Control of Surface Plasmon Substrates and Analysis of Near field Structure

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Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Electrical and Computer Engineering in the Graduate School of Duke University

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ABSTRACT

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Abstract

The electromagnetic properties of various plasmonic nanostructures are investigated. These nanostructures, which include random clusters, controlled clusters and particle-film hybrids are applied to surface-enhanced Raman scattering (SERS). A variety of techniques are utilized to fabricate, characterize, and model these SERS-active structures, including nanoparticle functionalization, thin film deposition, extinction spectroscopy, elastic scattering spectroscopy, Raman scattering spectroscopy, single-assembly scattering spectroscopy, transmission electron microscopy, generalized Mie theory, and finite element method.

Initially, the generalized Mie theory is applied to calculate the near-field of the small random clusters to explain their SERS signal distribution. The nonlinear trend of SERS intensity versus size of clusters is demonstrated in experiments and near-field simulations.

Subsequently, controlled nanoparticle clusters are fabricated for quantitative SERS. A 50 nm gold nanoparticle and 20nm gold nanoparticles are tethered to form several hot spots between them. The SERS signal from this assembly is compared with SERS signals from single particles and the relative intensities are found to be consistent with intensity ratios predicted by near-field calculation.

Finally, the nanoparticle/film hybrid structure is studied. The scattering properties and SERS activity are observed from gold nanoparticles on different substrates. The gold nanoparticle on gold film demonstrates high field enhancement. Raman blinking is
observed and implies a single molecule signal. Furthermore, the doughnut shape of Raman images indicates that this hybrid structure serves as nano-antenna and modifies the direction of molecular emission.

In addition to the primary gap dipole utilized for SERS, high order modes supported by the nanoparticle/film hybrid also are investigated. In experiments, the HO mode show less symmetry compared to the gap dipole mode. The simulation indicates that the HO modes observed may be comprised of two gap modes. One is quadrupole-like and the other is dipole-like in terms of near-field profile. The analytical treatment of the coupled dipole is performed to mimic the imaging of the quadrupole radiation.
Dedication

To my family.
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Chapter 1 Introduction

1.1 Surface Enhanced Raman Scattering

1.1.1 Raman Scattering

Raman scattering is an inelastic process that occurs when photons exchange vibrational quanta of energy with a molecule. The incident photon $h\nu_0$ is scattered at an energy shifted by an amount, $h\nu_r$, equal to a vibrational quantum for one of the molecule’s vibration modes. If the photon excites a molecule already in an excited vibrational level, the scattered photon may be shifted to higher energy $h\nu_0 + h\nu_r$. Raman scattering at higher frequency is called anti-Stokes scattering, in contrast to Stokes scattering at $h\nu_0 - h\nu_r$ that may occur when a photon interacts with a molecule in a ground vibrational level [1]. The Stokes signal is usually stronger than anti-Stokes because the probability that a molecule is in the vibration ground state at room temperature is typically $10^6$ higher than that it is in an excited state according to the Maxwell-Boltzmann distribution [2]. Raman spectra are highly useful because they provide detailed molecular structural information such as the presence of functional groups and the nature of their environment. The specificity of the information contrasts greatly with that provided by a fluorescence spectrum which is a broadband signal. In addition, there are several other advantages of Raman spectroscopy [3]:

1. The transparency in water: when using visible light excitation, the Raman scattering of water, whose dominant feature is $3400 \text{ cm}^{-1}$ from O-H stretch mode [4], is
on the order of $10^{-27}$ cm$^2$/molecule. This is a pretty weak signal. Thus, Raman spectroscopy is ideal especially for the studies of biological molecules in aqueous solution.

2. Non-destructivity and the lack of need for sophisticated sample preparation. This means that Raman spectroscopy has a real time detection capability.

![Figure 1-1. Energy level of IR, Raman, and Fluorescence spectra.](image)

3. Complementary to IR spectroscopy because the selection rules are different such that vibrational signatures can be detected from IR-inactive bonds such as the symmetric C-C, C=C, S-S and O-O bonds. Among spectral methods, Raman is
particularly useful for displaying the presence of di-sulfide (S-S) cross-linkages in biomolecules.

4. Higher spatial resolution for imaging because of laser source located in UV-NIR range.

5. The same optical setup can be used to detect vibration modes over an extensive spectral range, i.e. from the IR to the far-IR.

Figure 1-2. Raman spectrum (left) and fluorescence spectrum (right) [5].

1.1.2 Surface-Enhanced Raman Scattering

Although Raman spectroscopy has promising properties, its use is not widespread; Raman scattering is used infrequently compared with the lower spectral resolution fluorescence spectroscopy. The main reason is that the efficiency of Raman scattering is extremely low. The typical Raman cross section is between $10^{-31}$ and $10^{-29}$ cm$^2$/molecule whereas a fluorescence cross section can be as large as $10^{-16}$ cm$^2$/molecule [5]. However, in 1974, Fleischman [6] observed the unusual high Raman intensity from
pyridine adsorbed on an electrochemically roughened silver electrode. At that time, he explained that this high Raman signal is the result of an increased number of molecules owing to the high surface area of the rough substrate surface. In 1977, Jeanmarie and Van Duyne [7], and Albrecht and Creighton [8] re-examined the experiments and pointed out that unusual Raman intensity is due to enhanced Raman cross sections not the increased number of molecules. From then on, SERS became an attractive research topic.

1.2 Localized Surface Plasmon and SERS

After surface-enhanced Raman was observed and confirmed, extensive effort has gone into explaining the mechanism of this attractive phenomenon. Philpott [9, 10] and Moskovits [11] proposed that the enhancement may be the result of excitation of surface plasmons, the electromagnetic excitations of electrons in a conductor that occur at the interface of the conductor and a dielectric material. These electromagnetic surface waves arise via the coupling of the electromagnetic field to oscillations of the conductor’s electron plasma. According to discrete or continuous nature of the substrate on which they are supported, surface plasmons can be divided into surface plasmon polaritons (SPP) and localized surface plasmons (LSP). SPPs propagate parallel to the surface and are confined evanescently perpendicular to the surface.

It is generally believed that the first observed SERS was due to excitation of SPPs on the silver electrode. For compact gold and silver nanoparticles with sizes less than 100 nm, localized surface plasmon resonances are located within the visible region of the spectrum. As with SPP, field near to the surface can be strongly enhanced at excitation
frequencies close to the LSP resonance frequency [12]. The first experimental SERS from silver and gold colloids was done by Creighton et al. in 1979.

### 1.3 SERS enhancement

In the “normal” Raman scattering, the total Raman signal is proportional to the number of molecules in the detected volume $N_f$, the Raman cross section $\sigma_f^R$, and excitation laser intensity $I(\nu_0)$ [5, 13].

$$P_{\text{Raman}}(\nu_f) = N_f \sigma_f^R I(\nu_0)$$

When Raman active molecules are adsorbed on (or close to) a field enhancing substrate (usually metal nanostructures), the Raman cross section and the field intensity may need to be modified.

$$P_{\text{SERS}}(\nu_f) = N_{\text{ad}} \sigma_{\text{ad}}^R G^E M I(\nu_0)$$

where $N_{\text{ad}}$ is number of molecules in the detecting surface, $\sigma_{\text{ad}}^R$ is the cross section of the molecules which are attached or closed to the metal surface, and $G^E M$ is the field enhancing factor. These factors may contribute to the enhancement of the RS. Chemical enhancement (also called electronic enhancement or first-layer effect) is usually thought to arise from the modification of the Raman polarizability tensor of the molecules as a result of the formation of a complex between the adsorbed molecules and the metal. Basically, $\sigma_{\text{ad}}^R$ represents this effect, even though it is hard to quantitatively analyze. In most SERS experiments, it is generally believed that the most significant source of
enhancement is the electromagnetic enhancement, $G^E M$ [14] [15] [16] [17], which can be expressed as

$$G^E M (\nu_0, \nu_s) = \left[ \frac{E_{loc} (\nu_0)}{E_{inc} (\nu_0)} \right] \left[ \frac{E_{loc} (\nu_s)}{E_{inc} (\nu_s)} \right] \approx \left[ \frac{E_{loc} (\nu_0)}{E_{inc} (\nu_0)} \right]^4$$

$E_{loc} (\nu_0)$ is the local field at the position of the molecule which, in the presence of a metal structure may be enhanced relative to $E_{inc} (\nu_0)$, the incident field. The first factor characterizes the enhancement of the excitation field, whereas the second term characterizes the enhancement at the Raman-scattered frequency. The enhancement at the scattering frequency is often approximated by the enhancement at the excitation frequency. The quality of the approximation is dependent on the Raman band, i.e. function of the magnitude of the Stokes shift relative to the range of frequencies over which the field enhancement is large.

### 1.4 An overview of the thesis

In Chapter 2, we present the near-field calculation to explain the nonlinearity of the SERS signals versus the size of the nano-clusters. In Chapter 3, the controlled nanoparticle clusters is demonstrated by using the DNA-linker to tether 50 nm core particle and 20 nm satellite particles. The near-field simulation is used for quantitative comparison of SERS from this assembly and from single nanoparticle. In Chapter 4, the scattering properties and SERS signals of nanoparticle on different substrates are investigated. The particle on gold thin film shows strong enhancement and Raman
blinking is observed. In Chapter 5, we continue to study the properties of the nanoparticle/film hybrid structures. The high order gap mode is studied through the far-field scattering profile, and near-field distribution. The results show that the HO mode observed from experiments may result from the superposition of a dipole-like mode and a quadrupole-like mode. In the Chapter 6, we analytically calculated the quadrupole radiation on the image plane and compared with the dipole emission.
Chapter 2  Intensity Scaling of SERS from Random Nanoparticle Clusters

We report morphology correlated surface-enhanced Raman scattering (SERS) from molecules on the surface of individual clusters of gold nanoparticles of two types, and compare the signal from clusters of two, three, four and five nanoparticles with the signal from single particles. Cluster geometry and particle morphology are determined from transmission electron microscopy for both clusters of 78 nm to 133 nm nanospheres and clusters of ~250 nm etched cylindrical particles with crevices and sharp edges, formed in templates. Scattering from molecules on etched cylinders, but not spheres, is sufficiently strong to allow spectra to be collected from single particles illuminated at 632.8 nm. SERS intensities from clusters of cylinders are found to scale linearly with particle number, whereas, for nanospheres, the scaling is nonlinear. The linear scaling of SERS from cylinders is a reflection of the high enhancement provided by the sharp features of the individual particles, whereas the nonlinear scaling of SERS from clusters of spheres is found to be consistent with the near field enhancement from inter-particle coupling simulated for clusters of spheres arranged in representative observed geometries.

2.1 Introduction

More than 25 years ago, it was discovered that the localized surface plasmons on roughened metal surfaces could be used to couple to and enhance the Raman vibrational modes of molecules attached to or in the vicinity of those surfaces [6, 18, 19]. The discovery prompted the growth of a new field dedicated to understanding and exploiting surface enhanced Raman scattering (SERS). The substrates used for SERS—initially
roughened silver electrodes—have evolved over time and have been characterized recently in great detail. A variety of substrates have been investigated, including deposited metal films, arrays of and randomly dispersed metal nanoparticles [20-23], small aggregates of metal nanoparticles [24-32], and, recently, single metal nanoparticles [31, 33-35].

The sensitivity of molecular detection using surface enhanced Raman spectroscopy, which has steadily increased since the initial discovery of SERS, has progressed to the point that Raman spectra from single molecules can be observed, implying SERS enhancement factors of up to fourteen orders of magnitude. Although the contribution of a chemical enhancement is still debated, it is generally accepted that enhancement of the local electromagnetic fields by the localized surface plasmons [19, 35-38] provides the predominant contribution to SERS.

Substrates that support surface plasmons amplify the driving field over the incident field, and also enhance emission through modification of the local density of optical states [39]. For Raman scattering at low wavenumber, the net expected electromagnetic SERS enhancement scales with the fourth power of the field enhancement and is a spatial average of the local SERS enhancement over positions of the molecules. For Raman shifts that are significant relative to the width of plasmon resonance, local electromagnetic SERS enhancement scales with the product of the field intensity enhancements at the incident and scattered frequencies [40]. While particles with sharp features can provide high near field enhancement, most reports of the strongest SERS are from coupled plasmon resonant nanoparticles [24, 27, 36, 38]. Calculations of the field strengths in the coupling region between adjacent nanoparticles
typically reveal that interparticle field strengths are many orders of magnitude greater than the fields at the surface of a single nanoparticle. Junctions play a role, also, in the surface enhancing properties of fractal nanoparticle substrates [41] and semi-continuous 2D metal films [42]. While the field enhancing properties of fractal substrates are well studied, the electromagnetic contribution to SERS in few particle clusters is less well characterized than that for particle pairs. For example, in 2002, Futamata et al.[24] utilized finite-difference time-domain (FDTD) simulations to calculate the expected electromagnetic enhancement from dimer and trimer configurations of silver spheres, 80 nm in diameter. Their simulations showed that the addition of a third nanoparticle to a dimer aggregate reduced the maximum field amplitude at resonance in the gap region responsible for SERS enhancement. Systematic investigation of the surface enhancing properties of cluster substrates, however, is complicated by the high sensitivity of the enhancement to cluster geometry, the frequency dependence of enhancement, and the challenge of fabricating substrates with sufficient structural control.

Here, we investigate the scaling of SERS from molecules on gold nanoparticle clusters of two types as a function of cluster size using a single excitation wavelength. One type of cluster is comprised of approximately spherical colloidal particles of mean diameter either 78 nm, 98 nm or 133 nm in size, while the other clusters are comprised of cylinders roughly 250 nm in diameter and approximately 200 nanometers tall, formed in templates between sacrificial layers of silver. Clusters formed in solution or during deposition on a grid are imaged in an electron microscope following characterization of their individual Raman scattering properties. Structure/ SERS correlation is accomplished by collecting SERS and TEM, as well as darkfield, images of multiple clusters as
deposited on TEM grids. Colloidal clusters are expected to be strongly field enhancing in spectral ranges associated with their surface plasmon modes and plasmon band positions depend strongly upon cluster geometry. Thus the enhancement of Raman scattering from molecules on a cluster is expected to be dependent upon both cluster geometry and the wavelength of the laser line. However, while we can predict that the ideal excitation wavelength might vary between clusters, for measurements presented here excitation was fixed at 632nm, near the middle of the typical plasmon band for colloidal clusters, and un-polarized. In contrast with spheres, the relatively large cylinders with sharp features individually may form hot spots at many frequencies and, similarly to fractals, provide broader band SERS.

In this work, SERS from individual clusters is correlated with cluster geometry and particle morphology determined from transmission electron microscopy (TEM). Nanoparticle aggregates that provide a measurable SERS signal are identified by observing the sample under laser illumination. Once the SERS from all of the aggregates on a grid has been recorded, the structural characteristics of the aggregates are determined by viewing the same aggregates in the electron microscope. The correlated measurement process is repeated for hundreds of nanoparticles and nanoparticle aggregates.

The goal of the investigation is to identify trends in surface enhancement of Raman scattering as a function of the nanoparticle properties and nanoparticle aggregate size. While Raman data is collected from clusters individually, no attempt was made to optimize conditions for enhancement on the individual cluster substrates. Rather, we investigate the scaling of the total Raman signal with cluster size, using an un-polarized
laser excitation at a fixed wavelength and form statistics on mean scattering from clusters of like size. We are interested in discovering how the scaling of Raman signals from nanosphere clusters may differ from the scaling of Raman signals from structures that are comprised of particles that, even without clustering, are likely to support hot spots over a broad range of frequencies. The collection of single cluster excitation spectra required for understanding the scaling of SERS under single cluster optimized conditions remains as future work.

In this study, we observe SERS signals from Raman active molecules adsorbed on individual gold nanospheres and nanosphere aggregates and from gold nanoparticles with irregular shape (“cylinders”). The spherical nanoparticles are formed by colloidal methods [43] and have relatively smooth surfaces, while cylinders are formed via template methods [44] and terminate in jagged edges. For excitation at the laser wavelength of 632.8 nm, SERS intensity varies widely among clusters of like size. However, mean SERS from clusters of cylinders is found to increase linearly with cylinder number. In contrast, mean SERS from clusters of nanospheres is dependent upon inter-particle coupling and there is a huge increase in SERS intensity between single and dimer clusters and an intensity plateau for clusters of between 2 and 5 colloids. To understand the scaling of SERS from colloid clusters, surface-averaged electromagnetic SERS enhancement factors are calculated for representative cluster geometries. We find trends in the dependence of SERS intensity upon particle number in the clusters are in qualitative agreement with simulations of electromagnetic SERS enhancement.
2.2 Nanocluster Preparation

The gold nanoparticles used in the experiments were prepared with coatings of various Raman-active molecules, including 4-mercaptophenol (MP); mercaptobenzoic acid (MBA); quinolinethiol (QSH); 4,4’-dipyridyl (DPY). The nanoparticles were formed either by colloidal methods, resulting in predominantly spherical particles, or by template-based electroplating, which produces nanoparticles of relatively consistent size but irregular shape. Because the overall shape of the templated nanoparticles is roughly cylindrical, we refer to these particles as cylinders.

Colloidal gold nanospheres were produced by a standard preparation, in which a mixture of sodium borohydride and sodium citrate is added to previously mixed hydrogen tetrachloroaurate and hydroxylamine hydrochloride. An Au colloid solution results, with a total reaction time of less than 30 seconds. Sodium borohydride rapidly reduces the Au(III) to Au(0) providing very small Au clusters. Hydroxylamine hydrochloride then serves as the reducing agent for the growth of the clusters and citrate serves to stabilize the colloids. The size of the final Au colloid depends both upon the ratios of the various reagents and on the efficiency of the mixing of the various solutions. Three distinct colloid populations were prepared for this study, and their sizes were determined from T.E.M measurements of the selected SERS clusters.

The nanoparticle cylinders are made using a template-based approach, using alumina templates with pore sizes of approximately 250 nm (Whatman). Electroplating many tens of layers of alternating short gold and silver stripes and subsequently dissolving away the silver, leaves gold nanocylinders of the desired height. Following electrodeposition of alternating layers of gold and silver, gold segments were released in
a two step process. First, the silver is dissolved in nitric acid, followed by the alumina template dissolution in sodium hydroxide (NaOH). The Raman reporter molecule is added to the particle solution during template dissolution in NaOH.

2.3 Correlated structural characterization and SERS measurement

The nanoparticles were immobilized on Formvar coated 75 mesh, center-marked, copper TEM grids (Ted Pella) by placing a 25 µl drop of the nanoparticle solution on top of the grid while holding the grid with tweezers. After 5 minutes of incubation, the grid was placed face down on a 100 µl drop of double distilled water (DDH2O) for a 1 minute rinse. This rinse step is repeated 3 times, and the grid is then dipped in ethanol and allowed to dry before being characterized either optically or by TEM.

The darkfield/Raman optical microscope used to characterize the SERS nanoparticles is a hybrid construction utilizing Nikon darkfield microscope components, custom parts and commercially available accessories. A Prior motorized translation stage attached to a z-column (J-mar) with 12” vertical travel allows positioning of a selected nanoparticle cluster on the optical axis of the microscope. The darkfield microscope assembly of infinity corrected objectives (a Nikon 50x ELWD was used for all data in this paper), a vertical illuminator, a secondary imaging port (with CoolSNAP™ ES CCD detector), eyepieces and trinocular head are firmly mounted above the z-column. The vertical output port on the trinocular head leads to a custom designed, image plane, rotatable pinhole aperture with a monocular eyepiece and an in/out mirror used to select individual nanoparticles for spectroscopic study. Above the pinhole is a mirror and lens assembly that redirects and images the light onto the entrance slit of the Spectrometer (Acton 2300i, with Photometrics CoolSNAP™ HQ camera).
Darkfield illumination is achieved by directing the light from a collimated lamp source (75W Xenon, ORIEL) into the back of the vertical illuminator through the darkfield cube and into the darkfield objectives. SERS excitation laser illumination is from a HeNe laser beam directed onto the sample at an angle of ~60 degrees from the normal to the sample using a one-inch focusing lens, to a spot that is approximately 200µm diameter. For darkfield imaging, the laser is turned off and the white light scattered from the nanoparticles is collected. To collect SERS spectra, light from the lamp is blocked and the Stokes-shifted light from laser illuminated nanoparticles is collected. Specular scattering from the laser is removed by a holographic notch filter (Kaiser Optical Systems) placed in the path of the collected light.

Figure 2-1. Demonstration of Darkfield-SERS-TEM “mapping” correlation. (A) Darkfield scattering image of a selected field of nanoparticles. (B) The same region, but now illuminated with a laser (632.8nm excitation), and filtered to collect only Stokes shifted Raman band emission. (C) TEM image of the SERS nanoparticles.

The samples, prepared as described above, are first imaged in the darkfield microscope to identify the locations of nanoparticle clusters. One square of the nanoparticle-coated TEM grid is selected with a sufficient, but not too dense, number of nanoparticles. A darkfield CCD image of the grid is first taken (Figure 2-1A). The same region is then illuminated by the laser (632 nm HeNe), and a second CCD image taken.
The laser light was brought in under oblique, off-axis illumination, at an angle larger than that determined by the numerical aperture of the objective (50X, NA=0.55). The excitation laser power at the sample was measured to be 22 mW. The scattering light from the incident laser beam is removed by a holographic notch filter, allowing just the Stokes-shifted SERS emission to be collected (Figure 2-1B). The two CCD images can then be compared and randomly selected nanoparticles numbered for both spectral and TEM characterization. The SERS wide-field image can be used to search for and identify “hot” nanoparticles for further characterization. Once this is done, nanoparticles and clusters of interest can be positioned on the optical axis, and the pinhole aperture in the trinocular tube used to remove the light scattered from other nearby particles or artifacts on the slide. A SERS spectrum can then be acquired from the individual clusters.

