was used to wash the samples several times before resuspension in water to achieve concentration of 3-6 nM (particles per liter).

**Raman Spectroscopy Measurements.** Normal Raman spectroscopy was done with solutions of 10 mM of benzenethiol in basic NaOH (~6 M) where the molecule is in anion form and much more soluble. This was verified by the absence of any S-H stretching for solutions containing 1,4-BDT and 4-MBT. For these samples \(\lambda_{\text{ex}} = 514\) nm, \(P_{\text{laser}} = 4\) mW, \(t = 30\) s.

**Surface-Enhanced Raman Spectroscopy Measurements.** The Raman spectra were recorded from a solution phase using a Renishaw inVia confocal Raman spectrophotometer coupled to a Leica microscope with a 50× objective (N.A.=0.90) in backscattering configuration. The 514 nm wavelength was generated from an argon continuous wave (cw) laser and used with a holographic notch filter based on a grating of 1,200 lines per millimeter. The 785 nm excitation was from a
semiconductor cw diode laser and used with a holographic notch filter with a grating of 1,200 lines per millimeter. The backscattered Raman signals were collected on a thermoelectrically cooled (-60 °C) CCD detector. Sample cells were constructed by attaching the cap of a microcentrifuge tube to a glass slide. The cap acted as a vessel for the liquid sample, and a glass cover slip (0.17-0.13 mm) was carefully placed on top to eliminate solvent evaporation and to act as a reference point from which the focal volume was lowered to a depth of 200 µm into the sample. SERS data was collected with $\lambda_{\text{ex}} = 514$ nm, $P_{\text{laser}} = 3.1$ mW, and $t = 60$ s and $\lambda_{\text{ex}} = 785$ nm, $P_{\text{laser}} = 5.1$ mW, and $t = 60$ s.

Preprocessing of the Raman spectra and all data analysis was done with IGOR Pro software (Portland, OR). All data was baseline corrected before normalization. For the baseline correction a fourth order polynomial was fitted to the raw Raman spectrum and subtracted. For glucose detection peak normalization was used. The SERS spectral intensities were normalized using the peak at 706 cm$^{-1}$ from the 1-DDT SAM. The normalization factor for each spectrum was determined by subtracting the intensity of the peak at 706 cm$^{-1}$ from the background of the spectrum. This absolute intensity was then divided by the average absolute intensity calculated for each experiment. This allowed for complete removal of the 1-DDT SAM spectrum. For determining the effect of glucose on the 1-DDT, vector normalization was done by calculating the sum of the squared intensity values of the spectrum and using the squared root of this sum as the normalization constant. We found that this normalization method was most agreeable to interpreting the structural changes in the
1-DDT SAM, as most of the Raman peaks varied and were not suitable for peak normalization.
Figure 5.1 (A) SEM image of Ag nanocubes with an edge length of 111 nm. The scale bar corresponds to 300 nm. (B) UV-visible extinction spectra of the Ag nanocubes (NC) suspended in water before (black line) and after (red line) functionalization with the 1-DDT SAMs.
Figure 5.2 SERS spectrum from 1-DDT SAMs on Ag nanocubes suspended in water. \( \lambda_{\text{ex}} = 514 \text{ nm} \), \( P_{\text{laser}} = 4 \text{ mW} \), \( t = 60 \text{ s} \). The scale bar corresponds to 12 adu mW\(^{-1}\) s\(^{-1}\).
Table 5.1
Assignments of Raman bands and comparison of peak frequencies (cm⁻¹) for 1-DDT SAMs on Ag nanocubes and a smooth Ag surface, respectively.

<table>
<thead>
<tr>
<th>Assignment</th>
<th>Adsorbed Ag nanocubes</th>
<th>Adsorbed Ag surface&lt;sup&gt;a&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>ν(C-S)&lt;sub&gt;g&lt;/sub&gt;</td>
<td>632</td>
<td>632</td>
</tr>
<tr>
<td>ν(C-S)&lt;sub&gt;r&lt;/sub&gt;</td>
<td>706</td>
<td>706</td>
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<tr>
<td>CH&lt;sub&gt;2&lt;/sub&gt; rock</td>
<td>866</td>
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<td>870</td>
</tr>
<tr>
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<td>890</td>
<td>894</td>
</tr>
<tr>
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<td>-</td>
</tr>
<tr>
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<td>1065</td>
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<sup>a</sup>Assignments and frequencies taken from [9,11].
Figure 5.3 SERS spectra taken from the 1-DDT SAMs on Ag nanocubes after they had been mixed with aqueous glucose. The glucose concentrations were: (A) 250 mM, (B) 175 mM, (C) 100 mM, (D) 30 mM and (E) 0 mM. (F) Raman spectrum of a saturated aqueous solution of glucose. (G-J) Subtraction spectra displaying the presence of glucose in each sample. $\lambda_{\text{ex}} = 514$ nm, $P_{\text{laser}} = 4$ mW, $t = 60$ s. The scale bar corresponds to 12 adu mW$^{-1}$ s$^{-1}$.
**Figure 5.4** Structural changes of the 1-DDT SAMs upon adsorption of glucose. (A) The C-H region and (B) the C-C region. The broken line represents the 1-DDT SAMs on Ag nanocubes in contact with 250 mM aqueous glucose and solid line represents the 1-DDT SAMs on Ag nanocubes in contact with pure water. $\lambda_{ex} = 514$ nm, $P_{laser} = 5$ mW, $t = 2$ min. The scale bar corresponds to 12 adu mW$^{-1}$ s$^{-1}$. 
Table 5.2
SERS intensity ratios for 1-DDT SAM with glucose (250 mM) and without glucose (0 mM).

<table>
<thead>
<tr>
<th>Mode</th>
<th>Ratio$^a$</th>
<th>1-DDT SAM</th>
<th>1-DDT SAM/glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\bar{v}_{as}(\text{CH}_2)$</td>
<td>$v_{s}$(CH$_3$,FR)</td>
<td>$I_{2904}/I_{2936}$</td>
<td>0.59 ± 0.06</td>
</tr>
<tr>
<td>$\bar{v}_{as}(\text{CH}_3,FR)$</td>
<td>$v_{s}$(CH$_2$)</td>
<td>$I_{2936}/I_{2850}$</td>
<td>2.61 ± 0.05</td>
</tr>
<tr>
<td>$\bar{v}_{as}(\text{CH}_2)$</td>
<td>$v_{s}$(CH$_2$)</td>
<td>$I_{2904}/I_{2850}$</td>
<td>1.54 ± 0.05</td>
</tr>
</tbody>
</table>

$^a$Subscripts indicate frequency shifts in cm$^{-1}$. 
Figure 5.5 (A) Ag nanocubes and (B-D) Au-based nanocages used in this study. The scale bars are 500 nm and 100 nm for the SEM and TEM (inset) images, respectively. (E) Extinction spectra of the nanocubes and nanocages. The nanocages were prepared from the nanocubes in (A) with the galvanic replacement reaction and the LSPR peak was tuned to (B) 525 nm, (C) 620 nm, and (D) 790 nm. The vertical lines in (E) correspond to the wavelengths of the excitation sources used for SERS.
Figure 5.6 (A) The SERS spectra from 1-DDT SAMs on Ag nanocubes in water at four different solution temperatures with 514 nm excitation. The temperature of the solution was adjusted with a temperature-controlled stage. Each spectrum contains the gauche (G, at 1080 cm$^{-1}$) and trans (T, at 1125 cm$^{-1}$) carbon-carbon stretch of the 1-DDT SAMs (B) A plot of the temperature and peak intensities of the T and G bands, where an increase in the solution temperature causes the T band to attenuate and the G band to increase. (C) The SERS spectrum of aqueous 1-DDT nanocubes at excitation wavelengths 514 (red) nm and 785 nm (black). The scale bar corresponding to 10.8 adu mW$^{-1}$ s$^{-1}$. 