Only the brightest SERS emitting nanostructures provided a sufficient signal from which a measurable SERS spectrum could be acquired. For this study, however, we identified clusters that were detectable in the SERS wide-field images as Raman-active, and included them in the population for which SERS statistics were formed. As cluster morphology cannot be determined solely from the darkfield images, the same clusters were identified and characterized using TEM. Once the optical characterization of structures on a grid was complete, the grid was then imaged using a TEM, Figure 2-1. To find the optically characterized nanostructures, the same grid square used for the optical characterization is located, and the same nanoparticles are found by comparing the nanoparticle patterns observed in the TEM with the darkfield images (Figure 2-1C, Figure 2-1A). This mapping technique allows us to characterize the individual
nanostructures by TEM, darkfield and SERS emission; and to correlate particle size, shape, number, and cluster geometry with single cluster SERS emission.

Instead of relying on statistical analysis to determine the mean sizes of the three prepared gold colloid populations, each cluster of spherical gold colloids was physically measured from the TEM images and placed into one of three size bins. The mean particle diameter from the cluster dictated whether the cluster was considered a 78nm std=6.09nm, 98nm std=8.04nm, or 133nm std=8.46nm composition cluster (Figure 2-2A). The mean diameter of all the spherical colloids in this study is measured to be 101 nm std= 20 nm. While there is significant size and shape variation amongst the template prepared cylinders (Figure 2-2B), they are not individually measured and categorized. Rather, they are used for the purpose of comparison to their uniformly large size and rough surfaces.

Figure 2-2. Electron microscope measurements of SERS active gold A) colloid and B) template assembled cylinders. (A) Nanoparticle diameters are individually measured from each T.E.M. mapped and SERS characterized cluster. The mean NP diameter for each cluster determines which size bin is selected. The bin sizes (78 nm, 98 nm and 133 nm) are the mean diameters for all the NPs contained in that bin. Three bins are used which correspond to the three colloid populations prepared for this study (B) S.E.M. of template assembled cylinders confirms a wide range of shapes and sizes. The mapped cylinders are not individually measured. However, the surface roughness and large size stands in stark contrast to the colloidal spheres.
The Raman spectrum of a molecule provides a wealth of information on its vibrational modes and their interaction with the local environment. The Raman spectra for solutions of QSH, DPY and MBA are shown in Figure 2-3B (dashed lines). The spectra were acquired from cuvette samples. When the molecules are bound and immobilized on the nanoparticle surfaces, it is expected that the Raman lines will differ from those of the bulk solutions both in terms of position and strength. While Raman lines were not assigned, the Raman spectrum for each given molecular system was found to be reproducible with only slight shifts in the lines from sample to sample. Background spectra with similar acquisition times of the uncoated nanoparticles or nanoparticle aggregates revealed no Raman lines whatsoever, indicating minimal contamination during the preparation.

Figure 2-3. TEM images and corresponding SERS spectra of various SERS nanoparticles 1) single “cylinder” QSH, 2) dimer of “cylinder” MBA, 3) dimer of DPY spheres 4) single DPY sphere. Dashed line spectra represent cuvette based bulk Raman measurements from QSH, DPY and MBA.
2.4 Experimental results

In total, several hundred nanostructures were characterized, including single particles and clusters formed through aggregation in the course of the preparative process. While among the colloid samples several specific cluster geometries appeared to be favored, random sampling of a large number of clusters resulted in characterization of a broad cross-section of structures. Figure 2-3 summarizes the first conclusion of this study. A detectable SERS signal could not be measured, either in spectroscopic mode or wide-field imaging mode, from any of the individual nanospheres identified by correlated dark field imaging and TEM. However, every single larger template fabricated “cylinder” and every aggregate of either templated cylinders or spherical colloids were detectable using the wide-field SERS imaging and many were bright enough to provide SERS spectra. Many properties of the cylinders, including roughness, large surface area, and elongated shape, are likely to contribute to the intensity of single cylinder SERS. Single spheres in the 78 to 133 nm size range, in contrast, are neither resonant at the laser frequency nor shaped to support the high polarization charge that could otherwise produce hot spots. In a previous study, SERS emission sufficient for CCD detection [31] was detected from at least one apparently regularly shaped gold nanoparticle intermediate in size between the spheres and cylinders investigated here. However, relative to other particles in the sample, the particle was an outlier in cross section, and of unknown size in the third dimension. Here, consistently, ‘cylinders’ are found to provide detectible SERS, in contrast to the sub-threshold scattering properties of the smaller colloidal particles. Of 29 single colloids characterized in our study, the one colloidal particle that
exhibited a barely measurable SERS peak was found to have a cubic, as opposed to spherical, geometry.

Figure 2-4. SERS of DPY on colloidal nanosphere dimers. These generally have a measurable SERS spectrum. All 14 of the dimer “spheres” without the glass shell measured above the threshold of detection using wide-field laser illumination.

While single cylinders coated with any of the Raman active molecules consistently displayed detectible Raman signals, single nanospheres are not expected to produce a significant field enhancement (maximum field enhancement of ~5) [36]. By contrast, less symmetric nanoparticles, or nanoparticle aggregates, are expected to enhance fields strongly. Field enhancements of as much as two or more orders of magnitude will lead to many orders of magnitude larger SERS. As a result of the colloidal preparation, a fraction of the nanoparticles are aggregated into clusters. The “hottest” structures identified in the wide-field SERS images corresponded to dimers,
trimers, and larger aggregates. Molecules on clusters of cylinders, in general, scattered more strongly than molecules on clusters of comparable numbers of colloidal particles. However, aggregates of both types, not only were detectable using wide-field SERS imaging, but also sufficiently SERS-enhancing to produce measurable SERS spectra.

TEM profiles of six typical colloid dimers (coated with DPY) and their associated SERS spectra are presented in Figure 2-4. All of the colloid dimers observed (14 in total) yielded a detectable wide field SERS imaging signal. The SERS spectrum being observable for dimers is consistent with the expected large electromagnetic enhancement between pairs of nanoparticles. In addition to the dimers that were identified during the mapping of the colloidal samples with DPY, clusters of other sizes also were found, including twelve trimers, five 4-particle clusters and four 5-particle clusters. Images and SERS spectra of several larger nanoparticle aggregates are displayed in Figure 2-5. The DPY SERS spectra from all of these larger aggregates were detectable and could be acquired. The collection of both SERS and electron micrographs allows the single cluster SERS signal to be correlated with the number of nanoparticles in the cluster.
Many factors contribute, however, to variability in SERS intensity among clusters, including cluster shape and resonance location relative to the excitation laser line. For many clusters, such as dimers, excitation of the enhancement region further depends upon the polarization of the incident light. In our experiments, however, the output of the laser is unpolarized, so that there always will be a component of the field available to create a field within a gap. Because polarization is not controlled in these experiments, we might expect an increased spread in the distribution of the observed SERS enhancement. However, broad SERS distributions would be expected, even among clusters aligned with incident fields, if gap widths vary by even sub-nanometer amounts, given the extreme sensitivity of gap fields to interparticle separation. Variation in SERS signals may result also from variations in gap shape or length, or from blinking or non-uniform distribution of SERS active molecules in the regions of highest field.

In our study we have investigated, not only the Raman enhancing properties of single clusters, but also the overall SERS statistics of clusters classified by cluster size.
As spectra were not taken from all mapped nanoclusters, as an alternative measure of SERS activity, we tabulated total CCD counts from individual clusters under wide-field laser excitation. The correlation factor between this measure of single nanocluster SERS and amplitudes of molecule-specific Raman peaks from SERS spectra acquired from the same clusters was found to be 0.8. We attribute the imperfect correlation of the amplitude of the SERS as measured by spectral acquisition and by imaging as likely due to a combination of the lower detection threshold of our SERS microscope in widefield imaging mode and the phenomena of SERS “blinking”. As has been documented previously [45], the SERS emission for individual nanoparticles regularly cycles through unpredictable on/off periods that would make amplitude comparisons between finite (1 minute or less) exposures inaccurate.
SERS intensity data was collected not only from colloidal particle clusters, but also from 24 cylinders, 23 pairs of cylinders, and smaller numbers of cylinder clusters of larger size. The cylinders were coated with MBA and QSH. Mean SERS intensity as a function of number of particles in the cluster (Figure 2-6, solid squares) was found to differ significantly for clusters of cylinders as opposed to clusters of colloidal spheres. Mean counts from cylinders and clusters of cylinders scaled linearly with number of
particles in the cluster. The trend was observed for Raman scattering of either molecule. The simple scaling with particle number of SERS from cylinder clusters relative to dispersed cylinders indicates that single cluster SERS enhancement is sufficient to dominate any additional field enhancement from inter-cylinder coupling.

Average SERS counts from clusters comprised of 2 to 5 spherical particles was an order of magnitude higher than the noise level (~4000 counts) and, thus, at least an order of magnitude enhanced relative to the undetectable signal from single colloid. Comparison of the mean intensities for all clusters measured, of various size, (Figure 2-6, solid circles) revealed that the SERS intensity (for 632nm laser excitation) has a local maximum for aggregates composed of two to three particles; however, the variation in mean counts between clusters of 2 to 5 particles were within the standard deviations of the counts for clusters of any one size. Thus, the primary conclusion is that, under single wavelength excitation, per particle mean SERS enhancement decreases as colloid cluster size increases from 2 to 5. Much higher counts from several larger aggregates suggests that this scaling cannot be assumed to extend to larger clusters, however, it clearly is in contrast to the scaling properties of the more SERS enhancing cylinders. As the size of colloids varied by many tens of nanometers around the mean, we examined, also, the scaling of cluster populations subdivided by particle size. Curves, thus, are presented for aggregates comprised of particles of size 78 nm +/- 6.1nm, 98nm +/- 8nm, and 133nm +/- 8.5nm. While this further subdivision of the aggregates grouped by particle number into smaller groupings left groupings of large aggregates with poor statistics, mean enhancement as a function of particle number for these more refined groupings, in general, left the overall trends of SERS with particle number unchanged. For the colloid
and cluster sizes investigated here under excitation at 632.8 nm, aggregates composed of larger particles, in general, had higher total SERS. A four particle cluster with particularly high SERS was an extended, but highly non-linear, structure likely to provide high field enhancement in more than one hot gap.

### 2.5 Nearfield simulation for nanoclusters

While electron microscopy revealed clusters with an extensive range of geometries and not all relevant structural parameters could be determined from the TEM, the imaging, nonetheless, provided structural information that served as a basis for a computational investigation of possible electromagnetic contributions to the SERS. In particular, we were interested in determining the level of correlation between the scaling of SERS with cluster size and that associated with a surface averaged $E^4$. While field at Raman shifted emission frequencies play a role in detailed estimates of the electromagnetic (EM) surface enhancement of a single Raman line, an approximation for total EM SERS from Raman-active molecules near particle surfaces can be found by averaging the fourth power of the excitation field enhancement over the locations in which molecules are positioned. While simulation of the field enhancing properties of sharply featured structures such as etched cylinders would require an enormous amount of unavailable structural information, fields associated with colloids and colloid clusters can be determined readily using semi-analytic methods that describe the response of optically coupled particles in terms of the natural modes of spheres.

To compute the field enhancement for various configurations of nanospheres, the near fields of the nanospheres are expanded in terms of vector spherical harmonic functions (VSH). For a single particle, the VSH expansion equates to Mie theory and
represents an exact solution for a sphere. For clusters of optically coupled particles, accuracy is maintained by requiring convergence of the field in regions of high enhancement regions (the most sensitive region) as the number of VSH terms is increased [28]. For a 100 nm diameter single sphere two orders of vector spherical harmonics are sufficient to describe near field intensity. However, higher orders of VSH (up to N=25) are needed to provide a converged description of the gap fields of a pair of 100 nm gold spheres separated by 2 nm. The dielectric constant of Au used in the calculations was taken from Johnson and Christy [46]. To approximate the effect of illumination by unpolarized laser light used in the experiment, for each structure EM SERS enhancement was calculated for two orthogonal polarizations and averaged.
Figure 2-7. Calculated E field intensity plots (in logarithmic scale) for aggregates with 2nm spacing. The coupled multipole solution is for a mode driven with linear polarization at 632nm (corresponding to the excitation wavelength of our experiment).
Mean EM SERS enhancement was evaluated for nanosphere dimers and for several 3, 4, and 5 particle structures representative of those observed. In lieu of attempting simulations of an exhaustive set of unique observed structures, for each cluster size simulations were performed for up to four highly represented structures. Thus, for example, TEM revealed that surprisingly many 3-particle structures displayed a ~120 degree bend. Similarly, many 4-particle clusters also incorporated particles arranged with similar angles. Figure 2-7 shows calculated fields for a single gold nanoparticle and nanoclusters of varying number and geometry. Single nanoparticles illuminated at wavelengths longer than the wavelength of their dipole resonance support dipolar oscillations and relatively uniform surface fields. By contrast, for two closely spaced particles having an interparticle separation of 2 nm, there is a very large local field enhancement in the interparticle gap. Near fields were calculated for clusters of 80 and 130 nm particles, as well as clusters of mean sized (100 nm) particles, so as to more fully capture the properties of clusters of particles dispersed in size. Spectra of the structures were calculated to determine the offset of cluster resonances from the laser line. Gap fields even of off-resonant clusters were found to be significantly enhanced. For both on and off resonant clusters, \( E^4 \) enhancement as expected is even more highly localized in gaps. If we track the magnitude of \( E^4 \) at 1 nm from a particle surface at increasing lateral displacement from the center of a gap, we find a displacement of 5 nm is sufficient to drop the SERS EM enhancement by an order of magnitude.

**2.6 Comparison between SERS signals and nearfield enhancement for different aggregates**

In correspondence with the mean SERS statistics summarized in Figure 2-6, means of simulated EM enhancement as a function of number of particles in a cluster
also were compiled. Because the experiments record total SERS intensity from each structure, SERS enhancement for each structure simulated was evaluated by integrating $E^4$ over the surface of all nanospheres. The surface for integration was chosen arbitrarily as 1 nm away from the nanosphere surface. Because the calculated electric fields for larger aggregates are dependent upon aggregate shape and size, up to four distinct geometries were simulated for each particle and aggregate size. EM SERS enhancements for each aggregate size were evaluated by averaging these results over both cluster shape and particle size. In Figure 2-8, we plot the simulated enhancement factor (open circles-dashed curve) vs. aggregate size, for clusters comprised of 80nm, 100nm and 130nm diameter spheres. The peak amplitude of the simulated enhancement is normalized to the peak amplitude of the measured SERS (solid circles-solid curve). While a fairly small number of clusters near resonance at 632.8 nm provided a dominant amount of the total simulated SERS enhancement, the calculated EM SERS trends with the number of particles in a cluster is consistent with the SERS measurements summarized in Figure 2-6.
Figure 2-8. Open circles- The calculated electromagnetic enhancement (integrated surface $E^4$) for aggregates of gold spheres with 2nm spacing and mean diameter: 103nm (results for each size range averaged). The solution is for a mode driven with linear polarization at 632nm (corresponding to the excitation wavelength of our experiment). Enhancement factor is calculated and averaged for two predominant polarizations and as many as 4 different cluster shapes for each aggregate size. Solid circles- Experimental measurements of SERS (DPY) from 78nm, 98nm and 133nm mean diameter gold spheres (results averaged).

Absolute electromagnetic enhancement comparisons between simulation and experiment are not made in this work due to the uncertainty of the molecular orientation, surface coverage and overall coupling efficiency.
2.7 Discussion

Sub-threshold scattering from single colloids is consistent with the expectation that, for particles of regular shape, high fields are localized in junctions created by inter-particle coupling. The consistency with which SERS is detected from all clusters suggests that, in these experiments, cluster self-assembly creates junctions that are of narrow width. Nonetheless, SERS is highly variable among clusters, as expected given that not only alignment but also cluster shape and precise gap width are not controlled.

More remarkable, however, is that average scattering from clusters of 2-5 colloidal particles is relatively insensitive to cluster size. This is in marked contrast to scattering from molecules on large irregular structures with high per particle enhancement where scattering scales with particle number and to scattering from molecules on semi-continuous films where hot spots are strongly frequency dependent and, at a single frequency, spatially sparse [41]. Thus, in these experiments, colloid dimers are found to enhance SERS more efficiently than clusters with a few more particles.

The plateau of mean Raman scattering intensity between 2 to 5 colloid cluster sizes initially appears contrary to the expectation that SERS is sensitive to the cluster plasmon resonance and resonance is dependent upon cluster geometry. Specifically, field enhancement is expected to be highest when illumination is at a frequency within a cluster plasmon band. Dipolar cluster resonances will be red shifted when particles are larger or in clusters that incorporate more particles in linear arrays [47]. Many of the trimers observed in these experiments, while bent, are nonetheless sufficiently extended as to support a longitudinal resonance at a redder wavelength than the resonance...
wavelength of a dimer. Our simulations suggest, however, that for the range of particle sizes present, dimer, trimer, and quadrumer resonances span a broad range of wavelengths including the wavelength of the laser. Thus, among each cluster size, even at fixed excitation wavelength, hot spots are present in the gaps. Remarkably, for the small (2 to 5 particle) aggregates, whatever variation exists between SERS enhancement in hot spots on clusters of unlike size is roughly compensated for by the frequency with which hot spots are present.

Are there other explanations that rely less on fortuitous compensation of hotness in clusters and the frequency of occurrence of the hot spots? One possible explanation is that SERS intensity is most strongly determined by the width of individual interparticle gaps. Gaps of narrow width may occur with similar frequency in clusters of unlike size. Alternatively, slightly weaker gap fields in larger clusters may compensate for higher numbers of strongly field enhancing gaps. Such a situation would be expected to arise if gap capacitance was distributed among gaps aligned in series. Possibly, the EM enhancement may be less dependent upon plasmon band location than expected. Near field intensities have been shown in simulation to be high over a broader range of frequencies than the range that characterizes plasmon band width as detected in the far field [48]. Furthermore, field enhancement at frequencies detuned from the cluster plasmon maximum may result from excitation of a dimer within a larger cluster. Recently, the signatures of dimer dipoles have been observed in the polarization of Raman scattering from a close-packed colloid trimer [49]. A key step in distinguishing among these possibilities is more precise characterization of the geometry of gaps.
Precise measurement of gap widths is, however, difficult. Clarification of the role of variable gap width, thus, is left for future work.

2.8 Conclusion

Experimental and theoretical results are presented that illustrate trends in SERS enhancement as functions of cluster number, shape and size of the component nanoparticles. By employing a custom, wide-field Raman image capture system along with a reliable TEM mapping strategy, we have been able to correlate SERS enhancement with cluster morphology for two qualitatively different classes of gold nanoparticles. As expected, nanoparticle clustering increases SERS from Raman active molecules on 78 nm to 133 nm spheres. However, larger, highly structured nanoparticle substrates, that support single particle SERS, display negligible additional SERS per particle when grouped in clusters. Simulation of the coupled response and near fields of the nanosphere clusters provides an avenue to understand the electromagnetic component of the former trends.

Structure/SERS correlation is an inherently difficult endeavor even under circumstances in which variations in chemical enhancement may possibly be neglected. Electromagnetic surface enhancement of Raman scattering depends on nanometer and even sub-nanometer features that result in field gradients on the same order and are extremely difficult both to characterize accurately and control. Subtle imperfections in particle makeup, a practical reality in any reasonable scale nanoparticle synthesis operation, can cause broad variations in SERS intensities making generalizations on composition, cluster morphology, particle shape, and aggregate number, a difficult proposition. The large number of the variables, inherent to random clustering of colloids,
motivates the development of a more controllable SERS platform. Nonetheless, the surprising consistency in the average SERS enhancement provided by ensembles of self-assembling clusters of unlike size suggests that self-assembling plasmonic nanostructures may have a role in practical applications of SERS.
Chapter 3 Two-Step Enhancement of SERS from Cy5 on Isolated Particles and Particle Clusters

Metal nanoparticle assemblies of well-defined structure are investigated as substrates for quantitative surface enhanced Raman scattering (SERS). The ~100 nm structures are composed of oligonucleotide-functionalized gold nanoparticles of two sizes; a large particle serves as a template for assembly of multiple small particles. Satellite structure formation is driven by hybridization of linker oligonucleotides to thiolated single stranded DNA bound to the particle surfaces and is verified by transmission electron microscopy and Rayleigh scattering spectroscopy. Raman scattering spectra are collected from dispersed particles and core-satellite assemblies (CSA) formed with core particles that incorporate Cy5 in the core particle surface strands. Quenching of Cy5 fluorescence allows Raman spectra to be clearly detected both from dispersed and assembled core satellite structures. Raman scattering from Cy5 in the coupled assemblies is amplified by a factor of 8, relative to the Raman scattering collected from dispersed cores. The amplification of Raman scattering from Cy5 on the core particle surface is quantitatively consistent with the amplification expected based upon surface field intensities that increase when satellite particles assemble at controlled distance from the core. Raman scattering per core-satellite structure is determined by calibrating measured intensities using methanol as a Raman intensity standard. The number of Cy5 molecules per core-satellite structure contributing to the Raman scattering is determined from analysis of the spatial non-uniformity of the simulated core surface field distribution. The Raman cross section of the immobilized Cy5 species is estimated from the Raman scattering per CSA using the Cy5 coverage per core particle and the calculated mean electromagnetic enhancement on the surface of the CSA. While
uncertainties in Cy5 coverage, properties of the Raman standard, core-satellite geometry and concentration contribute modest errors in the Raman cross section estimates, weak electromagnetic enhancement allows the cross sections of the immobilized Cy5 molecules to be assessed without the usual order of magnitude uncertainties associated with strongly enhanced near fields.

3.1 Introduction

Enhancement of scattering at interfaces that support surface plasmons is a phenomenon of great importance for spectroscopic characterization of immobilized molecules. Raman scattering, for example, is a weak inelastic process with a wavelength dependence that is highly specific to the molecule. Since the early demonstrations [6, 8, 50] and theoretical studies [10, 11, 15, 51-53] of surface enhanced Raman scattering (SERS), extensive effort has been devoted both to developing enhancing substrates and to understanding enhancement mechanisms [38, 54-58]. Due to the high specificity of Raman spectra, SERS is a potentially useful technology for applications such as sensitive and highly selective molecular detection of dyes [59-61], nucleic acids [62], and proteins[63] and chemical imaging [64, 65].

Advances in knowledge of material plasmonic properties, chemical synthesis, and fabrication methods have enabled demonstration of SERS on a diverse set of substrates. The substrates include colloidal nanoparticles (nanoshells [66], nanoboxes [67], and nanorods[68]) and nanoparticle clusters [69], and surface structures made through patterning using e-beam lithography [70], nanosphere lithography [71], template methods [72, 73], or oblique angle deposition [74, 75], or through colloidal monolayer assembly [76]. The most highly enhancing substrates are important because of their potential to
support single molecule (SM) SERS [59, 60, 63]. Single molecule measurements are of special interest because they offer a detailed picture of the chemistry of molecules on surfaces, otherwise masked by averaging over an ensemble of molecules in diverse environments. Alternatively, it may be possible to detect intrinsic properties of molecules on surfaces by measuring SERS from molecules on substrates that provide known near field enhancement.

The phenomenon generally understood to provide the enhancement necessary to measure Raman scattering from molecules on surfaces is the near field enhancement associated with surface plasmons, which contributes the electromagnetic component of the surface enhancement factor [77]. Significant effort has been devoted to characterizing electromagnetic enhancement. Quantitative characterization of electromagnetic contributions to SERS [78], however, can be difficult, especially under conditions that support SM-SERS. Near fields of high intensity, such as those required for SM-SERS, are associated with narrow gaps between particles or vertices or other asperities [37]. However, highly structured features are the most vulnerable to transformation, and field enhancement in narrow gaps is extremely sensitive to small changes in separation. At fixed excitation and emission frequencies, enhancement of Raman scattering depends, furthermore, upon the spectral position of the substrate plasmon resonance; structural changes that shift plasmons can lead to significant variation in the intensity of SERS. Thus, the most enhancing substrates can be both difficult to fabricate reproducibly and susceptible to variations that interfere with quantitative understanding or application of SERS.
For applications that do not require single molecule data, it is useful to identify substrates that provide modest, but predictable, enhancement to a larger group of molecules. Requirements for predictability include control of geometry and substrate plasmons and, for multi-particle structures, reliable placement of molecules in interparticle gaps [79]. Here, we investigate SERS from Cy5 incorporated in nucleic acid oligomers that assemble on weakly field enhancing, dispersed 50 nm gold nanoparticles. Surface fields are enhanced further by assembly of a shell of smaller satellite particles that sandwich the reporter molecules in gaps between satellites and 50 nm cores. Satellite – core separation is controlled by the structure of the duplex DNA linkers. Prior investigation of closely related plasmonic substrates has shown that similar structures offer good control of plasmon resonance and thus, most likely also, interparticle spacing [80]. By incorporating chromophores within the molecules that stabilize core particles, the number of Cy5 species is quantitatively controlled. Prior measurements of networks of particles linked by similar molecules with similar oligonucleotide sequences [81] suggest that interparticle linking may leave the Cy5 – surface chemistry unperturbed. Here, core and sandwich structures provide two levels of electromagnetic (EM) enhancement and, thus, two measurements of the underlying, possibly chemically enhanced, Cy5 SERS. The work builds upon prior demonstrations of SERS from molecules in solution phase sandwich structures [82-84] but is novel in the extent to which geometry and enhancement in the coupled structures is controlled.

The target SERS structures are comprised of a core nanoparticle surrounded by many smaller satellite particles and, when assembled, are approximately 100 nm in size. 20 nm satellite particles are bound to 50 nm core particles through formation of duplex
DNA linkers, previously found to provide control of core-satellite separation [81, 85]. Cy5 is incorporated in the thiolated oligonucleotide [83, 84] that stabilizes core particles in 0.1M salt; the density of the molecular layer is bounded within a narrow range through the competing requirements of particle stability and linker hybridization efficiency. Prior measurements suggest that the efficiency with which core particle oligonucleotides bind linker oligonucleotides is controllable and manifest in mean satellite coverage per core [86]. Given the sphere-like shape of both the core particles and assemblies, field distributions on the core surface are minimally sensitive to orientation of the structures relative to the excitation field. The contribution to SERS from electromagnetic enhancement, thus, is well controlled by known or measurable properties of the full assemblies and the Cy5 labeled cores. Within the assumption that particles are spherical and that the plasmon perturbing effects of surface molecules are small, electromagnetic enhancement factors at the core surface, further, are easily derived from simulation of the coupled or single particle excitations of core satellite structures or dispersed cores.

Section 3.2 describes the preparation and characterization of both nanoparticle conjugates and coupled assemblies, including electron microscope imaging, elastic light scattering, and Raman scattering. Section 3.3 outlines the formalism used to describe the scattered power for a Raman band in terms of mean enhanced molecular cross section, and the assumption underlying factorization of the ensemble averaged scattering into separate integrals over molecular cross sections and over EM enhancements that vary with position. Section 3.4 describes the calculation of the nanostructure EM response and the mean EM enhancement over a core particle surface for both dispersed cores and assembled core - satellite structures. The effect of the Stokes shift on the enhancement
calculation is described. The ratio of simulated mean EM enhancement for coupled structures relative to core particles is compared with ratios of SERS from Cy5 on assembled structures relative to dispersed cores. Section 3.5 describes the estimation of SERS cross sections on a per nanostructure and per molecule basis through comparison with Raman scattering from a Raman scattering intensity standard. The distribution from simulation of enhancement factors over the core surface for the two structures is presented and the fraction of immobilized molecules contributing significantly to the SERS signal is discussed. Section 3.6 discusses the concentrations of CSA-immobilized Cy5 that can be detected, including under conditions of slightly stronger coupling, and summarizes the extent to which mean EM enhancement ratios describe the observed ratios of SERS from coupled structures relative to dispersed cores.

3.2 Fabrication and Characterization

3.2.1 Functionalization of particles

Citrate stabilized gold colloid (Ted Pella) of two sizes was functionalized with thiolated oligonucleotides (IDT DNA) using previously demonstrated methods and recognition sequences [87] as listed in Table 3-1.

Table 3-1. DNA sequences of the particle and linker strands. Recognition sequences are from ref [87].

<table>
<thead>
<tr>
<th>Core Strand</th>
<th>5’ Thiol-Cy5-AAA AAA AAA ACG CAT TCA GGA T’ 3’</th>
</tr>
</thead>
<tbody>
<tr>
<td>Satellite Strand</td>
<td>5’ TCT CAA CTC GTA-Thiol 3’</td>
</tr>
<tr>
<td>Linker Strand</td>
<td>5’ TAC GAG TTG AGA ATC CTG AAT GCG 3’</td>
</tr>
</tbody>
</table>

a, b, a*, b*
The strand used to functionalize the 50 nm template (core) particles incorporates the Cy5 reporter in between the terminal gold binding thiol group and an A$_{10}$ sequence demonstrated previously to have affinity for the surface of the gold particle upon which it is immobilized [88]. The A$_{10}$ sequence provides stability and constrains the position of the reporter, while also positioning the 12-base recognition sequence close to the Raman reporter, Cy5. The Cy5, thus, is situated in close proximity to the core particle, a geometry conducive to fluorescence quenching [89]. The linker strand $a^*b^*$ has affinity also for satellite particles functionalized with a thiolated 12-base strand with recognition sequence, $a$. Concentrations of both gold nanoparticle solutions were determined by absorbance at 520 nm and Beer’s law, both before and after functionalization with oligonucleotides. Particles were functionalized in phosphate buffer (pH 7) by gradual salting (to 0.1 M NaCl) and left on an orbital shaker overnight. The core solution was washed by multiple cycles of centrifugation at 3.2K rcf, removal of the supernatant, and re-suspension in PBS [85]. The partition of total oligonucleotide between the core surface and the bulk was determined by measuring optical density of the supernatants, (see Supporting Information). Relative concentration of bound oligonucleotides and core particles determined the mean number, 1781, of Cy5 labeled oligonucleotides, A$_{10}b$, per core. Surface coverage was 37 pmol/cm$^2$, a coverage similar to coverage observed previously on smaller nanoparticles in the same buffer with a higher concentration of salt [90]. The satellite particles were prepared similarly; however, separation of the smaller particles from the unbound oligonucleotides required a higher (8k rcf) centrifugation speed.
3.2.2 Characterization

Cy5 labeled cores and freshly prepared core-satellite assemblies (CSA) were characterized by their structural, plasmonic, and Raman scattering properties. To form assemblies, core particles mixed with linker strands were combined with satellite particles in a 1:400 ratio. The 1:400 ratio of core and satellite particles allows for termination of interparticle aggregation following formation of a single layer of satellite particles on cores [86]. Hybridization of satellites to cores occurred over a one hour interval; shorter assembly times resulted in assemblies with low satellite coverage. Two other solutions were prepared from the same functionalized ‘core’ nanoparticles as were used to prepare the complete CSAs. A solution comprised of core particles served as the substrate for dispersed particle Cy5 SERS. A solution comprised of functionalized cores and satellites without linker strands, $a^*b^*$, was prepared as a control. Final concentration of core particles in all three solutions was 1 pM. These dilute solutions had sufficiently low optical densities to allow light attenuation to be neglected in calculations of Raman cross sections from detected scattered light.
Figure 3-1. Substrates for Cy5 Raman scattering. (a) Core-satellite assemblies. (b) Functionalized core particles. (c) Control comprised of functionalized core particles and satellites, but without linker strand.

**Characterization of structures.** Satellite coverage and fraction of single-core structures were characterized by transmission electron microscopy (FEI Tecnai) for both assemblies and controls. As the electron microscopy was executed under vacuum, solution phase CSAs were deposited and dried on TEM grids. A 10 ul drop of the CSA solution was placed on a TEM grid (300 mesh, Ted Pella) and incubated for 10 minutes, after which excess solution was removed by wicking. Nanostructures in all three solutions were found to be discrete, as opposed to be aggregated into networks (Figure 3-2). Mean satellite coverage in the CSA sample was 12 satellites per core. Assuming a 6 nm separation between satellites and core particles in solution, the 12 satellites form a fairly sparse layer (area fraction, 18%) on each core. For the solution composed only of functionalized ‘core’ particles, all particles imaged on the TEM grid were fully dispersed.
On the grids prepared from the control solution that included satellites, a small fraction of core particles had one or two associated satellite particles; the majority of core particles, however, were dispersed. Among 84 imaged CSA structures, 80 were comprised of single cores and multiple satellites. Four structures were comprised of two cores, presumably as a result of one or more satellites binding to both cores. The ‘yield’ of single core assemblies, therefore, was greater than 95%.

![Figure 3-2. TEM images of (A) a CSA with 12 satellites (B) a functionalized core from core-only solution.](image)

**Plasmonic properties.** Although TEM images of dry samples provide valuable morphologic information, including 'yield' and coverage of satellite nanoparticles, details of the 3D structures of CSAs in solution are not discernable from micrographs of dessicated samples. Elastic light scattering spectra, that display particle and assembly plasmon resonances, therefore, were collected from solution phase assemblies for comparison with spectra from simulations that characterize scattering from model structures. In the elastic scattering experiment, a double monochromator (Olis® Hummingbird) selects the desired wavelength of incident light from a broadband source (150W Xenon). Light scattered elastically from the sample is collected at a 90 degree angle from the propagation direction of the incident light. Excitation and emission gratings move synchronously selecting wavelengths from 500 to 700 nm with 5 nm
bandpass at each slit. The integration time is 1s. The concentration of CSAs and dispersed cores is $1.0 \times 10^{-12}$ M and the scattering intensity of CSAs was corrected by subtraction of the scattering from excess (unbound) satellite nanoparticles. For dispersed cores (Figure 3-3), the peak intensity of the plasmon resonance was at 541 nm, whereas the peak of the spectral difference between the assembly mixture and excess satellites was red-shifted to 571 nm. Given the discrete nature of the structures observed in the micrographs and low concentration (one assembly per $\sim 10^3 \mu m^3$), interactions between assemblies are unlikely to influence the spectra. Thus, the observed red-shifting of the plasmon resonance upon assembly from the position of the core particle plasmon is the consequence of plasmon coupling within individual satellite structures.

To verify that spectra were consistent with a core satellite structure, a T-matrix coupled multipole method was used to simulate scattering from clusters of particles of known size and satellite number [91-93]. Spectra were simulated for model structures with mean properties comprised of perfect spheres. While polycrystalline nanoparticles have many features, such as grain boundaries, facets, and vertices, that distinguish them from perfect spheres, the plasmon band of an assembly of well separated particles is determined largely by the size of the aggregate and the density of particles within it and only weakly sensitive to minor eccentricity in the components. Model satellite structures were comprised of the mean observed number (12) of 20 nm satellites positioned at a fixed distance from the 50 nm core and evenly distributed within a satellite ‘shell’. The dielectric functions of the core and satellite particles were derived from a bulk dielectric function [94] corrected for additional losses associated with surface scattering of electrons in particles of small size [95]. The effect of oligonucleotide polarizability was
neglected due to the low dielectric contrast between the aqueous solvent and the mixtures of oligonucleotide and solvent on the particle surfaces. The effect of Cy5 polarizability on the nanostructure plasmons also was neglected, an approximation that can lead to errors in descriptions of the particle plasmons when molecular resonances overlap with plasmon bands. Core-satellite separation was assumed to be 6 nm, on the basis of small angle X-ray measurements of interparticle separation in networks of oligonucleotide-nanoparticle conjugates connected by duplex linkers with the same recognition sequences that differed only in their inclusion or exclusion of A₁₀ spacers on both ends of the tether [81]. While the network data was collected at a higher electrolyte concentration and separations are sensitive to charge screening, spectral simulations performed for core satellite structures with a 6 nm separation provided coupling strength consistent with the experimental spectra. Resonance frequencies from the simulations are 544 nm and 576 nm for isolated cores and CSAs, respectively. While both the core and CSA peaks from simulation are positioned several nanometers to the red of those observed, overall, the consistency of the scattering spectra with simulation based upon satellite coverage observed in the micrographs suggests that assemblies in solution have core-satellite structures close to the structures expected. Finite distributions in particle size and shape and in position of the satellites and number per core contribute broadening and possibly slight red shifting of the ensemble plasmon resonance relative to that of a single structure or a spectrum simulated for a mean structural model. Collecting scattered light at a wide angle, only, also could slightly offset the peak in the collected spectra to the red from expected peaks in total scattering. The slightly redder position of the simulated peaks relative to the peak positions in the data, thus, are most likely the result of neglecting in
the simulation the effect of the Cy5 layer on the frequency of the plasmon. The electronic transition of Cy5, centered at 647 nm (see supporting information), is to the red of core and assembly plasmons and, unlike the other unmodeled phenomena, could perturb the shorter wavelength core and assembly resonances slightly to the blue [96].

**Figure 3-3.** Elastic scattering spectra (left) and simulated spectra (right) of CSAs (red) and dispersed cores (black). Core satellite spectra are corrected for unbound satellite scattering, Supporting Information. Simulations are for a model structure comprised of 12 satellites evenly distributed around a template core particle and separated from the core by 6 nm. Also shown is the laser line and the wavelength of the Cy5 absorbance band at 647nm (arrow).

### Raman signal measurement

Raman spectra were collected with a Horiba LabRAM Raman microscope. An 8mW, HeNe laser, λ=632.8 nm, was used as the excitation source. The core concentration is constant (1pM) for all three solutions. Samples were prepared in quartz cuvettes to minimize background noise including fluorescence and Raman signals. Spectra spanning Stokes shifts from 500 cm⁻¹ to 1800 cm⁻¹ (emission from 652 nm to 713 nm) were collected; the lower limit of the spectrum was selected to avoid the Raman peak at 463 cm⁻¹ from the quartz cuvette. The full spectrum was collected in three ranges by shifting of the grating. Exposure time was 90s for each setting. For each sample, the spectrum was collected twice and averaged. The total collection time was 9 min. All spectra were corrected by the spectrum of the buffer
blank to remove the contribution from the buffer, cuvette, and other optical components in the Raman spectrometer.

Raman peaks are evident in all three spectra, but prominent only in spectra collected from the CSAs, **Figure 3-4A**. Sharp features are superimposed upon a fluorescent continuum, quenched to various levels in the spectra of the three samples. To confirm that the observed Raman spectrum was from Cy5, and not the oligonucleotide components of the linker, CSA structures were assembled, also, using a linker that lacked the Cy5-modification. Under identical conditions, Raman scattering was not detected from assemblies that lacked Cy5, see Supporting Information. In addition, the source of the observed Raman spectrum was verified by comparing with other surface enhanced Cy5 spectra in the literature [84].

![Figure 3-4](image)

**Figure 3-4.** (A) Raman spectrum of CSAs (red) and dispersed cores with (purple) and without satellites (green). Distinct Raman peaks are detectible in all spectra, however, Raman scattering from dispersed cores is very weak. The spectrum from core particles only (green) is offset down by 350 counts for clarity. (B) The same spectra as in A, along with a spectrum of free Cy5-modified DNA b. The concentration (2.9 nM) is similar to that of bound Cy5 (1.8 nM) and the spectrum was acquired under the same excitation and collection conditions. Fluorescence from the three nanoparticle solutions shows different degrees of fluorescence quenching. Fluorescence from the CSA, which sandwiches fluorophores in between particles, is partially quenched, whereas fluorescence from the sample comprised only of Cy5-oligonucleotide modified core particles is totally quenched.
The prominence of the Raman peaks in the spectrum collected from the CSAs was an expected consequence of field enhancement upon formation of junctions between core and satellite particles. Slightly increased scattering from the control with satellites but without linker, relative to that from dispersed cores, may result from a low level of nonspecific attractive interaction between oligonucleotide-functionalized satellites and cores. While spectra from mixtures of cores and satellites in the presence and absence of linker is the comparison of most relevance to the application of CSAs to oligonucleotide detection, the goal of our investigation is to evaluate the CSAs as quantitative SERS substrates. In the following, we focus, therefore, on a comparison of Raman signals from the CSAs and fully dispersed core particle solutions. Intensity ratios of peaks from CSAs and dispersed cores are 8.0 for the 556 cm\(^{-1}\) peak (\(\lambda_s\) at 655 nm) and 8.5 for the peak at 1190 cm\(^{-1}\) (\(\lambda_s\) at 683 nm). As the Cy5 is immobilized on the core through incorporation in the surface oligos, the intensity ratios provide a measure of the additional enhancement provided by the assembly relative to that provided by the core. These two measurements of the enhancement factor associated with gap formation were compared to the ratio of simulated mean electromagnetic enhancement (\(E^4\)) at the core surface for assemblies and for dispersed cores. Later, we will see that the experimental SERS and simulated EM ratios are remarkably similar.

To investigate the effect of immobilization on the fluorescence from the Cy5 labeled oligonucleotide, the spectrum from free floating core strand A_{10b} was acquired under the same excitation/collection conditions, Figure 4b. The concentration of the free floating Cy5-modified oligonucleotide is 2.87x10\(^{-9}\) M, determined by DNA absorbance at
260 nm and the known extinction coefficient. The concentration of immobilized strand $A_{10b}$ in CSA and control solutions, from fluorescence of the core supernatant, is $1.78 \times 10^{-9}$ M, a similar concentration. Comparison of the fluorescence of bulk and bound strands (Figure 3-4B) shows that quenching is dramatic for Cy5 on the metal core particle surface. The quenching is a good indication that the Cy5 is positioned close to the core particle surface as expected for a species incorporated in the backbone of the modified oligonucleotide in between the thiol and surface bound $A_{10}$. Interestingly, the fluorescence signal from the assembled CSA is stronger than fluorescence detected from the dispersed functionalized core particles. While a possible explanation is that linking of cores to satellites leads to displacement of a fraction of the $A_{10}$’s from the surface of the core particle, an alternative is that the Cy5 on the core particle interacts with the 6 nm distant metal satellite through a mechanism similar to that of a fluorophore near a single fluorescence-enhancing surface [89].