**Figure 5.7** (A-C) The SERS spectra of 1-DDT SAMs on Au-based nanocages in water with 514 nm (red) and 785 nm (black) excitation, respectively. The LSPR of the nanocages was tuned to (A) 525 nm, (B) 620 nm, and (C) 790 nm. (D) The SERS spectra of 1-DDT SAMs on nanocages (LSPR: 525 nm) with 514 nm (red) and 785 nm (black) excitation in continuous cycles, showing the reversible nature of the trans-gauche conformational change. (E) SERS spectra of 1-DDT SAMs on nanocages (LSPR: 525 nm) showing other bands associated with the 1-DDT SAM with 514 nm (red) and 785 nm (black) excitation. The scale bars correspond to 14.0 adu mW$^{-1}$ s$^{-1}$. For all spectra, $t = 120$ s and $P_{\text{laser}} = 4.5$ mW for 514 nm, and 5.2 mW for 785 nm.
Figure 5.8 (A) Optimized alkanethiol conformation of a 1-DDT SAM on an extended Au surface at three different temperatures as revealed by molecular dynamic (MD) simulations. The cartoons are looking down the chain toward the sulfur group where grey, white, and black colors represent carbon, hydrogen, and sulfur, respectively. When the temperature was increased, the torsion of the alkanethiols increased and there was a higher population of end-gauche and gauche conformations as evidenced by the increasing non-planar character of the alkanethiolate molecule. (B) A plot of the trans/gauche ratios of the 1-DDT SAM from experimental (square markers) and MD simulation (triangular markers) data. (C) Temperatures of the 1-DDT SAMs on nanocages derived from the fit in (B) for different excitation wavelengths and LSPR peak positions and the increase in surface temperature (ΔT).
5.7. Notes to Chapter 5


Chapter 6

Imaging with SERS

6.1. Introduction

The use of Raman scattering to create images has recently received attention as a viable imaging technique due to the explosion of nanotechnology research. The low cross sections inherent in Raman scattering can be enhanced by many orders of magnitude with metal nanoparticles. Molecules on a nanoparticle’s surface provide the signals that can be used to construct a SERS image and are often called “SERS tags”. SERS imaging takes advantage of the rich chemical information that is contained in a SERS spectrum to create images of metal nanoparticle distributions.\cite{1-6} When conjugated with targeting ligands such as monoclonal antibodies, peptides, or small molecules, nanoparticles can be used to target cells and tissue with high specificity and affinity for SERS imaging in addition to therapeutic and diagnostic applications \textit{in vivo}.\cite{4,5}

Some of the benefits of SERS imaging are its multiplexing capabilities, sensitivity, and real-time data feedback.\cite{3,7} For example, by simply changing the molecules attached to metal nanoparticle’s surface, many unique SERS probes can easily be fabricated.\cite{8} SERS vibrational bands are also much narrower compared with fluorescent bands, allowing for easier and more accurate data analysis and image construction.\cite{6} Finally, only a single excitation source is needed (unlike quantum dots and fluorescent molecules) to generate SERS from several different contrast agents,
and the excitation can be conveniently tuned to the near-infrared region (NIR) where soft tissue, blood, and water have minimum absorption. For these reasons, SERS continues to be developed as a novel imaging technique.

This Chapter explores the ability of our Raman system to generate SERS images with Ag nanocubes or nanospheres. In this work the SERS images are closely compared with their corresponding physical objects. This allows us to characterize several parameters of our Raman system like the blur and spatial resolution. In addition, phantoms are used to mimic the scattering and absorption of tissue, and the penetration depth of SERS imaging is determined. I also investigate the capability of our Raman system to create SERS images of three-dimension objects with micrometer dimensions.

6.2. Preparation of Phantoms and Silver Nanoparticles

The phantoms were made of poly(vinyl alcohol) (PVA) gels. An aqueous solution of PVA will form a gel upon standing at room temperature, but the gel has a low mechanical strength and cannot support its own weight. Reinforcement could be achieved by enhancing the cross-linking between the polymer chains by freezing and then thawing the gel. This method is based on physical cross-linking, which avoids additives and complex procedures that are often involved in chemical cross-linking. Simply freezing and thawing of an aqueous solution of PVA results in a gel whose mechanical strength progressively increases with the number of freezing and thawing cycles. Furthermore, the scattering and absorption properties of PVA gels have been reported, and by optimizing the number of freezing and thawing cycles, one can
easily obtain PVA gels with optical properties similar to those of soft tissue.\textsuperscript{[10]} It this study the gels were cast and then frozen for 12 h followed by a thawing period for 12 h. This counted as one cycle, and four cycles were used for the PVA-gel phantoms used in the experiments described in this Chapter.

The Ag nanoparticles were prepared as described in previous Chapters and were functionalized with the molecule 1,4-BDT. The particles were then suspended in water and their concentration was determined by ICP-MS analysis. For single-particle SERS studies the nanoparticles were deposited on a Si substrate, located with dark-field microscopy, and probed with our Raman system as detailed in Chapter 3. The 1,4-BDT molecule is non-resonant molecule, and resonance affects were not present with the 785 nm excitation used in these experiments. The SERS band at 1562 cm\textsuperscript{-1} was used to construct the SERS images unless otherwise noted.

6.3. The Spatial Resolution and Penetration Depth of SERS

In this section Ag nanocubes and nanospheres are used as contrast agents in SERS imaging experiments where the effects blur, spatial resolution, penetration depth, and nanoparticle aggregation to SERS imaging are determined.

6.3.1. The Blur and Spatial Resolution

To determine the spatial resolution of our Raman system we first characterized the blur value ($B_o$) associated with a SERS image. Blur takes into account that an image is a visual representation of a specific physical object.\textsuperscript{[11]} Ideally, each small point within the object would be represented by a small, well-defined point within the
image. In reality the image of each object point is spread or blurred within the image. The amount of blurring can be expressed as the dimension of the blurred image of a very small object point. Figure 6.1 shows the SEM of a single nanocube with a 100 nm edge length, the Rayleigh scattering image, and the SERS image of the same nanocube. The nanocube is small enough to be used as an object point from which $B_\nu$ can be determined for the images. A $B_\nu$ of 0.012 mm and 0.005 mm were determined for the Rayleigh scattering and the SERS image, respectively. In contrast, typical blur values are 0.15 mm for mammography and 0.5 mm for photoacoustic tomography.$^{[12]}$

Closely related to blur is spatial resolution, which describes the ability of an imaging system to distinguish or separate objects that are next to each other. The ability of our Raman system to resolve individual nanoparticles that were closely spaced was determined in order to evaluate the spatial resolution of the system. The resolving capability of a particular imaging system can also be inferred by the amount of blur. Based on the measurements of blur from a single nanocube above we expect to resolve nanocubes that are $\sim 1 \mu m$ apart.

To verify this, we designed an experiment where a linear array of nanocubes with different distances from one to another was formed on a Si substrate. Drop-casting a dilute suspension of nanocubes so that the outer edge of the meniscus slowly dried could easily form these linear arrays of nanocubes. Figure 6.2A shows the SEM image an array of five nanocubes and Figure 6.2B shows the Rayleigh scattering image from the same array. The red line in Figure 6.2B shows the path of the Raman microprobe acquisition, and the peak intensity of the 1562 cm$^{-1}$ band from 1,4-BDT was plotted along this line in Figure 6.2C. This graph shows that nanocubes with
more than 2 µm separation from each other can easily be resolved in the SERS image with the naked eye. However, greater resolution could be achieved by determining the area between the peaks plotted in Figure 6.2C, which corresponded the locations of the nanoparticles. As the area between the peaks ($P_a$) approached zero, the distance between the nanoparticles approached 1.1 µm, which represented the spatial resolution of this system. The Rayleigh scattering is not capable of achieving this resolution and nanocubes with a 1.1 µm separation appear as one object.