3.3 Theory of the Raman power ratio

For molecules in solution illuminated with light of frequency, $\nu_0$, the Raman scattered signal at a frequency, $\nu_s$, is proportional to the number, $N$, of illuminated Raman scatterers and the Raman cross section, $\sigma^R(\nu_s, \nu_0)$. The scattered power is a product,

$$P_{rs}(\nu_s) = N \sigma^R(\nu_s, \nu_0) I(\nu_0),$$

(3-1)

of the molecular number, cross section, and intensity, $I(\nu_0)$, of incident light. Molecules on or close to a metal surface may have perturbed Raman polarizabilities and will respond to surface enhanced fields and scatter surface enhanced light. Under the
assumption that the effect of field enhancement is separable from other surface effects on Raman scattering, the surface enhanced Raman scattered power [25],

\[ P_{\text{SERS}}(\nu_s) = N_{\text{ad}} \sigma^R_{\text{ad}}(\nu_s, \nu_0) G^{EM}(\nu_s, \nu_0) I(\nu_0), \]  

(3-2)

from an ensemble of \( N_{\text{ad}} \) molecules adsorbed on (or close to) an illuminated, field enhancing substrate is a function of modified mean quantities – a mean molecular cross section, \( \sigma^R_{\text{ad}} \), and a mean electromagnetic field enhancement factor, \( G^{EM} \). The modified Raman cross section is determined by the Raman polarizabilities of the molecules on the surface. The electromagnetic enhancement factor, \( G^{EM}(\nu_s, \nu_0) \), is the average over all molecules of the product of the local enhancement, \( E^2_{\text{loc}}(\nu_0)/E^2_{\text{inc}}(\nu_0) \), of the incident field and local enhancement of the re-emitted waves. In simulations of the electromagnetic enhancement, enhancement of the re-emitted wave is typically approximated by the local enhancement of an incident plane wave at the scattered frequency, \( E^2_{\text{loc}}(\nu_s)/E^2_{\text{inc}}(\nu_s) \).

Thus, electromagnetic enhancement is represented by the average over molecule locations of the product of the local field enhancements at excitation and emission frequencies,

\[ G^{EM}(\nu_0, \nu_s) = \left\{ \frac{E^2_{\text{loc}}(\nu_0)}{E^2_{\text{inc}}(\nu_0)} \right\}^{\nu_s} \left\{ \frac{E^2_{\text{loc}}(\nu_s)}{E^2_{\text{inc}}(\nu_s)} \right\}^{\nu_0}, \]  

(3-3)

Enhancement at the scattering frequency may be approximated by enhancement at the excitation frequency under circumstances where the surface enhancement varies slowly over the spectral range of emission and excitation frequencies. In this case the
electromagnetic enhancement factor is approximated by the fourth power of the field enhancement at the incident frequency,

$$G^{EM}(\nu_0) \equiv \left( \frac{E_{loc}(\nu_0)}{E_{inc}(\nu_0)} \right)^4 \right)$$

(3-4)

In our experiment, the number, $N_{ad}$, of Cy5 molecules contributing to the scattered power is held constant by maintaining constant experimental conditions and using constant concentrations of core particles from the same functionalized particle preparation. We proceed under the assumption that the mean surface-modified cross section, $\sigma_{ad}^{K}$, is not affected by assembly of satellites on cores, an assumption that will be supported if the Raman amplification associated with assembly formation turns out to be consistent with the anticipated amplification of $G^{EM}$. The assumption initially is based upon the position of the Cy5 modification in the core strand, in between the thiol and the surface binding $A_{10}$. Prior X-ray measurements of separation between oligonucleotide functionalized gold nanoparticles that form networks indicate that a thiol terminated $A_{10}$ sequence is largely adsorbed on the particle surface even after hybridization of the recognition sequences and formation of duplex DNA linked nanoparticle networks [81]. As particles stable in electrolyte necessarily have surface molecules uniformly distributed over the particle surface, the electromagnetic (EM) enhancement factor for the core-bound molecules is an area normalized integral of local enhancement factors over the surface of the core. Substrate reconfigurations, such as interparticle linking, that leave the
chemical enhancement unchanged (σ_{ad}^k constant) can be expected to display relative
Raman scattering intensities that are controlled by mean EM enhancement, G_{EM}^{}.

### 3.4 Theory of the Raman power ratio

To determine whether the ratio of Raman scattered power for CSAs, P_{CSA}, and
cores, P_{core}, is well described by the ratio of mean electromagnetic enhancement factors,

\[
\frac{P_{CSA}}{P_{core}} \approx \frac{G_{CSA}^EM}{G_{core}^EM} \approx \frac{\int_{core surface} \left[ \frac{E_{loc,CSA}(\nu_0)}{E_{inc}(\nu_0)} \right]^4 da}{\int_{core surface} \left[ \frac{E_{loc,core}(\nu_0)}{E_{inc}(\nu_0)} \right]^4 da}
\]

(3-5)

we calculated mean enhancement factors for both CSAs and cores. Local fields are
evaluated by summing fields associated with multipolar excitations of individual
particles, \(i\), as determined by solution of the isolated core or coupled particle response.
The total scattering field at position \(\vec{r}\) is a superposition

\[
\vec{E}_i(\vec{r}) = \sum_{i=1}^{N_a} \vec{E}_{i,i}(\vec{r}) = \sum_{i=1}^{N_a} \sum_{n=1}^{N_i} \sum_{m=-n}^{n} \sum_{p=1}^{2} c_{nmp}^i \tilde{N}_{nmp}(\vec{r} - \vec{r}_i)
\]

(3-6)

of fields, \(\vec{E}_{i,i}\), scattered from individual particles, \(i\), at positions, \(\vec{r}_i\). The scattered field of
each sphere is represented as a linear combination of vector spherical harmonics, \(\tilde{N}_{nmp}\);
each term describes the field associated with a multipolar mode identified by indices \(n\),
\(m\), and \(p\) [91]. Convergence of the coupled particle solution was monitored initially by
monitoring convergence of total far field scattering. However, convergence of the total
scattering is necessary but not sufficient for convergence of the near field within a CSA.
Convergence of the near field was achieved by monitoring the maximum fourth power of the surface field; for coupled CSAs, multipoles up to 15th order were required, whereas, for a dispersed core, multipoles up to 8th order were sufficient. The spatial integral for mean surface enhancement, likewise, was monitored for convergence as a function of the surface mesh. 8000 angular surface elements were found to be sufficient to accomplish convergence of the core surface integral for both the core particle and the model CSA structure.

Table 3-2. Ratios of SERS intensities from satellite structures relative to dispersed particles: comparison of measured ratios and ratios from simulations of core particle surface field enhancement.

<table>
<thead>
<tr>
<th>Raman band: ((\nu_0 - \nu_s)/c_s (\lambda_0))</th>
<th>556 cm(^{-1}) (655 nm)</th>
<th>1190 cm(^{-1}) (683 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental SERS ratio</td>
<td>8.0</td>
<td>8.5</td>
</tr>
<tr>
<td>Simulated EM enhancement ratio using (G^{EM}(\nu_0, \nu_s))</td>
<td>7.2</td>
<td>6.5</td>
</tr>
<tr>
<td>Simulated EM enhancement ratio using (G^{EM}(\nu_0))</td>
<td>8.0</td>
<td></td>
</tr>
</tbody>
</table>

From simulation, peak fourth order enhancement on the surface of the core particle is \(~8.3 \times 10^3\) for a CSA comprised of 12 satellites positioned 6 nm apart from a core. \(8.3 \times 10^3\) is 35-fold larger than the value, \(2.4 \times 10^2\), of the highest enhancement on the surface of a dispersed core. However, average fourth order enhancement, \(G^{EM}(\nu_0)\), over the core particle surface is only 8.0 fold larger for the CSA (521) than for an uncoupled core (65). Thus, the CSA-to-core enhancement ratio for a layer of molecules on the
surface of the core particle is very close to the intensity ratios \( \frac{P_{CSA}}{P_{core}} \) of measured SERS, Table 3-2.

To clarify the consequence of using the band-independent description, \( G^{EM}(\nu_0) \), of electromagnetic enhancement, we re-evaluated mean enhancement for the two Raman bands of interest using the expression for the EM enhancement, \( G^{EM}(\nu_0,\nu_s) \), that incorporates field enhancement at the Stokes-shifted frequency of the scattered wave, Eqn 3-3 and Table 3-2. Ratios of mean EM enhancement simulated with the band-specific enhancement model yielded slightly lower enhancement ratios than those derived from the more approximate, \( G^{EM}(\nu_0) \), description. These EM ratios are slightly lower, also, than the measured SERS ratios. A possible explanation is that polarization charge at facet vertices and eccentricities in particle shape increase the dispersed particle field enhancement above the level calculated for a perfectly spherical core. The same factors may have less effect on the field enhancements in assemblies, where enhancement is controlled primarily by distances between satellites and cores. Uncertainty in interparticle separation, also, leads to errors in enhancement ratio estimates; a low estimate of enhancement ratio could be a consequence of an overestimate of interparticle separation. Also noteworthy was the mismatch between the relative sizes of 556 cm\(^{-1}\) and 1190 cm\(^{-1}\) band ratios for SERS and simulated electromagnetic enhancement. Simulated \( G^{EM}(\nu_0,\nu_s) \) ratios decrease with increasing Stokes shift, as the emission frequency moves further to the red of the substrate plasmon resonance and the enhancement factor for the CSA drops more rapidly than the enhancement factor for a core. The effect of the
plasmon resonance on the shift dependence of the electromagnetic enhancement may be exaggerated in simulations that neglect inhomogeneous broadening of the near field spectrum. While the difference between the two measured assembly to core SERS ratios may be statistically insignificant given uncertainties in intensities of bands displayed by molecules on dispersed cores, differences in the band dependence of SERS and simulated EM ratios may reflect a band specific molecular response to higher or reoriented surface fields.
Figure 3-5. Calculated electromagnetic enhancement of CSA (left) and isolated 50 nm sphere (right). Enhancements are displayed for locations in a plane passing through the center of the core. The polarization direction of the incident field is parallel to the horizontal coordinate. NB. High enhancements (red) and low enhancements (blue) both appear dark when viewed without color.

3.5 Cross Section

Estimates of Raman cross sections. Raman cross sections, $\sigma_{\omega}^{R}(\nu_{s},\nu_{0})$, were determined from Raman intensities by comparing the intensities from Cy5-CSA with intensities from Raman scattering standard. For the specific immobilization conditions
through which Cy5 is bound to gold core particles, Cy5 cross sections were estimated by comparison of Cy5 scattering and with scattering from methanol. Under the same excitation / collection conditions as used to acquire the sample spectra, Raman intensity from methanol at 1034 cm$^{-1}$ was 27 fold larger than Raman intensity of Cy5 on CSAs at 556 cm$^{-1}$. From the concentration ratio, 2.47x10$^{13}$, of methanol to CSAs and the known differential cross section of methanol at 1034 cm$^{-1}$, 1.8x10$^{-31}$ cm$^2$/sr [13], we determined an effective differential cross section, $\lambda_0=632 \text{ nm}$, $\Delta \nu/c=556 \text{ cm}^{-1}$, of Cy5 per CSA. Here we use the term ‘effective’ to refer to cross sections that incorporate within them all surface enhancement effects. Methanol calibration yielded an effective differential cross section for all Cy5s on a CSA equal to 1.6x10$^{19}$ cm$^2$/sr. From the mean number, equal to 1781, of Cy5s per CSA, we also determined an effective differential cross section, $\lambda_0=632 \text{ nm}$, $\Delta \nu/c=556 \text{ cm}^{-1}$, per molecule, equal to 9x10$^{-23}$ cm$^2$/sr for each Cy5 on the surface of a core particle incorporated in a CSA. From simulation, the peak enhancement on a CSA is one order larger than the average value. Thus, the effective differential cross section, $\lambda_0=632 \text{ nm}$, $\Delta \nu/c=556 \text{ cm}^{-1}$, of Cy5 in the “hottest” surface location is approximately 9x10$^{-22}$ cm$^2$/sr. Factoring out the electromagnetic enhancement, the average differential cross section, $\frac{d\sigma_{ad}}{d\Omega}$, $\lambda_0=632 \text{ nm}$, $\Delta \nu/c=556 \text{ cm}^{-1}$, for immobilized single Cy5 molecules under the given excitation conditions is 1.7x10$^{-25}$ cm$^2$/sr. In the absence of knowledge of molecular orientation, we have made the assumption that cross sections are amplified by the enhancement factor associated with the total local field. The
cross section estimates derived from factoring out these overly large EM enhancement factors are, therefore, somewhat small.

**Spatial Distribution of Electromagnetic Enhancement.** To understand how lateral localization of field enhancement determines the number of molecules that contribute significantly to the surface enhanced Raman signal, surface elements from the field simulations were ordered by local field magnitude to form surface enhancement distributions for cores and CSAs. The ‘non-Stokes shifted’ enhancement elements, \( E^+da \), corresponding to the 8000 element mesh of the surface of a core particle, were sorted in terms of enhancement value into a descending sequence. Partial sums of enhancements and area were calculated, **Figure 3-6**, for the purpose of determining the localization of high field regions, ie ‘warm spots’, **Figure 3-5**. Partial sums are useful for identifying the fraction of molecules that contribute a given fraction of total enhancement. From the enhancement distribution for dispersed core particles, half of the electromagnetic Raman scattering enhancement appears to be from scattering from the 18% of molecules positioned in regions of highest field. Field enhancement at the surface of core particles in CSAs is distributed differently, such that, under polarized illumination, only 6% of molecules contribute 50% of the SERS. As near fields were simulated for structures fixed in space relative to the polarization direction of the incident field, the simulated fields are high only in junctions aligned with the incident field. For fixed structures, the ~107 molecules that from simulation contribute 50% of the SERS are localized in only four ‘warm’ gaps. The assemblies, however, are tumbling in solution.
such that molecules in gaps under all ~12 satellites contribute intermittently, ie whenever the gaps are properly aligned with the incident field. Thus, the number of molecules, ~320, contributing half of the Raman scattering is 3-fold higher, ie 18% of the number immobilized on each core.

![Graph](image)

**Figure 3-6.** Partial electromagnetic enhancement of the CSA (red, dot dash) and the dispersed core (green, dot) vs. the percentage of surface area. The EM enhancement elements, $E^4 da$, that collectively determine the surface mean enhancement are sorted in decreasing order of $E^4$.

**3.6 Discussion and Conclusions**

Using weakly field enhancing substrates, we have found Raman scattering to be amplified by a factor close to the ratio of electromagnetic enhancements calculated for structures in two states. The consistency with electromagnetic theory of the amplification of Raman scattering from core immobilized Raman scatters upon assembly of satellite
particles suggests that cross sections, $\sigma_{ad}^R$, of surface enhanced molecules can be reasonably estimated by factoring out EM enhancement factors from Raman scattering detected under weakly field enhancing conditions. We suspect that the resulting cross sections, and cross sections of other molecules measured similarly, will be useful in interpreting Raman scattering from species exposed to more strongly enhanced fields. The hybrid experimental and theoretical approach to estimating properties of immobilized molecules differs from purely experimental quantitative studies that determine total surface enhancement from all mechanisms [13]. Corroboration of the theory follows from the use of substrates that allow retuning of the strength of the electromagnetic component of the surface enhancement. Measurements of SERS from substrates under different levels of EM enhancement yield multiple ‘measurements’ of the intrinsic cross sections, $\sigma_{ad}^R$, of molecules interacting with substrates.

Here we consider also limits of ‘detection’ of Cy5 immobilized and detected as described in this work. From the estimates of effective Raman cross sections and signal to noise in the experiment, the detectible concentrations of Cy5 on CSAs are estimated for several variants of the system, assuming the same experimental geometry, components, source, and settings. From the derived concentration, 1.8 nM, of core-immobilized Cy5 and a signal/noise ratio of 10, the lowest concentration of core-immobilized Cy5 in the current core-satellite assemblies is 0.18 nM. The enhancement distribution can be used to predict a lower limiting concentration that could be detected if dye molecules could be localized within the interparticle gaps. From the 18% fraction
required for 50% of the signal, localization of molecules to warm spots could be expected to lower the concentration limit to 64 pM. Alternatively, one could consider the effect on detectibility of fabricating CSAs with different core-satellite spacings. As can be seen in Figure 3-7, which displays mean enhancement for a range of gap sizes, relative mean enhancement, $G_{EM}^{CSA}/G_{EM}^{core}$, is sensitive to the width of the gap. Reducing the gap from 6 to 3 nm would lead to a 10 fold increase in enhancement factor and a concomitantly lower detectible Cy5 concentration. However, further reductions in gap size ultimately lead, not only to higher enhancement, but also to the high sensitivities of field strength to geometry that result in large errors in estimates of molecule number, $N_{adv}$, or cross section, $\sigma_{adv}$.

Figure 3-7. Ratio of core surface-averaged electromagnetic enhancement for core particles in CSAs relative to isolated core particles.
The gap dependence of the electromagnetic enhancement ratios can be used to quantify errors in enhancement calculations that would be associated with errors in interparticle separations used in structural models. Estimated enhancement factors for structures formed from CSAs with satellites separated by ~6 nm are in error by 18-26% if the estimates of separation are low or high by 0.5 nm. An error of this level is unlikely to dominate the collection of other errors in field estimates, signal strengths, and other experimental variables such as coverage. This magnitude of error is in contrast to the 50-200% enhancement error associated with a 0.5 nm error in the estimate of separation for satellites ~2 nm from a core. While uncertainties in electromagnetic enhancement estimates for other coupled structures will be different, uncertainties in enhancement estimates for other structures comprised of plasmonic components with 1-2 nm separations can be expected to be large. Thus, the combined experiment/theory approach we have demonstrated for estimating cross sections of molecules on nanostructure surfaces is not recommended for molecules on substrates with 1 or 2 nm junctions.

In summary, we have observed enhanced Raman scattering from molecules immobilized on metal nanoparticles before and after formation of discrete multi-particle assemblies. Under conditions in which the contribution to enhancement from chemical interactions between the Raman-active Cy5 species and the metal particles is likely to be constant, amplification of the Raman scattering observed upon formation of assemblies is interpreted in terms of the electromagnetic enhancement mechanism. While the near fields of dispersed 50 nm particles under 8 mW HeNe illumination in a LabRAM are sufficiently enhanced to result in detectable SERS from a monolayer of molecules on a
picomolar solution of particles, plasmonic coupling between the metal nanoparticles in core satellite assemblies leads to higher local field intensities and stronger Raman scattering from molecules in the CSA. The amplification of enhancement observed in experiment agrees quantitatively with the prediction determined for CSA geometries based upon interparticle separations controlled by the double stranded DNA linkers. Substrates that provide controlled EM enhancement may prove useful in further analysis of the scattering properties of molecules in gaps. Substrates that are amenable to quantitative interpretation, further, may prove useful in applications for which quantitative outputs are more important than ultrasensitivity [97].
Chapter 4 Raman Scattering Profile Controlled by Particle-Film Surface Plasmons

Surface plasmons supported by metal nanoparticles are perturbed by coupling to a surface that is polarizable. Coupling results in enhancement of near fields and may increase the scattering efficiency of radiative modes. In this study, we investigate the Rayleigh and Raman scattering properties of gold nanoparticles functionalized with cyanine deposited on silicon and quartz wafers and on gold thin films. Dark-field scattering images display red-shifting of the gold nanoparticle plasmon resonance and doughnut-shaped scattering patterns when particles are deposited on silicon or on a gold film. The imaged radiation patterns and individual particle spectra reveal that the polarizable substrates control both the orientation and brightness of the radiative modes. Comparison with simulation indicates that, in a particle-surface system with a fixed junction width, plasmon band shifts are controlled quantitatively by the permittivity of the wafer or the film. Surface enhanced resonance Raman scattering (SERRS) spectra and images are collected from cyanine on particles on gold films. SERRS images of the particles on gold films are doughnut shaped as are their Rayleigh images, indicating that the SERRS is controlled by the polarization of plasmons in the antenna nanostructures. Near field enhancement and radiative efficiency of the antenna are sufficient to enable Raman scattering cyanines to function as gap field probes. Through collective interpretation of individual particle Rayleigh spectra and spectral simulations, the geometric basis for small observed variations in the wavelength and intensity of plasmon resonant scattering from individual antenna on the three surfaces is explained.
4.1 Introduction

Raman scattering from molecules positioned close to metal surfaces is well known to be enhanced when the substrates support surface plasmons and molecules are within the near fields of surface excitations [59-63]. The surface plasmon modes that provide the field enhancement may also facilitate outcoupling of Raman emission [15, 98]. The high specificity of Raman spectra and their sensitivity to the local environment have motivated extensive investigation of the Raman scattering enhancing properties of structures that support surface plasmons [66-68, 70, 72-74, 76, 99, 100]. Desirable properties of substrates for surface enhancement of Raman scattering (SERS) include geometries that support large polarization charge, provide robust control of resonances, and allow resonances to be tuned. Among plasmonic substrates that provide high field enhancement are structures with junctions such as pairs of particles or clusters [59-63, 99, 101, 102] and particles on polarizable films [103-112]. Whereas structures designed to enhance fluorescence must balance field enhancement against quenching [113], SERS signals will be brightest when molecules are closer to substrates or in extremely narrow junctions that support intense gap fields. However, in these systems, SERS is strongly sensitive to junction width [101, 102, 107]. Furthermore, quantitative excitation and enhancement requires not only control of junction width but usually also alignment of the SERS substrate relative to the polarization direction of the incident field. We previously have reported quantitative SERS at the ensemble level from solution phase assemblies that used molecular tethers to control junction width [114]. In this bulk phase system the problem of aligning structures with the incident field direction was circumvented through
use of symmetric satellite structures that incorporate inter-particle junctions oriented in all directions. However, it would be desirable to control both junction width and orientation in structures on surfaces where the properties of single structures can be observed.