We also examined the effect of increasing the acquisition step size to the spatial resolution. A larger step size will decrease the number of acquisitions over an area and also reduce the acquisition time. In Figure 6.3, a $9.6 \times 9.3$ µm$^2$ area was mapped with a 300 nm, 700 nm, 1.5 µm, 3 µm, and 5 µm step size. As the step size increases from 300 nm to 2 µm the resolution does not change dramatically. However, when the step-size increased beyond 2 µm, the resolution decreased, intensities decreased, and the nanocubes were not identifiable in the SERS image. In addition, we measured $P_a$ as a function of step size, as shown in Figure 6.3H, which also shows a sharp decline in resolution beyond 2 µm. This value is close to the estimated diameter of the acquisition volume (approximately 1.8 µm) of our SERS system. Step sizes smaller than ~1.8 µm would result in the SERS acquisitions from overlapping areas. This can explain why the resolution is fairly constant for the smaller step sizes, but decreases significantly for step sizes larger than 2 µm.

### 6.3.2. The Penetration Depth

Optical methods, including SERS, can be greatly influenced by strong tissue
scattering and blood absorption.\textsuperscript{[13,14]} Therefore, it is important to determine the effects of scattering/absorption on SERS imaging.\textsuperscript{[4]} The penetration depth is an important parameter which describes how deep light can penetrate into a material and still be used for image construction. To evaluate the penetration depth we made a phantom consisting of a tygon tube filled with an aqueous suspension of 1,4-BDT functionalized nanocubes. The tube was then embedded in a PVA-gel. The experiment is schematically shown in Figure 6.4A. The distance between the top of the tygon tube and the top of gel ($d_c$) was varied from 1.5 mm to 2 cm. In Figure 6.4B, the SERS intensity for the nanocubes is plotted as a function of distance into the PVA gel with four different $d_c$ values: 1.5, 10, 12.5, and 15 mm. The step size of the acquisition was 100 $\mu$m. The graphs clearly show the ability to resolve the tube up to 15 mm, beyond which the signal becomes difficult to separate from the noise. The peak values were calculated at 1.7, 10.1, 12.8, and 14.3 mm, which correspond well to $d_c$.

Additionally, we determined the spatial resolution of the SERS system in the PVA-gels by comparison of the peak widths in Figure 6.4B to the actual diameter of the tube ($\sim 900 \mu m$). With $d_c = 1.5$ mm, the full-width half max (FWHM) of the peak was calculated to be $\sim 950 \mu m$ which is in close agreement with the diameter of the tube. In this case the resolution is not affected, probably due to the large size of the tube. However, as $d_c$ is increased to 15 mm the peak intensity decreased significantly and the FWHM increased to 2.2 mm. We found that beyond 10 mm the spatial resolution is significantly decreased due to the increased scattering from the PVA-gel phantom. The gel effectively spreads the image of objects into the surrounding
background resulting in reduced resolution.

In Figure 6.4C, the SERS peak intensity for three different concentrations of nanocube solutions are plotted as a function of $d_c$. We used nanocube concentrations of 10 nM ($6 \times 10^{12}$ particles/mL), 1 nM ($6 \times 10^{11}$ particles/mL), and 1 pM ($6 \times 10^9$ particles/mL). These are concentrations that are typically used intravenously for in vivo imaging.\cite{15,16} Decreasing the concentration to the pM region dramatically reduced the penetration depth to $d_c = \sim 4$ mm. With higher concentrations ($\sim 10$ nM) the Raman system could resolve the tube to 2 cm. The relationship between concentration and penetration depth was measured based on the values determined in Figure 6.4C, and was linear ($R^2 = 0.968$). The data showed that as the concentration is reduced a factor of 5 the penetration depth will be reduced by 2.2 mm. This results in large dynamic range for SERS imaging, where the same acquisition parameters can be used for a broad range of nanoparticle concentrations.

Nanoparticles used in vivo accumulate at tumor regions based on the enhanced permeability and retention (EPR) mechanism,\cite{17} or they are targeted to specific regions via monoclonal antibodies.\cite{18} They are not expected to be present as concentrated suspensions in vivo. SERS imaging studies typically do not investigate the morphology or the aggregation of the nanoparticles in their images,\cite{4,7,19} even though it is well known aggregation affects the SERS signals dramatically.\cite{20} The relationship between SERS imaging and nanoparticle aggregation and/or morphology is therefore not clear. It is very likely SERS contrast agents do aggregate in vivo, as many studies have indicated that when cells uptake nanoparticles, the nanoparticles are localized to endosomes where aggregation may occur.\cite{1,2,5,21} We used nanocubes,
nanospheres, and their aggregates, to determine what impact aggregation has on SERS imaging.

In Figure 6.5 nanocubes and nanospheres were deposited onto a Si substrate and a PVA-gel was placed on top of them during the SERS measurements. Figure 6.5A shows a typical phantom where $d_s$ is the distance from the top of the gel to the Si substrate. The SERS signals from nanocubes and nanospheres with different morphologies (a single nanoparticle, a dimer, and a trimer as seen in Figure 6.5C) were recorded with the techniques described in Chapter 3. Figure 6.5B plots the intensity of the 1562 cm$^{-1}$ band from 1,4-BDT for $d_s$ values of: 1.5, 3, 4, and 5 mm. With $d_s = 1.5$ mm the SERS signals were detectable for all the different nanoparticle morphologies. However, when $d_s = 3$ mm, the SERS for the single nanoparticles were attenuated to undetectable levels. Ultimately, only the trimer morphologies had SERS signals that could be measured at $d_s = 5$ mm, and it did matter if the trimers were composed of nanocubes or nanospheres.

This clearly shows the role aggregation plays in SERS imaging and suggests many studies using Au and Ag nanostructures rely on their aggregation even though this is not explicitly stated.$^{[1-5,7,21]}$ A nanoparticle’s ability for generating SERS images should be determined by how well the particle aggregate, and not necessarily the SERS activity of a single nanoparticle.$^{[4]}$

6.4. Three-Dimensional Imaging with SERS

A SERS image of a three-dimensional object could provide valuable information about the distribution of nanoparticles in a cell, organ, or organism.$^{[22]}$ While the back
scattering mechanism of SERS is generally expected to limit its use in vivo, the sensitivity of SERS and its ability to identify a broad range of molecules (like fluorescent probes) continues to push the development of SERS imaging forward. In particular, the use of SERS during surgery (i.e., intraoperatively) eliminates the attenuating effects from the penetration depth because the regions of interest are surgically exposed. SERS can therefore be extremely useful in locating tumor periphery and evaluating tumor resections during surgery, due to the real-time and sensitive signals capable with SERS.\cite{23,24} Imaging with SERS over millimeter regions intraoperatively is therefore expected to contribute to diagnostic and therapeutic cancer procedures.\cite{23}

We developed a phantom experiment to determine the ability of our Raman system to image a three-dimensional structure over an 8 mm$^3$ region. The phantom consisted of two glass micro-capillary tubes (700 µm in diameter) filled with a 1 nM solution of 1,4-BDT functionalized nanocubes. The tubes were embedded 5 mm into a PVA gel and were crossed to form an “X” shape. In Figure 6.6A, a large SERS image (step size 500 µm, acquisition time 10 min) of the phantom is shown with the tubes labeled i and ii. The white broken line in the image represents the cross section mapped of the SERS image in Figure 6.6B, which clearly resolves the two tubes along the z axis (step size 500 µm in z and x).

A 4.0 × 6.1 mm$^2$ area that encompassed the tubes was chosen as the region where several x,y-plane sections were mapped at discrete z intervals. Values of 1.5 ≥ z ≥ -1.5 were chosen based on the cross sectional SERS image of the phantom in Figure 6.6B. In total, 15 sections were generated with a 200 µm distance between sections.
WiRE software was used to create the images from these sections using the 1562 cm\(^{-1}\) peak from 1,4-BDT. These images were then saved as 24-bit RGB images in tiff file format. The files were then combined into a single file and the 15 SERS images of the x,y-plane section were stacked and combined to generate an image of the three-dimensional phantom using IMOD software. Figure 6.6C shows the SERS image of the tubes generated in IMOD. The total acquisition time for the image was 43 min. The image has excellent resolution and can clearly resolve the diameter of the tubes (~675 µm). Decreasing the individual acquisition time (15 s) and increase step sizes could significantly decrease the overall acquisition time.