Recently some of us have demonstrated control of plasmon resonance and polarization in a format that consists of a metal particle on a dielectric spacer layer on a metal film [115]. This format provides intrinsic control of the orientation of the observable dipolar excitation, as evident from the doughnut shape of the particle image. The reproducible dependence of the plasmon resonance frequency of single nanoparticles on the thickness of the spacer layer points to good control not only of the plasmon resonance, but also of the separation between the particle and the film. As junction width is the variable to which gap fields are most sensitive, control of junction width implies control of field enhancement and suggests that the particle – film system is promising for quantitative SERS. Here we investigate both the directionality of scattering and the influence of surface composition on the scattering efficiency of particles on films.

Prior studies have documented control of plasmon resonance in particle on surface systems using thin films comprised of lithium fluoride [116], silica [117-119], polymers [104], and self-assembled monolayers [107]. These and other studies document red shifting of nanoparticle plasmons when metal nanoparticles are deposited on dielectric or metal surfaces [115, 117, 119-124]. The polarization of scattering from nanoparticle -surface systems has been characterized both by angle scanned detection [117, 118] and, more recently, by imaging [115, 119]. The role of discrete nanostructures
in controlling the directionality of scattering has been studied implicitly through investigation of scattering from coupled structures under polarized illumination [125-129]. Ongoing research is devoted to understanding how these optical antenna systems control the spatial properties of emission. Several groups have studied how single metal nanostructures modify emission from single fluorescent molecules. Modification of both the rate and polarization of single molecule emission has been reported as a function of the molecule’s relative position and proximity to a metal nanodisk [130]. Redirection of emission from a molecule with a horizontally oriented transition dipole has been demonstrated using a vertical nanorod [131]. The emissive properties of quantum dots and molecules positioned in junctions between nanoparticles also have been investigated on the single nanostructure level [63, 101, 102, 132-135]. These investigations of how antenna systems control molecular emission build upon prior studies of two photon photoluminescence (TPPL) [129, 136-138] and inelastic scattering [121]. Studies of both discrete coupled structures and particles on films provide compelling evidence of the effectiveness with which plasmonic nanosystems direct emission.

Here we adopt the cyanine functionalized particles used previously as templates for assembly of bulk phase satellite structures [114] for use in the planar format of a particle on a film. Under resonant illumination, cyanine in the near field of the most field enhancing coupled system displays surface enhanced resonance Raman scattering (SERRS). We investigate the ability of metal, semiconducting, and dielectric surfaces to control frequency, intensity, and alignment of the brightest radiative plasmon mode and the role of the plasmon in controlling the spatial properties of SERRS. The antenna are
gold nanoparticles positioned on one of two highly polarizable surfaces, gold and silicon; particles on quartz serve as a control.

4.2 Results

Gold nanoparticles were functionalized with cyanine- and thiol-modified oligonucleotides as described previously [114]. Fluorescence of the initial solutions and supernatants were monitored to quantify final coverage of cyanine (Cy5) on the particles. From the molecules adsorbed per particle, the area density of Cy5 molecules is one per 4 nm$^2$. Gold films were deposited on quartz by e-beam evaporation. Polyelectrolyte, poly(allylamine) hydrochloride (PAH) was deposited on silicon and quartz wafers and on gold films. Prior to deposition of the cyanine-functionalized particles, the films and spacer layers were characterized using ellipsometry. The thickness of the native oxide layer on silicon was 1.83 nm. In the region of the antenna resonance, relative to bulk values [94], the dielectric constant of the gold film displayed a larger imaginary part. Thicknesses of 0.56, 0.13 and 2.9 nm were found for the PAH, on gold, silicon and quartz. Thin PAH layers have been observed previously [139] for PAH on silicon titanium oxide. However, uncertainties in the thickness estimates for PAH on silicon and on quartz are large due to low contrast between the PAH and either glassy or crystalline SiO$_2$. 

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Figure 4-1. Illustration of the optical setup for collecting Rayleigh or Raman signals. White light excitation through the objective and two laser excitation schemes and the image collection path are shown. For collection of spectra, light is directed through a pinhole to a spectrometer (not shown). Inset: Antenna system comprised of a gold nanoparticle electrostatically coupled to a gold film. The Raman scatterers, Cy5, are tethered to the nanoparticle. The antenna gap width is controlled by a polyelectrolyte film.

Dark field color images were collected from nanoparticles deposited on the polyelectrolyte spacer/adhesion layer on all three surfaces, using a customized Nikon darkfield (DF) microscope, Figure 4-1. A description of the setup is in the methods section. Nanoparticles were illuminated by collimated white light passed through and collected by the same objective (100x DF, numerical aperture (NA), 0.9). Exposure times were 1.6 s for nanoparticles on gold and silicon and 5 s for nanoparticles on quartz because of the dependence of the cross section upon the polarizability of the substrate. Similar coverages of particles were observed on all substrates. Scattered light was red, yellow-green, and green from particles on gold, silicon and quartz respectively, Figure 4-2. Red-shifting of the plasmon band is a signature of longitudinal particle – surface
coupling. The colors, thus, provide a qualitative measure of the strength of interaction between nanoparticle plasmons and plasmons or more localized excitations at the surfaces, respectively, of gold, silicon, and quartz.

Figure 4-2. Dark field images of gold nanoparticles on (A) a gold thin film, (B) a silicon wafer, and (C) a quartz wafer. Plasmon band position is displayed in the color of the images. The doughnut shape of the images of particles on gold and silicon indicates that, on high permittivity surfaces, scattering is dominated by a vertically polarized mode.

Gold nanoparticles on gold films and on silicon appear in dark field Rayleigh images as doughnut shapes. The doughnut shape of the image indicates that radiation from each nanoparticle is associated with an oscillation oriented normal to the wafer or the film [140]. Coupling of the nanoparticles to a surface with high permittivity thus creates a population of nanoantennas whose orientations are well defined. As the exciting field has components both perpendicular and parallel to the film, the doughnut shaped profile and darkness of the doughnut center indicates enhanced scattering from vertical relative to horizontal modes. Enhancement of emission from a vertical electric dipole (VED) relative to a horizontal electric dipole (HED) previously has been observed both from molecules [130, 141] and metal nanoparticles close to a metal surface [115]. Whereas the antenna resonance of nanoparticles on silicon is less red shifted than the
resonance of nanoparticles on gold, the polarizability of silicon is sufficient to produce an image with a doughnut shaped point spread function. Images of nanoparticles on quartz do not display a spatial distribution characteristic of a VED, consistent with limited interaction with the surface, as indicated by their color, green.

Figure 4-3. Single particle scattering spectra and spectral simulations of gold nanoparticles on a polyelectrolyte layer on quartz and silicon wafers and on a gold thin film. (A) Single particle spectra. Illumination and collection are through a 100x objective lens. Also shown is the absorption spectrum of the Cy5-modified oligonucleotide (shaded) and the laser line used for SERRS. (B) Simulated scattering spectra of a gold nanoparticle on each surface. Thicknesses of the dielectric spacer layers and the dielectric function of the gold film are as derived from ellipsometry.

To characterize more completely the properties of the nanoantennas, dark field Rayleigh spectra were collected from the individual particles on all three surfaces and compared with simulation. Dark field spectra were acquired using the same optical setup as was used for imaging except that output was directed through a different output port and dispersed by a spectrometer (Acton Spectrapro 2300i) onto a cooled CCD detector (Photometrics CoolSnap HQ). To allow collection of single particle spectra, a 200 µm pinhole aperture is used. The integration time is 15 s for all spectra; thus, intensities
reflect the relative scattering intensities of single particles on the three surfaces. The scattering signal from a single nanoparticle is corrected by the background collected from a nearby region without particles. Spectra from a single particle with a scattering intensity closest to the mean intensity from particles on each surface are shown in Figure 4-3(a).

Scattering band positions are 530 nm, 561 nm, and 668 nm for particles on quartz, silicon, and gold, consistent with the colors of the Rayleigh images. Relative scattering intensities are approximately 1:5:25 for particles on the three surfaces, thus brightness is correlated with red shifting of the dominant mode. Previous studies of gold particles on dielectric surfaces have correlated the magnitude of the red shift of the resonance with the permittivity of the surface by invoking the image dipole approximation [122]. Thus the longer wavelength of the resonance of particles on silicon (dielectric constant, $\varepsilon \approx 16.3$ at resonance, 561 nm) relative to that of particles on quartz ($\varepsilon \approx 2.13$) is as expected. However, the further redness of the primary scattering resonance of particles on a gold film relative to particles on silicon indicates that the radiative mode of a nanoparticle antenna is more strongly coupled to the gold film ($\varepsilon_{668\ nm} \approx -14.1 + 1.02i$) than to silicon. The plasmon band of the gold on gold antenna displays some distortion, most likely due to Cy5 absorption [142-144].

Inelastic scattering spectra in the Stokes range were collected from particles on all three surfaces. Inelastic scattering spectra were collected also from control nanoparticles on the gold film, using gold particles functionalized with oligonucleotides lacking the Cy5. For these measurements, a commercial Raman micro-spectrometer was used. 632.8 nm laser light (8 mW) on resonance with the Cy5 molecule was used to
illuminate nanoparticles on all three surfaces. The laser line falls within the absorption band of Cy5 and within the primary scattering band of the gold nanoparticles on the gold film. Representative spectra are shown in Figure 4-4. Under red illumination, a Raman spectrum was collected from the substrate with the red surface plasmon, the substrate comprised of gold particles on a gold film. The spectrum matches a bulk SERRS spectrum collected previously from nanoparticle cluster structures formed of the same Cy5-modified oligonucleotide nanoparticle conjugates [114], with no polyelectrolytes (PE) present. At this red excitation wavelength, Raman scattering from yellow-green or green particles on silicon and quartz substrates was not observed. Spectra collected from control particles with oligonucleotides lacking the Cy5, did not display detectible Raman peaks when deposited on another region of the PE on gold film. Thus neither DNA nor PE contributes to the Raman scattering observed.

Figure 4-4. Raman spectra (λ₀=632.8 nm) of Cy5 functionalized gold nanoparticles on three surfaces: silicon, quartz, and a gold thin film. Raman features are detected from the particles on gold, only. Scattering from control particles lacking Cy5 on a gold film is also shown. The spectrum of the nanoparticles on the Au film is offset by 30 counts.
Rayleigh and Raman laser light scattering images were collected from nanoparticles deposited on the polyelectrolyte spacer/adhesion layer on a gold film on quartz. A total internal reflection (TIR) excitation configuration was used owing to the small working distance of the 100x objective lens. Laser light (30 mW) at the same frequency used to collect Raman spectra was directed through the prism and sample substrate at 45 degrees and focused to a 100 µm spot. Raman images (integration time, 20 s) were collected using a long pass (LP) filter to remove the Rayleigh scattered light. Dark field Rayleigh images were taken using a shorter integration time (1s) under attenuated illumination to prevent saturation of the CCD. In the laser scattering image, Figure 4-5, as in the color image, Figure 4-2, gold nanoparticles on gold films have profiles with doughnut shapes. Under TIR illumination, as with excitation through the objective lens, the exciting field has components both perpendicular and parallel to the film [145]. Nonetheless, under monochromatic as well as broadband illumination, the scattering pattern has the signature of a VED.
In the LP-filtered image, scattering is observed from the same locations as the sites from which Rayleigh scattering is observed. The Raman images, also, display a doughnut shape. This matching of spatial properties indicates that Raman scattering is governed by the radiative properties of the particle – surface plasmon. On a substrate that tightly localizes field intensity within a particle – surface gap, the molecules contributing to the Raman scattering will be the small number of molecules in the region of each gap with the highest electric field. Blinking of the Raman signals from many particles was observed (Figure 4-6). Blinking may signify motion of the particle or molecule or signify intermittency in Raman scattering from a single Cy5 molecule. As the duration of an on-state is comparable to the period required to collect a Raman signal, not all blinking antennas displayed blinking as clearly in a time trace as in the real time image or in a video (Supporting Information 1). A control experiment was performed using particles
functionalized identically with a thiolated oligonucleotide that lacked the cyanine. Under the same excitation and collection conditions, surface-enhanced Raman scattering (SERS) signals were not observed (Supporting Information 2).

![Figure 4-6](image)

Figure 4-6. Intermittent Raman signal from a single nano-antenna. Time series of inelastic scattering from the Stokes shifted frequency range and series of images spanning several blinks.

The doughnut shaped point spread function of the Raman images of the SERS active substrate, comprised of gold nanoparticles on a gold film, demonstrates that, in this nano-antenna system, Raman emission has a scattering profile similar to the profile of a VED. To clarify the role of the vertical mode for Raman excitation, inelastic scattering spectra were collected in the custom microscope with the LP-filter using s- and p-polarized and unpolarized (30 mW) laser light. To control the incident light polarization more completely than possible using evanescent excitation, the laser beam is directed at the sample from the side and slightly focused to a $7.8 \times 10^3 \, \mu \text{m}^2$ spot. Unpolarized incident power density thus was 380 W/cm$^2$. To accommodate the laser beam, a 50x (0.55 NA)
objective with a longer working distance is used. Using the lower NA objective, scattering from a vertically oriented antenna is less efficiently collected; however, under unpolarized and p-polarized illumination both Raman spectra and Raman images were observed. Figure 4-7. P-polarization results in slightly lower signals than unpolarized excitation. However, all the brighter Raman active particles displayed Raman scattering under p-polarized excitation. S-polarized excitation did not produce any observable SERRS. In this configuration SERRS was collected also from smaller groups of scatterers under unpolarized illumination. Integrated intensity of the band at 1191 cm$^{-1}$ collected by the 50x (NA 0.55) objective from 3 particles was 37.8 cps. Correction for the efficiencies of the optical components and the CCD yielded a corrected photon count of 750 photons per second (Supporting Information 7).

Figure 4-7. SERRS spectra and images of the nanoparticle on gold film under different excitation states: unpolarized (blue), p-polarized (red), and s-polarized (black). Spectra and images were
collected through a 50x objective. The dimensions of the displayed images are 5.7 µm x 5.7 µm. Exposure times were 120 s for Raman spectra and 25 s for Raman images.

### 4.3 Discussion

Overall, the experimental data confirm that surface properties are of key importance in determination of the band positions of the radiative resonances of metal particles on planar surfaces, and that the primary radiative mode of the gold particles is vertically polarized when the particle is positioned 1 or 2 nm from a gold or silicon. In addition, we observe that, for Raman scattering as for fluorescence, the directionality of scattering from molecules in surface enhanced fields is controlled by the scattering properties of the radiative antenna surface plasmon modes. Prior fundamental studies of the coupling of particle and surface modes indicate that, at close spacings, the particle – surface system may support multiple coupled modes [146, 147]. However, not all modes are radiative and band overlap may preclude detection of closely spaced modes. Rayleigh spectra collected from the gold nanoparticle on gold film, Figure 4-3(a), do reveal, however, not only a primary mode more than an order of magnitude brighter than the dipole mode of a nearly free particle on quartz, but also a much weaker, higher energy mode at ~560 nm.

While the polarization of the brightest modes can be determined directly from the images, the character of the less bright modes may be inferred from their excitation by polarized light. Here we address the character of the radiative modes using simulation. Particle – surface coupling was calculated and scattering spectra simulated using the electrodynamic method of Bobbert and Vlieger [148]. In this method, the vector spherical
harmonic (VSH) modes of a sphere are coupled through the sphere’s response to reflection by the surface of the VSH scattered waves, Supporting Information 3. Surface plasmon modes of like orientation thus are mixed. In the model, particle size was 50.8 nm. PAH and oxide layer thicknesses were as determined from ellipsometry. Initially, we reproduce spectra collected under unpolarized excitation. To simulate excitation from the dark field objective lens, an incident wave 70° from normal is used. To simulate collection through the 100x objective, the angular range of scattering included in the calculation is matched to the acceptance cone of the lens. Simulated scattering spectra for gold particles on the three surfaces are shown in Figure 4-3(B). Plasmon band positions, widths, and relative intensities are reproduced extremely well. For all particle – surface systems, the average difference in band position between simulation and experiment is no more than several nanometers. Some cancellation of error may underlie this match, as the thickness and refractive index of the dessicated oligonucleotide particle-capping layer was not known and the dispersive property of the cyanine was neglected. However, the match of the scattering simulation to the observed spectra for particles on surfaces of all types confirms that, for a given antenna geometry, the complex permittivity of the surface controls both the intensity and wavelength of the primary scattering band. To clarify the effect on spectral features of the wider gap of the antenna on silicon compared with the antenna on the gold film, a spectrum was simulated also for a nanoparticle positioned as close to silicon as the nanoparticles are to the gold films (Supporting Information 4). The gap width variation accounted for only ~10% of the difference in band position. A spectrum was calculated also for a nanoparticle on a substrate with the permittivity, $\varepsilon_0$, of
free space using the same excitation/collection geometry. The calculation revealed that even a low permittivity substrate such as quartz enhances scattering from the nanoparticle several fold, and that, relative to a free particle, scattering from the nanoparticle on a gold film is two orders of magnitude more intense. For the gold on gold film system, furthermore, the simulated spectrum includes the low intensity, shorter wavelength (~570 nm) band detected in the experimental spectrum, Figure 4-3(A).

![Simulated spectra of nanoparticles on three surfaces: (A) gold (B) silicon (C) quartz under excitation with unpolarized (dash), p-polarized (solid) and s-polarized light (dot). Angle of incidence is 70° from normal; collection is of the light cone within sin⁻¹(NA), NA=0.9.](image)

While, the widths of resonances of nano-antenna composed of gold are too large to allow many bands to be resolved, the simulation capability provides an opportunity to investigate the substrate dependence of scattering from overlapping or low dipole moment modes. The response of the particle-film system under s- and p-polarization was simulated, although, in the experiments, only unpolarized light was used. On quartz, Figure 4-8(c), the horizontal and vertical dipole modes are minimally split; however, compared with a nanoparticle on an ε₀ substrate, only the p-component is enhanced. On silicon, Figure 4-8(b), while under unpolarized illumination only a single band is
observed, the vertical dipole mode is both red-shifted and 100-fold more intense than the horizontal mode. For a nanoparticle on a gold film, Figure 4-8(a), both modes observed in experiment are modes excited almost exclusively by the p-polarized component. Thus the higher energy mode is most likely a higher order mode possessing dipole character by virtue of sphere mode hybridization mediated by the film [146, 147]. Response to the s-polarized component consists of a weakly scattering mode at 530 nm and an even weaker mode at ~563 nm. In nanoparticle – film systems where the uncoupled particle modes are all lower energy than the interacting continuum of the film, theoretical studies indicate that hybridized particle modes retain their spectral order as coupling to a substrate is increased, such that the longest wavelength mode of a given azimuthal character will be the mode with the greatest dipolar character [146, 147, 149]. In the gold nanoparticle – gold film system investigated here, it appears that, while s-wave excited modes scatter too weakly to be readily detected, at least one higher order mode can be observed.

The dominance of the spectrum by the brightest vertically oriented mode contributes to the spectral control offered by the simple geometry of a particle on a planar film. The match between representative (mean intensity) single particle Rayleigh spectra and simulated spectra provides strong evidence that the plasmonic properties of these junction bearing substrates are well controlled. Nonetheless, a better understanding of the robustness of the system requires consideration of the ensemble of individual particle spectra. Thus, a full set of single particle Rayleigh scattering spectra from each substrate are displayed, Figure 4-9(a)-(c). Spectra of particles on each surface are remarkably reproducible with respect to both intensity and peak position. Variations in intensity
relative to mean intensity are 31%, 18%, and 25% for particles on gold, silicon, and quartz, respectively. Standard deviations in peak wavelength are 7 and 6 nm for particles on gold and silicon, and 1.6 nm for particles on quartz.

Figure 4-9. Single particle scattering spectra and spectral simulations of gold nanoparticles on three surfaces. (a)-(c) Rayleigh scattering from single gold nanoparticles functionalized with Cy5-modified oligonucleotide on a polyelectrolyte layer on (a) a gold film, (b) a silicon wafer, and (c) a quartz wafer. Single particle spectra are colored. The average of the single particle spectra is identified by an arrow and shown in black. (d)-(f) Simulated spectra for nanoparticles of mean size and $+\sigma/-\sigma$ variations. (g)-(i) Simulated spectra for nanoparticles on the three surfaces with spacer layers of varying thickness. For the (g) gold and (i) quartz surfaces, the layer thickness varied is the thickness of the polyelectrolyte layer. For the (h) silicon surface, the thickness varied is the combined thickness of the polyelectrolyte and oxide layers. For all simulations, the nominal thickness value is the thickness measured by ellipsometry. Note that, in simulations, gap widths
are larger than spacer thicknesses by the thickness, 0.3 nm, assigned to the nanoparticle dielectric shell.

To gain insight into the geometric origins of the observed spectral variations, scattering spectra simulations were performed for particles of variable size or variable separation from the three substrates. On all surfaces, scattering intensity depends strongly upon particle size, Figure 4-9(d)-(f). For size variations within one standard deviation, variation in intensity scales nearly linearly with the sixth power of the size (Supporting Information 5). The dependence of scattering intensity on size for particles on a surface thus matches that of spherical particles in homogeneous media. The sensitivity of band position to size varied significantly among the substrates. Variation in peak position with a $+\sigma/\sigma$ change in particle size is $+11/-8$ nm, $+4/-4$ nm, and $<1$ nm for particles on gold, silicon, and quartz, respectively. Thus, antenna plasmons of particles of variable size on the more highly polarizable surfaces display positively correlated dispersion in scattering intensity and peak wavelength. On these substrates, positive correlation between variation in intensity and band position is the signature of size dispersion. The result is expected to be general except when confounded by wavelength dependent interband transitions.

Gap width plays a key role in determining plasmon resonance and a critical role in field intensity in a metal-insulator-metal gap. We are interested in both the signatures of gap width variation and sensitivity of simulation results to errors in measurements of thicknesses of spacer layers. To test the sensitivity of the scattering to variations in polyelectrolyte and/or silica thickness, spectra were simulated for nanoparticles separated
from surfaces by dielectric layers with thicknesses different from the thicknesses derived from ellipsometry. Representative simulated spectra are shown in Figure 4-9(g)-(i). On gold and silicon a 1 Angstrom increase in thickness of the polyelectrolyte spacer layer results respectively in a 4 or 0.7 nm blue shift in the nanoparticle scattering band position. On quartz, low contrast of the polyelectrolyte relative to quartz renders the spectrum insensitive to changes in thickness of the PE layer. The sensitivities to dielectric layer thickness on gold and silicon are consistent with the expectation that plasmons in strongly coupled structures will have resonances highly sensitive to gap width. While intensity also is sensitive to gap width, the change in intensity relative to the change in band position is much less if due to variation in gap width than if due to variation in particle size.

Knowledge of the sensitivities of antenna scattering to particle size and spacer thickness leads to hypotheses about the geometric basis both for variations and invariance observed in the single antenna spectra. Minimal variation in peak position among the antenna spectra of particles on quartz, Figure 4-9(c), is explained by the insensitivity of the band position to either particle size or particle – surface separation displayed in the simulations, Figure 4-9(f) and (i). For the weakly coupled gold – quartz system (Figure 4-9(c)), the invariance of peak position further indicates low dispersion in particle shape. As the intensity of scattering from gold particles on quartz is insensitive to the thickness of the polyelectrolyte layer, we interpret the observed variation in intensity as a manifestation of dispersion in particle size. From the sensitivities displayed in simulation, the observed variation of 25% in intensity corresponds to a variation in particle size of
~6%. Scattering spectra from gold nanoparticles on silicon (Figure 4-9(b)) display a similar variation in intensity and also variation in band position. As the band position for nanoparticles on silicon varies only slightly with size dispersion, the observed variation in band position is most likely a manifestation of variation in particle – surface separation. This hypothesis is strengthened by the minimal variation in band position observed for particles on the most weakly interacting surface, quartz, as low dispersion in resonance wavelength rules against the alternative explanation, heterogeneity in particle shape. On silicon, nanoparticle – silicon separation is determined by the combined thicknesses of the optically similar polyelectrolyte and oxide layer, and variation in gap width cannot be attributed to a specific layer. Correlation of peak intensity and band position observed among single particle spectra of gold particles on a gold film (Figure 4-9(c)) is displayed also in simulations of spectra of particles dispersed in size. The variation in intensity is consistent with the size distribution displayed by the nanoparticles on quartz. In the spectra of gold particles on gold film, the correlation between intensity, \( I \), and peak location, \( \lambda^* \), is somewhat scrambled relative to what would be expected from size variation alone. This suggests that the single nanoparticle spectra may reveal the variation among nano-antenna gap widths. From comparison with simulations of the gap width dependence of Au nanoparticles on gold, Figure 4-9(g), scrambling of the \( \lambda^*-I \) correlation in the single structure spectra is consistent with a modest (~12%) variation in spacing between the nanoparticles and the gold film, Supporting Information 6.

The resonant Raman scattering enhancement provided by the coupling of the nanoparticle to the gold film enables not only Raman imaging but also spectral collection
from a small number of molecules in gaps between a small number of nanoparticles and the film. Having determined previously a Raman cross section of Cy5 [114], it should be possible to use the molecules to probe gap fields. As the Raman scattered signals are too weak to allow collection of a single molecule spectrum, ensemble data may nonetheless be useful if the variation in gap widths and, thus, gap fields is not large. In the PE coupled system, variations in gap width are remarkably small. Thus variation in gap fields and electromagnetic SERS enhancement also will be unusually small. If Raman signals are strong enough to allow Raman bands to be collected from several molecules in gaps, the integrated bands may provide meaningful estimates of antenna gap fields. In our experimental system, the excitation power density, collection angle (defined by NA), number of nanoparticles illuminated, and density of Cy5 on each nanoparticle are well defined. Prior understanding of the tight confinement of high fields within a particle–film gap [110, 150, 151] combined with low Cy5 coverage (1 per 4 nm$^2$) collectively suggest that a SERS enhancement factor for molecules in the gap may be determined under the assumption that Raman scattering is from one molecule per particle. Using the Raman cross section determined previously (order $10^{-25}$ cm$^2$/sr after correction for electromagnetic enhancement) for the 1191 cm$^{-1}$ Cy5 Raman band ($\lambda_0=632.8$ nm) from the functionalized nanoparticles in bulk [114] and the efficiency of collection of the scattering signal from a particle on a film, we obtain a total apparent Raman cross section of $1\times10^{-17}$ cm$^2$ from a spectrum collected from three nano-antenna (Supporting Information 7). Assuming isotropic scattering for the solution phase Raman scattering, the electromagnetic SERS enhancement in the gold particle–polyelectrolyte–gold film
system is estimated to be $8 \times 10^6$. This electromagnetic SERS enhancement factor is surprisingly similar to a mean gap enhancement factor reported for a much larger number of molecules in a silver dimer gap ($2 \times 10^7$) [152] and a peak enhancement factor for much smaller silver nanoparticles on a thicker gold film [110]. However, we have neglected unknown effects of differences in on-state lifetimes for molecules in electric fields of unlike strength.

**4.4 Conclusion**

We have demonstrated that nanoantennas comprised of gold nanoparticles on spacer layers on diverse surfaces radiate light at frequencies that differ significantly and depend predictably upon the complex permittivities of the materials of which the surface is comprised. Plasmon band position sensitivity to permittivity has been observed previously when metal nanoparticles are deposited on dielectric films [122, 123]. The wavelengths of the scattering resonance of the nanoantenna, however, are very different on gold and silicon despite similar permittivity magnitudes and differences in gap width insufficient to explain the spectral variation. The electronic character of the materials, therefore, plays an important role; at optical frequencies only the metal has a negative permittivity and supports surface plasmon waves, whereas the response of silicon is dominated by electrons that are bound. While we have not tested the match of simulation to resonance of a metal particle on a metal film under TIR excitation, the implemented treatment of reflection captures intrinsically, not only coupling among localized plasmon modes, but also the interplay between localized modes and radially propagating surface waves.
It further has been shown that, for the particle on film geometry, not only band positions, but also Rayleigh scattering intensities are both enhanced and well controlled. On quartz, silicon, and a gold film, scattering from the brightest mode of a 50 nm gold nanoparticle is enhanced by approximately 5-, 25-, and 125-fold, respectively, relative to scattering from a free nanoparticle dipole mode. While we collect scattering primarily from vertical modes that do not scatter particularly efficiently into even a high NA objective, enhancement of vertical modes by the substrates allows VED scattering from single particles to be readily detected. From simulations and Rayleigh scattering characterization of single nanoantennas on all three surfaces, the intensity of VED scattering is found to depend, not only upon the composition of the surface, but also sensitively upon nanoparticle size.

In junction-bearing near field enhancing nanostructured substrates, the spectral signatures of variations in geometry are in general not unique. We therefore have used simulations to determine the distinctiveness of the plasmonic signatures of geometric parameters of key importance to this system. For particles on surfaces that shift the primary plasmon band position to the red region, where surface plasmon band positions of gold nanostructures are highly sensitive to perturbations in the resonance conditions, intensity variations associated with dispersion in particle size are accompanied by correlated small shifts in band position. Gap width variations in contrast are manifest primarily as variation in peak position. Whereas, in many coupled structures band position variation reflects variation in shape and orientation, as well as size and junction width, in the particle - surface system studied here, deviation from the particle-size
dependent $I-\lambda^*$ correlation provides a measure of variation in gap width. This assignment is made possible by the insensitivity of the scattered signal to other geometric variables such as shape dispersion by virtue of the vertical orientation of the brightest plasmon mode. The observation is of relevance to quantitative detection of SERS (and SERRS), as variations in SERS enhancement from nanostructure to nanostructure will be amenable to correction if single structure spectral data can be used to identify gap widths and gap fields.

From imaging we find that the scattering is dominated by a vertically oriented plasmon excitation when the nanoparticle is deposited on either highly polarizable material, gold or silicon. For the Raman-active substrate examined in this work, Raman images, like Rayleigh images, are doughnut-shaped, indicating that the directionality of SERRS is controlled by the orientation of the radiative plasmon mode. The result extends control of polarization of molecule emission previously observed for fluorescence [130, 131] to Raman scattering. In the Raman active system comprised of nanoparticles on a gold film, blinking of the Raman images of cyanine functionalized gold nanoparticles was observed. SERRS was not observed from particles on surfaces other than gold. However, the polarization control provided by the metal nanoparticle on silicon system suggests that nanoparticles will play a role in directing light in integrated optoelectronic systems.

Polarization control is critical also in both fundamental studies of plasmons and their interactions with molecules and in applications that require quantitative measurements of SE(R)RS. Control both of orientation and of gap width allows not only
excitation and emission, but also efficiency of collection, to be controlled. We observe not only control of antenna surface plasmon resonance intensity and band position, but also control by the substrate of the polarization of both Rayleigh and Raman emission which enables the fraction of emitted light collected to be controlled. On a gold film, nearfield enhancement and radiative rates are sufficiently large to allow Raman scattering images and spectra to be collected from a small number of blinking molecules. The few molecule measurements allow an electromagnetic SERS enhancement factor ($8 \times 10^6$) to be derived using a Raman cross section determined previously for Cy5 in a weaker field [114]. While the enhancement value is in the same range as values determined for junction-bearing noble metal nanostructures investigated by others [152], differences in on-state lifetimes in fields of different strengths will significantly affect enhancement estimates. We nonetheless suspect that the brightness of nanoparticle antenna and their sensitivity to surface permittivity will enable nanoparticle antennas to serve as reporters of both surface microstructure and the properties of surface molecules.

4.5 Methods

Sample preparation

Gold particles (BBI, mean size 50.8 nm, maximum standard deviation 4 nm) are functionalized with Cy5 modified 22-base oligonucleotides terminated with a 5’ thiol. (IDT DNA) The cyanine species, Cy5, is adjacent to the terminal alkane thiol. The adjacent 10 bases (adenines) have affinity for gold. The absorbance of the nanoparticles and fluorescence of the oligonucleotide solution and supernatants from successive rinses were recorded and used to quantify the concentrations of particles, excess cyanine, and
immobilized cyanine and the ratio of concentrations of immobilized cyanine and particles. The coverage of oligonucleotides is ~2000 strands per nanoparticle, Supporting Information 8. Detailed functionalization procedures can be found in previous reports [84, 114].

The quartz substrate was cut from a wafer (SQI) and cleaned with piranha solution (H$_2$SO$_4$;H$_2$O$_2$=3:1). The semiconductor substrates are p-type silicon wafers; the silicon is cleaned using acetone. The gold film on quartz was prepared by depositing a 5 nm chromium adhesion layer and a 45 nm gold thin film using e-beam evaporation (CHA Industries). Cationic polyelectrolyte (PE) is deposited on all three surfaces using the same self-limiting procedure [139]. Substrates are immersed in a 0.003 moles-of-monomer/L solution of poly(allylamine) hydrochloride (PAH) /1 M NaCl for 30 minutes, immersed in DI water for 1 min, rinsed with DI water, and dried under high purity nitrogen. A 100 µl drop of diluted functionalized gold particle solution (~10$^{-11}$ M) is placed on the PE surface of each surface for 1 min, rinsed with DI water, and dried with nitrogen.

**Thin film measurement**

Select layer indices and thicknesses were characterized by ellipsometry (J.A. Woollam M-88). Amplitude ratios and phase shifts are acquired at incident (and collection) angles 65°, 70° and 75° with wavelength scanning from 277.5 to 763 nm. Indices of refraction for the gold thin film and the thickness of the native oxide on silicon are determined prior to deposition of the polyelectrolyte, PAH. The refractive index of PAH is determined after deposition by fitting parameters in the Cauchy expression for normal dispersion to reflectivity data collected from PAH on gold (Supporting
Information 9) while simultaneously determining the thickness of the PAH thin film. The measurement and data analysis are performed using the software WVASE32. The refractive indices of silicon and quartz are from the literature [153, 154].

Dark field and Raman image setup

All images, Rayleigh spectra and some Raman spectra are acquired in a custom microscope equipped with two output ports; typically, output from one port is relayed to a spectrograph and output from the other is used for imaging. A commercial digital camera (Nikon D90) captures color dark field images. Raman images and gray level images of elastic scattering are recorded on a CCD (Photometrics CoolSnap ES). Signals are collected through a 50x (Nikon CF Plan NA 0.55) or 100x (Nikon CF Plan NA 0.9) objective lens. A long pass filter (Semrock LP02-633RU-25) is inserted between the objective lens and camera to remove the laser line when collecting Raman signals.

Spectrum acquisition setup

For Raman and Rayleigh scattering spectra, the scattering light is collected through the other port of the microscope and then relayed to a TE-cooled CCD (Photometric CoolSnap HQ). A pinhole is usually added to reduce background noise. For acquisition of Raman spectra, a high resolution grating (1800 grooves) is used. For Rayleigh scattering, a lower resolution grating (50 grooves) centered at 650 nm and spanning from 300 nm to 900 nm is used to obtain the broadband spectrum. Raman spectra ($\lambda_0=632.8$ nm) also were collected using a commercial Raman spectrometer (Horiba LabRam ARAMIS) equipped with an 8mW HeNe laser and 100x objective lens.

Excitation methods
For Rayleigh scattering spectra or images, the light source is a 75W Xenon lamp (OSRAM XBO); white light is directed to the nanoparticles through the objective lens. To acquire Raman signals, 30mW HeNe laser is used to excite the particles directly from the side or introduced through a prism at a 45° angle that leads to total internal reflection. Side excitation allows illumination of opaque substrates, and polarization of the incident laser is easily controlled by positioning a polarizer in front of the sample. To acquire the highest resolution images and characterize the point spread functions, TIR excitation of the particles on the gold film was used. TIR excitation allows collection of low angle scattering through a high magnification objective (100x) with a very small working distance. In this work spectra and images were collected in the TIR configuration from nanoparticles on a gold film.

Simulations

Scattering by the antennas into a cone determined by the objective was simulated using the method of Bobbert and Vlieger [148] (BV). The BV method describes scattering from spheres on planar surfaces and is a multipolar generalization of Sommerfeld’s method for evaluating scattering from a dipole on a planar surface [155]. We use the implementation of Germer [118, 156] which incorporates a dielectric shell on the particle and a multi-layered planar surface. Scattering spectra were computed initially for a series of particle-surface geometries in which a planar spacer layer atop the PE layer is assigned a width of decreasing thickness. Subsequently, the spacer thickness was assigned to the nanoparticle as a shell. In our system, this shell corresponds to space partially occupied by the modified oligonucleotides that cap the gold nanoparticles. As
the properties of the capping layer in air are not known, in our simulations the shell was assigned a thickness, 0.3 nm, and effective index, 1.22, consistent with the Rayleigh data. For sensitivity studies, the optical constants and dielectric functions used to describe the gold, silicon, and quartz were from Johnson and Christy,[94] Green and Keevers [157], and Palik [153] respectively. For comparison with experiment, optical constants and dielectric functions from the ellipsometric measurements were used.
Chapter 5 Modal Content of High Frequency Particle-Film Surface Plasmons

5.1 Introduction

The plasmonic nanoparticles can be treated as optical nano-antennas. These nano-antennas can concentrate the far-field excitation into small region even up to few nm$^2$, forming hot spots. These hot spots can strongly interact with the molecules or active materials to enhance the scattering or absorption, e.g. SERS. Also, these nano-antennas can modify the far-field properties of the emission from the materials interacting with them.

The particle/film hybrid structure is also formed an optical antenna. This structure has been used for SERS. This structure has been studied theoretically (e.g. B-V theory) and experimentally. According to Nordlander’s study, in addition to dipole mode, high order modes could exist. We have observed a spectral band to the blue of the primary gap dipole mode in single antenna spectra collected from gold particles on gold films. From comparison with simulated spectra, the band appeared to correspond to a mode excited most efficiently by p-polarized incident light.

In this chapter, we report further characterization of scattering from this higher frequency band. Spectra collected under polarized illumination confirm that the dominant higher order (HO) mode is excited by p-polarized radiation at a longer wavelength than the wavelength of the brightest mode observed under s-polarized illumination. Filtered images of scattering at the wavelength of the short wavelength band reveal that light focused through a high NA microscope objective scatters with a doughnut shaped profile.
similar to the profile of the brightest vertical electric dipole (VED) mode. Simulations of scattering of an HO-frequency incident plane wave to the far field reveal that, at this shorter wavelength, scattering has lower symmetry than a VED. Finite element calculations reveal that the gap field of the higher frequency mode has lower symmetry than the near field of the VED.

Further analysis of the character of the high frequency band is performed using a dielectric function with reduced loss which allows resolution of the underlying HO modes. The band is found to be comprised of two modes, one excited only under conditions of p-polarized excitation and another that is excited by either p- or s-polarized light. Both modes display nodes in the gap near field; under p-polarized illumination, both modes scatter primarily laterally. Whereas the mode excited only by a p-polarized field has a far field scattering pattern similar to a VED; under p-illumination, scattering from the mode excited by either an s- or p-polarized field, scatters directionally.

5.2 Method

BV method: The far-field scattering spectra are calculated by the method developed by Bobbert and Vlieger (BV). This method which includes the multipolar reflection of the particle scattering, extends the Sommerfeld’s method calculating the dipole interacting with a planar surface. The computer code based on this BV method is implemented by Germer. For the computational model, a 50 nm gold particle is placed above a dielectric film with 0.7 nm thickness and refractive index 1.65. Additional 0.3 nm spacing is inserted between the particle and film for the convergence purpose. The gold substrate with refractive index from Johnson and Christy’s (J-C) experimental data
is used below the dielectric film. The scattering intensity is integrated over the light cone spanning 64° around the +z axis. This angle is corresponding to maximum collection angle of an objective lens with numerical aperture of 0.9.

FEM: To calculate the scattered field in the near-field region, the finite element method (FEM) based full wave calculation is performed (COMSOL 3.5a). The excitation field is predefined by calculating the total field in the same model without the nanoparticle. In this case, this total field is obtained by solving a problem of a multilayer reflection system with incident wave at oblique angle. The incident angle is at 70° from normal in all following FEM calculation. The perfect match layer (PML) is used as boundary condition.

Figure 5-1. The scattering spectrum of the particle-film structure under p-polarized (left) and s-polarized (right) excitation with incident angle at 60°, 70° and 80°.

5.3 Dark field spectra and images

In the previous study, we use white light through objective lens to probe the scattering spectra of particle-film structures. However, this excitation configuration is not suitable for controlling the polarization and incident angle of the excitation light. Here, we used external (relative to microscope) white light source whose polarization can easily be adjusted by polarizer. Also, to make the incident angle well-defined, the white light is
only slightly focused on the film. The incident angle is limited within 70° to 90° from normal direction because the high NA (0.9) objective lens may block the incident light with smaller incident angle. As shown in Figure 5-1, the scattering spectra excited by p-polarized and s-polarized light have different features. The p-polarized excited spectra have a primary peak around 660 nm and a secondary peak around 575 nm. The primary peak may result from the gap dipole mode and the secondary peak may come from high order gap modes. The properties of these gap modes are investigated in more details in the following sections. For s-polarized excited spectra, the primary peak is located around 530 nm.

![Figure 5-2](image)

Figure 5-2. (UP) The dark field images filtered at 560 nm (left) and 650 nm (right). (DOWN) Cross section of the images from 15 selected particles.

The scattering images are collected to investigate the scattering profiles of the gap dipole and the HO modes, Figure 5-2. The white light through objective is used to obtain the better signals. The bandpass filters with 10 nm bandwidth centered at 650 nm and 560 nm are used to collect the scattering of gap dipole mode and HO modes respectively. The
integration time is 3 s. The images collected at 650 nm and 560 nm both have doughnut shape with a primary inhomogeneous intensity distribution around polar angle. In addition, the direction of this inhomogeneous distribution is consistent for all the particle images where the lowest intensity is distributed along the line at 60° away from the horizontal line. Therefore, this abbreviation may results from the imperfect alignment of the imaging system and further verification is needed. In order to study the intensity profile, we extract the intensity profile along the line 30° from the horizontal direction which is perpendicular to the line with lowest intensity. As shown in the Figure 5-2, for the dipole gap mode collected at 650 nm, the intensity is symmetric with respect to the line cross lowest intensity point. In addition, the secondary peak is also observed in the cross section. However, for the intensity profile collected at 560 nm, the profile is less symmetric. Also, the ratio between highest peak and center lowest point is much lower compared to the profile at 650 nm. The purely dipole may have pure doughnut shape. Therefore, the profile we observed at 560 nm implies that it may be contributed from more than one mode.