6.5. Summary

This work represents the first characterization of the imaging capabilities and limitations of a Raman system using well-defined nanoparticles. With our system we could resolve individual nanocubes that were ~1.1 µm away from each other on a Si substrate. Raman mapping step sizes below 2 µm did not dramatically increase the resolution, and can serve as the upper limit for SERS imaging of nanoparticle distributions. In phantom experiments SERS could penetrate up to 2 cm into PVA-gel phantoms to detect aqueous nanoparticles and the penetration depth increased linearly with nanoparticle concentration.

The experiments comparing single nanocubes and nanospheres to their aggregates clearly demonstrated the importance of aggregation in SERS imaging. Single nanostructures did not have an appreciable SERS signal when a 3 mm PVA-gel was placed on top of them. The nanocubes and nanospheres used in this study have large
EFs and are comparable (or better) than many nanoparticles. Therefore, we expect aggregation to play a large role in SERS imaging, particularly in situations where scattering from tissue is present.

Finally, we showed that our system is capable of imaging three-dimensional structures and can differentiate features of ~700 µm. The main limitations are the large acquisition time (~40 min) and data processing. However, additional developments in data analysis software and acquisition hardware could dramatically reduce time needed for data acquisition and image construction.

6.6. Experimental Section

**Instrumentation.** The Raman mapping was accomplished with a high speed encoded stage (HSES) system capable of step sizes of 100 nm in x,y, and z dimensions at speeds of 80 mm/s and a range of 112 mm in x and 76 mm in y. This system was combined with a Renishaw inVia confocal Raman spectrophotometer coupled to a Leica microscope with a 50× objective (N.A.=0.90) in backscattering configuration. The 785 nm excitation was from a semiconductor cw diode laser and used with a holographic notch filter with a grating of 1,200 lines per millimeter. The laser power was set at 3.1 mW. The backscattered Raman signals were collected on a thermoelectrically cooled (-60 °C) CCD detector.

**Data Analysis.** The two-dimension SERS images were generated with WiRE Mapping Review software. The peak value at 1562 cm⁻¹ and the x,y coordinates, were used to create data sets which could be mapped into two-dimension images of the nanoparticle locations and further modified with the WiRE software, or converted
into a matrix with Origin software for additional mapping and determination of peak-to-peak areas.

In the penetration depth experiments, the spectra were baseline corrected and normalized. For the baseline correction a fourth order polynomial was fitted to the raw Raman spectrum and subtracted. Vector normalization was done by calculating the sum of the squared intensity values of the spectrum and using the squared root of this sum as the normalization constant. For the single-particle studies (Figure 6.5), the SERS data was normalized to the Si peak centered at ~950 cm\(^{-1}\).

**Preparation of PVA gel phantoms.** PVA with an average molecular weight (MW) of 85,000 was used to prepare aqueous solutions. A PVA concentration of 20% by weight in solution was obtained by heating the appropriate amounts of PVA and demineralized water over a temperature bath at 95 °C for 2 h. Continuous gentle stirring is required to ensure homogeneity and promote dissolution of the PVA. The solution was allowed to stand for a few hours to allow any air bubbles to migrate to the surface from where they can be skimmed off. The solution was then cast in the required moulds and refrigerated at 20 °C for 12 h. Subsequently, the frozen solution was thawed at room temperature for 12 h. This constituted one freezing-thawing cycle. The gels in this study were prepared with four freeze and thaw cycles as this gave the gels optical characteristics similar to tissue.\(^{[10]}\)
Figure 6.1 (A) SEM image of a Ag nanocube with an edge length of 100 nm. (B) A dark-field image from the Rayleigh scattering of the same nanocube in (A). The diameter of the image in (B) 1.2 µm. (C) SERS image of the nanocube in (A). The SERS image had a diameter of 0.5 µm. $\lambda_{ex} = 785$ nm, $t = 2$ s, $P = 3.1$ mW.
Figure 6.2 (A) SEM image of Ag nanocubes (edge length ~100 nm) on a Si substrate and their corresponding dark-field image in (B). The red line corresponded to the path of the Raman acquisition with included the five nanocubes labeled in (A). Data was acquired over this red line with a step size of 200 nm. (C) A plot of the SERS intensity from 1565 cm\(^{-1}\) along the red line shown in (B), which clearly resolves the nanocubes. The distance between (i) and (ii) was 2.8 µm; (ii) and (iii) was 1.7 µm; (iii) and (iv) was 1.4 µm; and (iv) and (v) was 3.9 µm. \(\lambda_{\text{ex}} = 785\) nm, \(t = 2\) s, \(P = 3.1\) mW. (D) Plot of the area between the peaks in (C) as a function of the distance between neighboring nanocubes. As the distance between neighboring nanocubes approached 1.1 µm, \(P_a \approx 0\), which is the spatial resolution.
Figure 6.3 (A) SEM image of Ag nanocubes functionalized with 1,4-BDT with the white box corresponding to the SERS mapping area (9.6 × 9.3 μm²). (B) The corresponding dark-field scattering image from (A). In (C-G) the SERS intensity of the 1562 cm⁻¹ peak from the nanocubes is mapped with different step sizes as indicated in the SERS map. λex = 785 nm, t = 2 s, P = 3.1 mW. (H) A plot of the area between the SERS peaks for nanocubes (P_a) as a function of the acquisition step size. The scale bar corresponds to 5 μm.
Figure 6.4 (A) Schematic of the experimental setup used to determine the penetration depth of SERS in a PVA-gel. Aqueous nanocubes (NC) were suspended inside a tygon tube with diameter of ~900 µm. The distance $d_c$ was measured from the top of the PVA-gel to the top of the tube. (B) Plots of the SERS intensity from the peak at 1562 cm$^{-1}$ as a function of distance (z) into the PVA gel. The value of $d_c$ is shown at the top of each plot. The peaks correspond to the SERS from the nanocubes in the tube. (C) A plot of the SERS intensity from the peak at 1562 cm$^{-1}$ as a function of $d_c$ for three different nanocube concentrations. $\lambda_{ex} = 785$ nm, $t = 30$ s, $P = 3.1$ mW.
Figure 6.5 (A) Image of a PVA-gel on top a Si substrate that supported nanocubes or nanospheres. The distance from the top of the gel and the Si substrate is labeled \( d_s \).

(B) The relative SERS intensities from nanocubes (black) and nanospheres (red) with various morphologies. The SERS was recorded from single nanoparticles, dimers, and trimers with a \( d_s \) of 1.5, 3, 4, and 5 mm. (C) Typical SEM images of the nanocubes and nanospheres and their dimer and trimer configurations studied in (B).

\[ \lambda_{ex} = 785 \text{ nm}, \; t = 5 \text{ s}, \; P = 3.1 \text{ mW}. \]
Figure 6.6 (A) SERS image of two micro-capillary tubes (labeled i and ii) that contain a 1 nM suspension of Ag nanocubes functionalized with 1,4-BDT. The tubes had a diameter of 700 µm and were embedded in a PVA gel phantom. (B) SERS image of a cross section (x,z-plane) of the same phantom shown in (A) along the white broken line. The image shows that both tubes can be clearly resolved. (C) SERS image of a three-dimensional 8 mm³ region of the phantom which contained the tubes crossing each other as shown in (B). The step size was 200 µm for all dimensions. $\lambda_{ex} = 785$ nm, $t = 15$ s, $P = 3.1$ mW.
6.7. Notes to Chapter 6


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