5.4 Far-field calculation

The previous experimental results indicate that the HO gap modes exist and it has different scattering properties compared to the gap dipole mode. Therefore, in the following section, the simulation based on B-V method and FEM are used to explore the far-field and near-field properties of these gap modes.
Figure 5-3. The B-V method simulated scattering spectra excited by different polarization.
The scattering spectrum and profile simulated by B-V methods are shown. In Figure 5-3, for the scattering spectrum of un-polarized excitation, the gap dipole mode and the HO mode are mainly contributed from p-polarized component of excitation. The gap dipole mode is located at 660 nm and the HO is at 560 nm. However, for p-polarized excited spectrum, the HO mode is at 562 nm. This slightly peak sift is due to the s-polarized excited spectrum peaked at 534 nm. The s-polarized excited spectrum also has a shoulder peak at 585 nm. This spectrum shape is different from the isolated particle
scattering spectrum. This indicates that not only p-polarized excitation but also s-polarized excitation can result in the interaction between the particle and film.

We choose three plasmonic bands: 534 nm, 562 nm, 660 nm to observe the scattering profile, Figure 5-4. These wavelengths are chosen from the peaks of scattering spectra with p-polarized only and s-polarized only excitation. For p-polarized excitation, the gap dipole mode and HO mode have different scattering profiles. The gap dipole shows doughnut shape but HO mode has asymmetric pattern around z-axis both at 534 nm and 562 nm. For s-polarized excitation, the far-field scattering profiles are all symmetric around z-axis and the maximum is in the center.

To identify these gap modes, first we tried to find the most efficient configuration to excite them. Although we already know p-polarized component is dominant, the s-polarization excited spectra are included for comparison, Figure 5-5. The incident angle is tuned from 0° to 89°. The intensity for gap dipole mode reaches the maximum at 75°. For the HO modes, at the small angle (~0° to 30°), the free-particle-like mode is excited and then red shifted to the higher order plasmonic gap mode. This HO mode reaches the maximum at 70°. For s-polarized excitation, there are two peaks one is also a free-particle-like mode at 531 nm and the other is around 585 nm. The intensity of these two modes decreases as the angle of incident increases from normal direction. These simulation results are consistent to the experimental results, Figure 5-1.
Figure 5-5. The angle dependence of the plasmonic modes for P-polarized excitation (left) and S-polarized excitation (right).

Figure 5-6. (Down) The scattering spectra simulated by FEM (red) and BV (black dashed) method. (Up) The scattering profile calculated by FEM at the plasmonic bands.
5.5 Near-field calculation

FEM is utilized to calculate the scattered field from near-field region to far-field region. The electric field around the particle/film gap describes the plasmonic mode profile. To verify this scattered field calculation, the scattering spectrum calculated by FEM is compared with the spectrum calculated by the B-V method, Figure 5-6. According to the previously simulation, we know that only p-polarized excitation contributes to these gap modes. Therefore, in the following FEM calculation, only p-polarized excitation is considered. As shown in the Figure 5-6, the FEM calculated spectrum has similar features to the B-V method calculated spectrum except for the slightly blue shift of the peak wavelength. The gap dipole mode has 13 nm shift and HO mode has 1 nm shift.

In addition to spectral features, the FEM calculated far-field scattering profile is also compared to the B-V calculated profile, Figure 5-6. For the FEM calculated dipole gap mode at 647 nm, the far field scattered field is consistent to the B-V calculation which has clear doughnut shape, Figure 5-4. For the HO modes at 561 nm, the scattering also has unsymmetrical profile, which has maximum at the opposite side of the incident wave. The profile at 545 nm is also shown to compare with the profile mode appearing when less lossy dielectric function is used in the following simulations.

The near field distribution on two surfaces is demonstrated. One is on X-Z plane which is the incident plane and the other is on the particle surface facing the film. As shown in Figure 5-7, for all three wavelengths, Ez is dominant and Ex is roughly one order smaller. For the gap dipole mode at 647 nm, the field is highly concentrated in the
dielectric layer, roughly 1 nm by 1 nm in this cross section. The enhancement of Ez for gap dipole mode is around 200, which is five times larger than the one for HO mode at 561 nm. The highest Ez field is located in the air gap between particle and the film instead of dielectric layer for the HO modes at 561 nm. In addition, the distribution is not symmetric to the Z-axis. For HO modes at 545 nm, the Ez field has concentrated spots with opposite phase located in the dielectric film.

In Figure 5-9, for the scattered field on the particle surface, the gap dipole mode has one lobe on the surface, which is symmetrical around z axis. For the HO mode, two lobes with different phase are observed.

![Figure 5-7. The near-field distribution of three plasmonic bands on X-Z plane. The rainbow colorbar is used and the set of number indicates the maximum and minimum value of the field.](image)
Figure 5-8. The near-field distribution of three plasmonic bands on the particle surface facing the film. The rainbow colorbar is used and the set of number indicates the maximum and minimum value of the field.

5.6 Far-field calculation with removal of interband loss

From the previous experimental scattering profiles, calculated spectra, and near-field distribution, it indicates that the HO modes may be contributed from more than one mode. To clarify this, in the following simulations, we replace the J-C dielectric function into Drude model to remove the interband loss. The removal of interband loss can magnify the plasmonic modes, especially for the modes between 500 nm to 600 nm. The parameters we used for the Drude model are listed below: $\epsilon_\infty = 9.5, \omega_p = 8.9488, \gamma = 0.01$.

Both p-polarized and s-polarized can excite these plasmonic modes except that gap dipole mode at 656 nm and the mode at 542 nm can only excited by p-polarized light, Figure 5-9. These two modes have similar doughnut shape profile, Figure 5-10. For the mode at 568 nm, which can be both excited by p-polarized and s-polarized light, the
mode has maximum along z-axis. However, the p-polarized excited mode at 568 nm is slightly tilted because of the coupling from the 542 nm mode. The s-polarized excited 568 nm mode provide a clean profile of this mode. According to the scattering spectrum, the original HO mode observed from experiments and simulated spectrum may possibly result from band at 542 nm and 568 nm in this scattering spectrum calculated based on Drude dielectric function.

Figure 5-9. (Left) the scattering spectra under JC dielectric function and Drude dielectric function. The excitation is p-polarized at 70°. (Right) The scattering spectra by JC and Drude dielectric function under s-polarized excitation at 70°.

Figure 5-10. The scattering profiles of three plasmonic bands under p- and s-polarized excitation.
5.7 Near-field calculation with removal of interband loss

With the same Drude dielectric function, the FEM is used again to calculate the near-field profile of these plasmonic modes. Same as in the previous steps verifying the FEM calculation, in Figure 5-11, the FEM calculated spectrum is compared with the B-V method calculated spectrum. The number of distinguishable modes is the same but there is blue shift for the modes at 508 nm, 557 nm and 644 nm. In addition, the modes are more distinct in the B-V method calculated spectrum. Figure 5-11 also shows the scattering profile of the plasmonic modes at 545 nm, 557 nm and 644 nm. Same as in the B-V calculated profile in Figure 5-4, the gap dipole at 644 and HO mode at 545 nm both have doughnut shape profile. The HO modes at 557 nm is slightly different from the mode at 568 nm in Figure 5-10, which may comes from more coupling between to the mode at 545 nm according to the spectrum in Figure 5-11.

The near-field profile of gap dipole mode at 644 nm and HO modes at 557 nm and 545 nm is demonstrated in Figure 5-12 and Figure 5-13. In general, the model profile of the modes at 644 nm and 545 nm are the similar to the corresponding ones by using J-C dielectric function except that the maxima Ez is increase by the factor of 5 due to removal of the interband loss.

In Figure 13, owing to the decoupling from the adjacent mode, for the HO mode at 557 nm, the two lobes of Ez across the z-axis becomes more obvious. This Ez contribution corresponds to the quadrupole distribution of the charge density.
Figure 5-11. (Down) The scattering spectra calculated by FEM (red) and BV method (black dashed) with Drude dielectric function. (Up) The scattering profile calculated by FEM at the plasmonic bands.

Figure 5-12. The near-field distribution of three plasmonic bands on X-Z plane. The rainbow colorbar is used and the set of number indicates the maximum and minimum value of the field.
Figure 5-13. The near-field distribution of three plasmonic bands on the particle surface facing the film. The rainbow colorbar is used and the set of number indicates the maximum and minimum value of the field.

5.8 Conclusion

In conclusion, the gap dipole mode and HO mode observed in the experiments can be clarified by the spectrum and scattering profile either by BV method or FEM. These two plasmonic modes can be distinguished by far-field scattering spectrum and scattering profile. Under our collection scheme, these modes are mainly excited by the p-polarized light illuminating at 70° from normal direction. With the help of less lossy dielectric function, the HO modes can be decomposed into the two gap mode at 545 nm and 557 nm. These two gap modes have different properties. In terms of near field distribution of Ez, the 545 nm mode is more dipole-like and 557 nm mode is more quadrupole-like. The damping and then coupling of these two modes may lead to the unsymmetrical far-field scattering profile shown in the experiments and simulations.
Chapter 6 Analytical Formulation of Imaging of an Antiparallel Dipole Pair

In this letter, we use closely coupled dipoles to calculate the projected images of quadrupole radiation from nano-antennas. The intensity distribution of quadrupole mode is compared with the dipole mode. The effect of numerical aperture on calculated intensity and approximation methods is also investigated. This intensity pattern provides a directly way to characterize the quadrupole modes of nanostructures.

6.1 Introduction

Plasmonic nanoparticles have received a lot of attention in the past decades. With the advances in fabrication and characterization methods, the particles can be tailored for specific purposes. Also, the optical properties of nanoparticles can be predicted with the help of increasing computational capacity. Plasmonic nanoparticles are well-known for the excitation of localized surface plasmon, which leads these nanoparticles have extraordinary properties both in near field and far field. In the near field region, the external excitation can be concentrated around the particles. These near field properties results in the surface enhancing technology, e.g. surface enhanced Raman scattering, surface enhanced IR etc. Also, the enhanced field can strongly interact with active materials and results in the realization of active devices e.g. nanolaser, spaser etc. Recently, the strong coupling between plasmonic structures and quantum dots has been proposed. In the far-field region, the large scattering and absorption cross section of nanoparticles lead various applications, e.g. bio-imaging, thermal therapy, data storage
etc. Therefore, to characterize the properties of these nanoparticles becomes an important topic.

However, it is usually challenging to use near field method to characterize the optical properties of nanoparticles because the size of these nanoparticles are normally smaller or close to the size of scanning tips. Therefore, the far field methods, e.g. scattering or extinction spectrum or scattering images are normally used to identify the optical properties of nanoparticles. These methods not only can identify assemble behavior of nanoparticles but also single nanoparticles under the proper optical setup. These optical measurements may also depend on the parameters of the system. Therefore, to relate the experimental results to the microscopic properties of the nanoparticles, these system parameters need to be considered.

In this letter, we use two out-of-phase dipoles to simulate the quadrupole radiation from the single nanoparticle and compare with the dipole radiation. For the nanoparticles, the dipole is usually dominant. However, the higher order modes can be significant if the particles have larger size, aspheric shape, compositions with less loss or inhomogeneity, or interact with other nanostructures.

6.2 Method

The simulation scheme is shown in Figure 6-1. The radiation sources are placed in the coordinate O and the images are projected to the coordinate O’. The surface of the collection lens and imaging lens are shown. The focus length is \( f_1 \) and \( f_2 \) for the
collection lens and imaging lens respectively. The ambient refractive index is \( n_1 \) around the sources, \( n_2 \) around images and \( n_3 \) between two lenses.

![Simulation scheme of coupled dipole radiation projected on the imaging plane.](image)

We start from volume integral equations. In this equation, \( \vec{G}(\vec{r}, \vec{r}'), J(\vec{r}'), \omega, \mu \) represent the Green’s function, current source, angular frequency of the source and permeability respectively.

\[
E(\vec{r}) = \imath \omega \mu \int \vec{G}(\vec{r}, \vec{r}') J(\vec{r}') d^3r
\]

If a dipole source is located at \( \vec{r}_0 \), then the current density can be expressed into:

\[
J(t, \vec{r}') = \dot{n} \hat{ll} \delta(\vec{r}' - \vec{r}_0) = \hat{n} \frac{d(\epsilon^{1-\imath} \imath)}{dt} l \delta(\vec{r}' - \vec{r}_0) = (-\imath \omega) e^{\imath \omega t} \hat{n} \bar{Q} l \delta(\vec{r}' - \vec{r}_0)
\]

\[
J(\omega, \vec{r}') = (-\imath \omega) \hat{n} \bar{Q} l \delta(\vec{r}' - \vec{r}_0) = (-\imath \omega) \hat{p} \delta(\vec{r}' - \vec{r}_0)
\]

Therefore, the field generated by an oscillating dipole becomes

\[
E(\vec{r}) = \frac{\omega^2}{\epsilon_0 c^2} \vec{G}(\vec{r}, \vec{r}_0) \cdot \vec{p}
\]
We use free space Green’s function \( \bar{G}(\vec{r}, \vec{r}_0) \) in the far-field region because the working distance of the collection lens is usually larger than the wavelength of the radiation.

\[
\bar{G}(\vec{r}, \vec{r}_0) = \frac{e^{ikr} e^{-ik(\vec{r},\vec{r}_0)}}{4\pi r} \begin{bmatrix}
1 - \sin^2 \theta \cos^2 \phi & -\sin^2 \theta \sin \phi \cos \phi & -\sin \theta \cos \theta \cos \phi \\
-\sin^2 \theta \sin \phi \cos \phi & 1 - \sin^2 \theta \sin^2 \phi & -\sin \theta \cos \theta \sin \phi \\
-\sin \theta \cos \theta \cos \phi & -\sin \theta \cos \theta \sin \phi & \sin^2 \theta
\end{bmatrix}
\]

Now, the quadrupole configuration we considered is decomposed into two out-of-phase dipoles pointing to \( z \) direction. The spacing between two dipoles is \( d \), which is much smaller than the wavelength, and the field becomes:

\[
\bar{E}(\vec{r}) = \frac{\omega^2}{\varepsilon_0 c^2} \mathcal{F} \left( \frac{\bar{G}(\vec{r}, +\frac{d}{2}, \vec{r}_0) - \bar{G}(\vec{r}, -\frac{d}{2}, \vec{r}_0)}{d} \right) \left( d \mu_z \right)
\]

\[
= \frac{\omega^2}{\varepsilon_0 c^2} \frac{\partial \bar{G}(\vec{r}, \vec{r})}{\partial x_1} \bigg|_{\nu=0} \left( d \mu_z \right)
\]

\[
= \left( -ikd \right) \frac{\omega^2}{\varepsilon_0 c^2} \frac{e^{ikr} \sin \theta \cos \phi}{4\pi r} \begin{bmatrix}
1 - \sin^2 \theta \cos^2 \phi & -\sin^2 \theta \sin \phi \cos \phi & -\sin \theta \cos \theta \cos \phi \\
-\sin^2 \theta \sin \phi \cos \phi & 1 - \sin^2 \theta \sin^2 \phi & -\sin \theta \cos \theta \sin \phi \\
-\sin \theta \cos \theta \cos \phi & -\sin \theta \cos \theta \sin \phi & \sin^2 \theta
\end{bmatrix}
\]

The field on the surface of collection lens becomes

\[
\bar{E}_0(\vec{r}) = \left( -ikd \right) \frac{\omega^2 \mu_z}{\varepsilon_0 c^2} \frac{e^{ikf} \sin \theta \cos \phi}{4\pi f_1} \begin{bmatrix}
-\sin \theta \cos \theta \cos \phi \\
-\sin \theta \cos \theta \sin \phi \\
\sin^2 \theta
\end{bmatrix}
\]

The field denoted as \( \bar{E}_1 \) after the collection lens, and \( \bar{E}_2 \) after the imaging lens.

The \( \bar{E}_0(\vec{r}) \) is decomposed into s-polarized and p-polarized field.
\[
\bar{E}_2 = \left\{ T_x T_y \left[ (\bar{E}_0 \cdot \hat{n}_y) \hat{n}_y \cdot \hat{n}_y \right] + T_n T_m \left[ (\bar{E}_0 \cdot \hat{n}_y) \hat{n}_y \cdot \hat{n}_y \right] \right\} \sqrt{\frac{n_1 \cos \theta}{n_2 \cos \theta}}
\]

We assume that the transmittance on collection lens and imaging lens are equal to one and field can be simplified into:

\[
\bar{E}_2(\theta, \phi) = \frac{(ikd) \omega^2 \mu}{\varepsilon_0 c^2} \frac{8\pi f_1}{\sin^2 \theta \cos \theta \sin 2\phi} \left[ \sqrt{\frac{n_1 \cos \theta}{n_2 \cos \theta}} \right]
\]

Based on the field right after the imaging lens \( \bar{E}_2 \), we can calculate the field on the focal plane by introducing the angular spectrum method. The field on the focus plane becomes

\[
\bar{E}_1(\rho, \phi, z) = \frac{ik f_2 e^{-ik f_2}}{2\pi} \int_{0}^{2\pi} \int_{0}^{\rho_{\text{max}}} \bar{E}_2(\Theta, \Phi) e^{ik z \cos \theta} e^{ik \rho \sin \theta \cos (\phi - \varphi)} \sin \theta d\Theta d\Phi
\]

\[
= \frac{ik f_2 e^{-ik f_2}}{2\pi} \int_{0}^{2\pi} \int_{0}^{\rho_{\text{max}}} \bar{E}_2(\Theta, \Phi) e^{ik z \cos \theta} e^{ik \rho \sin \theta \cos (\phi - \varphi)} \left( \frac{\rho_{\text{max}}}{\rho} \right)^2 \sin \theta \cos \theta d\rho d\phi
\]

The integration in term of \( \theta \) is switched to \( \varphi \) by using the relation

\[f_1 \sin \theta = f_2 \sin \varphi\]

and the assumption of \( \frac{\rho_{\text{max}}}{\rho} \ll 1 \). The \( \bar{E}_1 \) can be calculated by introducing the expression of \( \bar{E}_2 \).
If the focal length of imaging lens, \( f_2 \) is large, then \( \theta \) will be small. We can use the assumption \( \sin \theta = 0, \cos \theta = 1 \). The \( S, Q_a, Q_b \) is defined as following:

\[
S = \frac{ik' \omega \mu \epsilon_0 c^2}{2\pi} \int_0^{\theta_{\text{max}}} e^{ik'z \sin \theta} \left( \frac{f_1}{f_2} \right) \sqrt{n_1}{n_2} d\theta
\]

\[
Q_a = \int_0^{\theta_{\text{max}}} \sin^3 \theta \cos^2 \theta J_0(k \rho \frac{f_1}{f_2} \sin \theta) e^{ik'z \sin \theta \rho \sin \theta \cos \phi} d\theta
\]

\[
Q_b = \int_0^{\theta_{\text{max}}} \sin^3 \theta \cos^2 \theta J_0(k \rho \frac{f_1}{f_2} \sin \theta) e^{ik'z \sin \theta \rho \sin \theta \cos \phi} d\theta
\]

The focal plane is set to be on the \( z = 0 \) so the integration in \( Q_a \) and \( Q_b \) can be further simplified. In the next step, the measurable intensity is expressed as:

\[
I_s = \left| \tilde{E}_1(\rho \theta) \right|^2
\]

\[
= (kd)^2 S(Q_a^2 - 2Q_a Q_b \cos 2\phi + Q_b^2)
\]

\[
= (kd)^2 S_p_\theta(\rho \theta)
\]
Here we define a quadrupole intensity profile function \( p_Q(\rho, \varphi) \). The following figures will be plotted based on this function. The last step is to calculate the integration of \( Q_a \) and \( Q_b \). If the numerical aperture of the collection lens is small \((\theta_{\text{max}} \sim \sin \theta_{\text{max}})\), then the integration of \( Q_a \) and \( Q_b \) will be greatly simplified.

\[
Q_a = \int_{0}^{\theta_{\text{max}}} \theta^4 J_0(k \rho \frac{\theta}{\theta_{\text{max}}} \sin \theta) d\theta = \theta_{\text{max}}^4 \left\{ \frac{J_1(\rho \frac{\theta_{\text{max}}}{\sin \theta_{\text{max}}})}{\rho \frac{\theta_{\text{max}}}{\sin \theta_{\text{max}}}} - 2 \frac{J_2(\rho \frac{\theta_{\text{max}}}{\sin \theta_{\text{max}}})}{(\rho \frac{\theta_{\text{max}}}{\sin \theta_{\text{max}}})^2} \right\}
\]

\[
Q_b = \int_{0}^{\theta_{\text{max}}} \theta^4 J_2(k \rho \frac{\theta}{\theta_{\text{max}}} \sin \theta) d\theta = \theta_{\text{max}}^4 \left\{ \frac{J_3(\rho \frac{\theta_{\text{max}}}{\sin \theta_{\text{max}}})}{\rho \frac{\theta_{\text{max}}}{\sin \theta_{\text{max}}}} \right\}
\]

Therefore, the intensity profile function \( p_Q(\rho, \varphi) \)

\[
p_Q(\rho, \varphi) = \theta_{\text{max}}^8 \left\{ \left[ \frac{J_1(\rho \frac{\theta_{\text{max}}}{\sin \theta_{\text{max}}})}{\rho \frac{\theta_{\text{max}}}{\sin \theta_{\text{max}}}} - 2 \frac{J_2(\rho \frac{\theta_{\text{max}}}{\sin \theta_{\text{max}}})}{(\rho \frac{\theta_{\text{max}}}{\sin \theta_{\text{max}}})^2} \right]^2 + \left[ \frac{J_3(\rho \frac{\theta_{\text{max}}}{\sin \theta_{\text{max}}})}{\rho \frac{\theta_{\text{max}}}{\sin \theta_{\text{max}}}} \right]^2 \right\}
\]

\[-2 \cos 2\varphi \left[ \frac{J_1(\rho \frac{\theta_{\text{max}}}{\sin \theta_{\text{max}}})}{\rho \frac{\theta_{\text{max}}}{\sin \theta_{\text{max}}}} - 2 \frac{J_2(\rho \frac{\theta_{\text{max}}}{\sin \theta_{\text{max}}})}{(\rho \frac{\theta_{\text{max}}}{\sin \theta_{\text{max}}})^2} \right] \left[ \frac{J_3(\rho \frac{\theta_{\text{max}}}{\sin \theta_{\text{max}}})}{\rho \frac{\theta_{\text{max}}}{\sin \theta_{\text{max}}}} \right] \]

Following the similar procedures, we can get the expression of electric field on the image plane for the single dipole radiation.

\[
E_j(\rho, \varphi, z) = (\sqrt{S}) \begin{bmatrix}
(-2iD_a \cos \varphi) \\
-2iD_a \sin \varphi \\
0
\end{bmatrix}
\]

\[
D_a = \int_{0}^{\theta_{\text{max}}} \sin^2 \theta \cos \varphi J_1(k \rho \frac{\theta}{\theta_{\text{max}}} \sin \theta) e^{i k z [1 - \frac{1}{2} \rho^2 \sin^2 \theta]} d\theta
\]

The field intensity on the image plane becomes:
\[ I_d = | \tilde{E}_d(\rho, \phi) |^2 = S(2D_e)^2 = S p_d(\rho, \phi) \]

Similar to the previous derivation, if the numerical aperture of the collection lens is small, the distribution function will become

\[ p_d(\rho, \phi) = \left[ \theta_{\text{max}}^3 \frac{2I_s(\rho, \phi_{\text{max}} \sin \theta_{\text{max}})}{\rho \sin \theta_{\text{max}}^2} \right]^2 \]

### 6.3 Results

The intensity profile function of quadrupole and dipole are shown in Figure 6-2. The quadrupole and dipole modes have totally different intensity distribution. The quadrupole mode has maximum intensity in the center but dipole mode has a minimum in the center. The dipole image has uniform distribution in the azimuthal \( \phi \) direction. Therefore, the doughnut shape of dipole radiation has been observed experimentally. On the contrary, the quadrupole mode has maximum value in the center with the significant part of \( \cos 2\phi \) terms at the larger radical distance. Therefore, the center peak is surrounded by a partial ring which peak intensity is half of the center peak.
6.3.1 Effect of collection angle on intensity distribution

In the following section, we investigate the effect of numerical aperture (NA) on the intensity profile function of dipole and quadrupole modes. In the Fig3, the cross section of profile function at $\phi = 0$ is plotted under three different NA. The solid line and dashed line represent the calculation based on exact integration and paraxial approximation respectively. For the dipole mode, the relative peak intensity is on the order of $10^4$ between NA=0.13 and NA=0.55 and $10^1$ between NA=0.55 and NA=0.9. For the quadrupole mode, the relative peak intensity is on the order of $10^5$ between NA=0.13 and NA=0.55 and $10^2$ between NA=0.55 and NA=0.9. Therefore, the NA value more seriously affects the collection intensity of the quadrupole mode than the dipole.
mode. However, the relative intensity between peak intensity and secondary peak is independent of the numerical value both for the quadrupole mode and the dipole mode. For the quadrupole mode, the peak intensity is two-fold larger than outer partial ring. For the dipole mode, the peak value is 10 times larger than the outer ripple. If we compare the total intensity between quadrupole and dipole modes under the same NA, in addition to the intensity profile function, the extra term $(kd)^2 = \left(\frac{2\pi nd}{\lambda}\right)^2$ in the quadrupole mode needs to be considered. If we assume $d = 50\text{nm}$, $\lambda = 632\text{nm}$, $n_t = 1$, this factor is around 0.25. Therefore, combined with profile intensity function, for NA=0.13, the total peak intensity of dipole intensity is larger than quadrupole by the factor of 500. With the increase of the NA, the peak intensity difference between these two modes decreases. For NA=0.55, dipole intensity is larger than quadrupole by the factor of 25 and by the factor of 10 for NA=0.9.

6.3.2 Effect of collection angle on approximation calculation

In Figure 6-3, we also compare the profile function based on the exact calculation and based on the paraxial approximation. For NA=0.13, the paraxial approximation have a good agreement with the exact expression for both dipole and quadrupole modes. However, for NA=0.55, the paraxial approximation has 25% intensity deviation from exact integration calculation and the position of secondary peaks start shifting. For largest NA=0.9, the paraxial approximation is three times larger than the value calculated by exact expression and the peak value in dipole is obviously shifted. Therefore, the intensity calculation is seriously affected by the paraxial approximation. However, if the
magnitude of the profile is normalized to one, the profile derived from the paraxial approximation can still described the feathers of the dipole and quadrupole images well, Figure 6-3 (inset). The paraxial approximation can largely decrease the computation time.

Figure 6-3. The intensity profile function of dipole and quadrupole under different NA.

6.4 Conclusion

In this study, the two coupled dipole scheme is used to simulate the quadrupole radiation in the nano-antenna and compared to the dipole radiation. The angular spectrum
method is used to project the radiation on the image plane. The total distribution is expressed in terms of microscopic parameter (dipole moments) and macroscopic parameter (NA, focus length). The quadrupole and dipole modes have totally different image pattern and should be easily distinguished in experiments. Under the same condition, the dipole mode is intense than quadrupole mode but the difference is decreasing with the increasing of NA. Compared to the dipole mode, the intensity of quadrupole is more sensitive to NA. However, the relative intensity between primary peak and secondary peak is independent of the NA. Finally, we show that paraxial approximation may seriously deviate from exact intensity calculation under the high NA but still can be used for description of the normalized intensity profile. The intensity profile calculated here may apply for fitting the experimental data to extract the microscopic values.
Chapter 7 Conclusion

This study demonstrates that the combination of near-field calculation and nano-structure fabrication can provides deeper insight of the physical phenomena at nano-scale. The near field properties and SERS properties of nanoclusters, controlled assemblies and particle/film hybrids are studied experimentally and theoretically.

In Chapter 2 and Chapter 3, the near field calculation based on generalized Mie theory described the SERS enhancement of these nano-structures. This may help to design the efficient SERS substrate under specific illumination condition. As shown in the Chapter 2, the dimer and trimer nanoclusters are efficient as SERS substrate compared to the larger clusters. In chapter 3, the controlled nanoparticle clusters provide the specific high field regions for SERS enhancement. This structure can serve as a platform for quantitative SERS detection. Furthermore, the rotational symmetry of this controlled cluster can provide the SERS enhancement free from the specific polarization of the excitation.

In the Chapter 4, the gold nanoparticle on gold thin film shows a clear Raman doughnut shape. The particle/film hybrid serves as nano-antenna which modifies the emission properties of the molecules. This may provide a platform for the active plasmonic devices. In addition, the Raman blinking is observed in this structure, which implies that the high field region is very confined that only single molecule contributes the Raman signals at the same time.

In Chapter 5, the high order modes in the particle/film hybrid are further identified. This high order mode has different far-field scattering profile and near field
distribution compared to the primary gap dipole mode. This high order mode can be decomposed into one quadrupole-like mode and dipole-like mode. The characterization of these modes can provide information for engineering the near-field distribution or far-field scattering properties of this particle/film nano-antenna.

In the Chapter 6, the coupled dipoles and single dipole radiation on the image plane is demonstrated and compared. The radiation from coupled dipoles has a peak in the center and two side peaks on the plane of couple dipoles located compared to the single dipole with azimuthally symmetrical doughnut shape. Compared to the dipole mode, the intensity of coupled dipoles is more sensitive to NA. The analytical expression of these mode images in terms of microscopic parameter (dipole moments) and macroscopic parameter (NA, focus length) may provide a way to investigate the properties of the nano-antenna through the far-field scattering images.

While this body of work presents the SERS from random structures to controlled structures, further advances is necessary for controlled and stable and easily-fabricated SERS substrates. The carefully nano-antenna modeling can provide the predictable interaction between plasmonic structures and active materials. This can extend the plasmonic devices into broader applications.
Appendix A

Calculation of DNA b coverage: The amount of Cy5-modified oligo b bound to the core particles was determined from the starting concentration by subtracting the amount separated from the core solution by centrifugation as determined by fluorescence. Free DNA b of known concentration (6.43x10^-9 M) is used as a reference. The intensity of peak fluorescence at 664 nm is used to obtain the concentration of the unbound DNA b in the supernatant.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Intensity at 664 nm</th>
<th>Dilution factor</th>
<th>Concentration (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>56979</td>
<td>1</td>
<td>6.43x10^-9</td>
</tr>
<tr>
<td>Supernatant 1</td>
<td>121721</td>
<td>2.22</td>
<td>3.05x10^-8</td>
</tr>
<tr>
<td>Supernatant 2</td>
<td>77361</td>
<td>2.22</td>
<td>1.94x10^-8</td>
</tr>
<tr>
<td>Supernatant 3</td>
<td>33202</td>
<td>2.22</td>
<td>8.33x10^-9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Concentration(M)</th>
<th>Volume(ul)</th>
<th>DNA b(mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA b added</td>
<td>3.07x10^-8</td>
<td>8.07x10^-11</td>
</tr>
<tr>
<td>Supernatant 1</td>
<td>3.05x10^-8</td>
<td>1.07x10^-11</td>
</tr>
<tr>
<td>Supernatant 2</td>
<td>1.94x10^-8</td>
<td>6.79x10^-12</td>
</tr>
<tr>
<td>Supernatant 3</td>
<td>8.33x10^-9</td>
<td>2.91x10^-12</td>
</tr>
<tr>
<td>Left-over</td>
<td>8.33x10^-9</td>
<td>2.29x10^-12</td>
</tr>
<tr>
<td>bound DNA b</td>
<td></td>
<td>5.8x10^-11</td>
</tr>
</tbody>
</table>
The total core particles added is $3.255 \times 10^{-14}$ mol. Therefore, the coverage of the DNA is $5.8 \times 10^{11} / (3.255 \times 10^{-14}) = 1781$ per core particle. Per area coverage for particles 50nm diameter is 37 pmol/cm$^2$.

Figure 7-1 Fluorescence spectra of reference solution and three supernatants.
**Scattering Data:** The CSA scattering spectrum shown in Figure 2-3 is a difference spectrum of the CSA spectrum and a satellite spectrum collected from satellites at the same concentration as in the final assembly solution. The spectra before differencing are included here. Free satellites contribute the majority of scattering in the control solution, also, because the core particles are dilute. However, once the CSAs are formed, the intensity of their scattering is twice as large as scattering from the much more concentrated satellites.

![Scattering spectra, all solutions.](image)

Figure 7-2. Scattering spectra, all solutions.
Absorption spectrum of strand b (Cy5 modified): The absorption peak is at 647 nm and the peak that forms a shoulder is at 600 nm.

Figure 7-3. Absorption spectrum of strand b (Cy5 modified)
Control to confirm that Raman signal is from Cy5: To confirm that the enhanced Raman signals were from Cy5, and not from DNA, CSAs were formed using DNA linkers without the Cy5 modification. As can be seen, the CSA without Cy5 shows no obvious Raman peak, other than the peaks from the disposable cuvettes used in this preliminary experiment. Small variations in the thickness of the cuvettes are most likely responsible for dips in the control spectrum following background subtraction. However, at the Stokes shifted frequencies of interest (556 cm\(^{-1}\), 1190 cm\(^{-1}\), marked by vertical lines), scattering from the plastic cuvette was negligible.

![Figure 7-4. Raman spectra of CSA with (green) and without (red) Cy5.](image)
Appendix B

1. **Temporal variation in Raman scattering from single nanoparticle junctions.** Blinking of the Raman images of gold nanoparticles on a PAH layer on a gold film under TIR excitation was captured in a movie. 100 frames are captured; the exposure time is 200 ms for each frame. The movie is compressed from 20 s to 5 s.

2. **Cy5 Raman control.** To verify the SERS signals were from Cy5 and not from the oligonucleotides or the PAH spacer layer, control particles with oligonucleotides lacking the Cy5 modification were prepared in parallel with particles with oligonucleotides incorporating Cy5. Cy5-functionalized and control particles were deposited in two spots on the same Au film. The plasmon properties of the control particles (images, Fig S1, and single particle spectra, Fig S2) are the same as the respective properties of particles with Cy5, Figure 3-2(a) and Figure 3-9(a).

Figure 7-5. Dark field image of the control particles
3. **Simulation Method.** Scattering from a sphere on a layered substrate was simulated using the method of Bobbert and Vlieger (BV). The BV method uses Debye potentials expanded in spherical harmonics to describe fields in a shell around the particle. If $W$ and $V$ are vectors of expansion coefficients for the Debye potentials that respectively describe currents flowing inside and outside the sphere, the coupled problem is defined by scattering by the sphere,

$$W^S = B \cdot (V^I + V^{IR} + V^{SR})$$

and the mapping (two changes of basis and reflection) back to the sphere of the sphere scattered waves,

$$V^{SR} = A \cdot W^S$$

where $W^S$ represents the wave scattering from the sphere, $V^I$, $V^{IR}$, and $V^{SR}$ represent respectively the incident wave, the reflection of the incident wave without the sphere, and the reflection by the substrate of the waves scattered by the sphere. $B$ is a matrix of coefficients from Mie theory that describe the response of the sphere to each partial
wave. A is the matrix that characterizes the expansion in sphere centered harmonics of reflection by the substrate of outgoing spherical waves. Scattering from the sphere can be expressed directly in terms of the incident and reflected incident wave as

\[ W^E = (1 - B \cdot A)^{-1} \cdot B \cdot (V^i + V^{iE}) \]

4. **Effect oxide thickness on NP-silicon coupling.** In order to verify that the weaker coupling of the nanoparticles to silicon relative to their coupling to the gold film results from the intrinsic properties of silicon rather than the thickness (1.83nm) of the native oxide layer, we simulated scattering from an NP-silicon system with a spacer thickness (oxide+PAH) equal to the thickness of the PAH space layer on gold (0.56nm). As in all other cases, total gap width is equal to substrate spacer (PAH or PAH on oxide) and a 0.3 nm dielectric shell on the NP. As shown in the Figure S5, the peak wavelengths are 553 and 568 nm, respectively, for the original geometry (oxide thickness of 1.83 nm, PAH 0.13nm) and the thinner spacer (0.43 nm oxide, 0.13 nm PAH). Comparison of the band position (peak at 668nm) of the NP on gold film with the band position (peak at 568) of an NP comparably spaced from silicon reveals that only a small fraction of the difference in peak wavelength can be attributed to the difference in dielectric spacer thickness on the two substrates.
Figure 7-7. Simulated scattering spectra of the NP on silicon with different oxide thicknesses. 1.83 nm is the oxide thickness from ellipsometry. 0.43 nm is the oxide thickness which together with a PAH layer 0.13 nm thick would result in the gold NP – silicon gap being equal to the gap between the gold NP and gold film.

5. Intensity – Particle Size scaling from simulation of scattering spectra. To clarify the relationship between scattered intensity and size of a nanoparticle on a dielectric substrate or metal film, scattering intensity was calculated for particles with different sizes (mean, ±σ, ±2σ). Simulated peak intensities for particles on each substrate are normalized by the simulated peak intensity of a particle of mean size (50.8 nm). Normalized simulated peak intensities are plotted vs particle size, where particle size, also, is normalized by the nominal particle size. As shown in the figure S3, simulated intensity scales linearly with the sixth power of the normalized particle size.
Figure 7-8. Simulated intensity versus sixth power of particle size.

6. Estimation of spacer thickness variation for the AuNP on gold film based upon deviations from the peak wavelength – intensity correlation due to variations in NP size. Peak intensities and wavelengths from single particle spectra (blue ◊s) were found to have a positive correlation consistent with the correlation displayed by simulation for plasmon bands of Au NPs on Au film for NPs of varying size (mean, ±σ/σ, red □s). To quantify the role of variations other than NP size, we fit a correlation line and compile statistics on the deviation. As we have identified spacer thickness as the second geometric variable of significance along with NP size, we use thickness sensitivity results to determine thickness variations from the mean deviation. Here we find a mean value of 3.8 nm for the difference between the trend line and the experimental points. From simulation, the sensitivity of peak wavelength to PAH thickness is roughly 17 nm /0.28 nm. Therefore, a 3.8 nm dispersion of peak wavelength from the trend line suggests 12% variation in Au NP – Au film gap width.
7. Estimation of Cy5 Raman scattering cross section and electromagnetic (EM) enhancement.
   
   a. The laser (HeNe 632.8 nm) power is 30 mW and the laser spot is 7788 µm$^2$. Thus, the incident intensity is 0.385 kW/cm$^2$ and is converted to $1.23 \times 10^{13}$ photon/s-µm$^2$.
   
   b. From the Raman spectrum, we integrate the 1191 cm$^{-1}$ peak from 1158 cm$^{-1}$ to 1213 cm$^{-1}$ and normalize the value by the number of particles (in this case, three) and integration time (120 s). Therefore, the collected Raman signal for the 1191 cm$^{-1}$ peak is 12.6 cps/NP.
c. The quantum efficiency of CCD at 1191 cm\(^{-1}\)(684 nm) is around 30%. The grating efficiency is around 40%. If we assume that the combined efficiency of the rest of the optical component is 50%, the efficiency of the whole optical setup is 5%. Therefore, the corrected Raman signal is 252 photons/sec-NP.

d. If we assume that only one molecule from the hot spot contributes to the Raman signal for one NP, from (a) and (c), the Cy5 Raman cross section will be \(2 \times 10^{-19}\) cm\(^2\).

e. If we assume the spatial distribution of the Raman emission is the same as an ideal dipole, based on the known numerical aperture (0.55), the fraction of the total cross section we collect is 2%. Thus from (d), the total cross section, \(\sigma_T\), is \(1 \times 10^{-17}\) cm\(^2\). For comparison with ‘differential’ cross sections measured in solution we define a cross section per steradian equal to \(\sigma_T / 4\pi\) which equals \(8 \times 10^{-19}\) cm\(^2\)/sr.

f. Previously [114], we have determined a differential Raman cross section of the Cy5 to be on the order of \(10^{-25}\) cm\(^2\)/sr. This value includes any chemical enhancement of the Cy5 on the NP but does not include EM enhancement. The cross section was determined under the assumption that our measurement of Raman scattering from bulk methanol (which we used as a standard) collected the same partial cross section as was collected by the apparatus used to establish the standard [13]. From comparison with the cross section determined here in (e), we estimate that the SERRS EM enhancement is \(8 \times 10^6\).
Figure 7-10. Raman spectrum of Cy5 in the NP-gold film system. The signal was collected from three isolated particles. The integration time is 120 s.

8. **Determination of coverage of Cy5 modified oligonucleotides on particles.** The amount of Cy5-modified oligonucleotide bound to the gold particles was determined from the starting concentration by subtracting the amount separated from the particle solution by centrifugation as determined by fluorescence. Free oligonucleotide of known concentration (2.488x10^{-9} M) is used as a reference. The intensity of peak fluorescence at 664 nm is used to obtain the concentration of the unbound oligonucleotides in the supernatant. The background noise is around 535 counts per second (cps).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Intensity at 664 nm(cps)</th>
<th>Dilution factor</th>
<th>Concentration(M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Referent</td>
<td>334730</td>
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<td>2.488x10^{-8}</td>
</tr>
<tr>
<td>Supernatant 1</td>
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<td>2.42</td>
<td>6.317x10^{-10}</td>
</tr>
<tr>
<td>Supernatant 2</td>
<td>2038</td>
<td>2.42</td>
<td>1.903x10^{-10}</td>
</tr>
</tbody>
</table>
The amount of total particles added is $5.5 \times 10^{-14}$ mol as determined from absorbance at 520 nm. Therefore, coverage of Cy5-modified DNA is $1.10 \times 10^{-10}/(5.5 \times 10^{-14}) = 2000$ Cy5 per particle. The area coverage is 42 pmol/cm$^2$, i.e. one molecule per 4 nm$^2$.

9. Parametric form of wavelength-dependent refractive index model used in ellipsometric characterization of PAH. Amplitude ratios and phase shifts from measurements of specular reflection of polarized light from surfaces with PAH layers were fit using the Cauchy parametrization[115] of the refractive index

$$n(\lambda) = A + \frac{B}{\lambda^2} + \frac{C}{\lambda^4} \text{ where } \lambda \text{ in } \mu m$$

$$k(\lambda) = \alpha e^{0.22409(\frac{\lambda}{\text{nm}} - 1)} \text{ where } \lambda \text{ in } \lambda,$$

Fit values were $A=1.45$, $B=0.01$, $C=0$ and the imaginary part was set to 0.
References


Biography

Shiuan-Yeh Chen received BS and MS degrees in Physics from National Taiwan University in 2001 and 2005. He received MS degree in Electrical and Computer Engineering from Duke University in 2008.

List of Publications:


