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Single Nanoparticle Spectroscopy:
Plasmonic Properties and Biosensing Applications

by

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ABSTRACT

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Single particle dark field spectroscopy has been combined with high-resolution scanning electron and atomic force microscopy to study the scattering spectra of individual gold nanoparticles. This technique has been applied to single gold/silica nanoshells, and single gold nanostars. For nanoshells, the plasmon resonant peak energies match those calculated by Mie theory based on the nanoshell geometry. The resonance line widths fit Mie theory without the inclusion of a size-dependent electron surface scattering term, which is often included to fit ensemble measurements. Single particle spectroscopy has also been applied to star-shaped gold nanoparticles which are ca. 100 nm in diameter. The gold nanostars were fabricated by a modified seed-mediated, surfactant-directed synthesis which is similar to a method known to produce gold nanorods in high yield. The yield, monodispersity, and initial investigations into the growth mechanism of the nanostar synthesis are described in detail. Through correlated structural characterization by electron microscopy, each scattering component can be assigned to different points on the nanostars. The plasmon resonances were also found to be extremely sensitive to the local dielectric environment, yielding sensitivities as high as 1.41 eV photon energy shift per refractive index unit. These properties suggest that gold nanostars may be highly valuable for certain biosensing and microscopic imaging
paradigms. To test their properties as molecular sensors, single nanostar spectra were monitored upon exposure to alkane thiols (mercaptohexadecanoic acid) and proteins (bovine serum albumin) known to bind gold surfaces. The observed shifts are consistent with the effects of these molecular layers on the surface plasmon resonances in continuous gold films. The results suggest that localized surface plasmon resonance sensing with single nanoparticles is analogous to the well developed field surface plasmon resonance sensors, and will push the limits of sensitivity.
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Chapter 1: Introduction and Background

1.1 The Plasmon Resonance in Nanoscale Gold

The brilliant colors that result when gold and silver are mixed with glass have been known for centuries. For example, the Lycurgus cup from 4th century Rome contains embedded gold nanoparticles and appears either red or green depending on the illumination.\textsuperscript{1} It was not until the 19th century that Faraday systematically studied this effect and found that small particles of metallic gold (colloid) were responsible for the colors. The origin of the plasmon resonance was mathematically described by Mie nearly a century ago using Maxwell’s equations. For gold the result is a peak in the absorption and scattering at around 520 nm. Today, this effect is referred to as a localized surface plasmon resonance (or “plasmon resonance” for brevity) and is due to a collective oscillation of the free electrons in a metallic nanoparticle. Plasmon-resonant noble metal nanoparticles have attracted tremendous interest within the past decade for biological, biomedical, and optoelectronic applications.\textsuperscript{2,3}

Plasmon resonances can cause optical extinction through both absorption

\textbf{Figure 1.1} The Lycurgus cup (pictured here) appears red when illuminated from within and green when illuminated from the outside. This is due to the plasmon resonance (520 nm) of the nanoscale gold particles in the glass. Picture reproduced from Wagner, F. E.; Haslbeck, S.; Stievano, L.; Calogero, S.; Pankhurst, O. A.; Martinek, P., Before striking gold in gold-ruby glass. \textit{Nature} \textbf{2000}, 407, (6805), 691-692.
and scattering. Figure 1.1 shows the Lycurgus cup illuminated from within (left) and from the outside (right). The cup appears red when it is illuminated from within because the green light is scattered by the nanoparticles in the glass, transmitting only the red light. When the cup is illuminated from the outside, the glass appears green due to the scattering of green light (at 520 nm) by the gold nanoparticles embedded in the cup.

1.2 Mathematical Origin of the Plasmon Resonance: Mie Theory

The mathematical origin of the plasmon resonance was described by Mie in 1908. Mie solved Maxwell’s Equations for a metal sphere interacting with an incident plane wave of white light. For a metal sphere with a given radius, dielectric function and surrounding medium he calculated the scattering and extinction cross sections. The particle will remove light from the incident wave by an amount proportional to its extinction cross section. The extinction cross section is the size of the particle that would be required to physically block the light removed from the incident plane wave. To

![Diagram](image_url)

**Figure 1.2** Mie theory describes the interaction of a metal sphere with a plane wave of incident light. For a metal sphere with a given radius, dielectric function, and the index of refraction of the surrounding medium, Mie theory describes the scattering and extinction cross sections.
mathematically find the extinction cross section Mie solved Maxwell’s equations for a plane wave of light incident on a spherical nanoparticle in a dielectric medium with appropriate boundary conditions. This solution is then expanded in a basis set composed of the vector spherical harmonics \( \mathbf{M} \) and \( \mathbf{N} \). \( \mathbf{M} \) and \( \mathbf{N} \) are modified by the expansion coefficients \( a_L \) and \( b_L \) which depend only on physical constants—the dielectric function, particle radius, and surrounding medium. The solution is a sum of the expansion coefficients and the \( \mathbf{M} \) and \( \mathbf{N} \) basis set. The resulting external fields yield the scattering cross section and the resulting internal fields yield the absorption cross section.

Thus, we find that the extinction cross section is:

\[
\sigma_{\text{ext}} = \frac{2\pi}{k^2} \sum_{L=1}^{\infty} (2L+1) \operatorname{Re}\{a_L + b_L\}
\]

where \( k \) is the wave vector of the incident light, \( L \) is the index (dipole, quadrupole, and higher orders) that we sum over. More complete descriptions of Mie’s derivation of the scattering and extinction cross sections may be found elsewhere by the interested reader.\(^5\) \(^6\)-\(^8\) Only the salient details necessary to understand how the cross sections vary with particle size and shape are discussed here.

1.3 Very small spherical particles: Dipoles and the Quasistatic Approximation

The extinction cross-section for a metal sphere interacting with light can be described as in equation (1.1). This equation is a summation of the dipole (\( L=1 \)), quadrupole (\( L=2 \)), and higher order terms. For a small nanoparticle (radius much smaller than 100 nm) the primary contribution will be from the dipole term, so much may be
learned by only taking the first term in the expansion. This is known as the dipole approximation.\textsuperscript{6}

\begin{equation}
\sigma_{\text{ext}}(\omega) = 9 \frac{\omega}{c} e_m^{3/2} V \frac{\varepsilon_2(\omega)}{[\varepsilon_1(\omega) + 2e_m]^2 + \varepsilon_2(\omega)^2}
\end{equation}

In this equation $\omega$ is the frequency, $V$ is the particle volume, $\varepsilon_1(\omega)$ is the real part of the dielectric function of the particle material, $\varepsilon_2(\omega)$ is the complex part of the particle material, and $e_m$ is the dielectric function of the embedding medium. This approximation is only valid when the nanoparticle is significantly smaller than the wavelength of light. For these small particles the extinction cross section is essentially the same as the absorption cross section. For small particles this is because dipolar scattering is very small and proportional to $(R/\lambda)^6$, where $R$ is the radius of the particle and $\lambda$ is the wavelength of light. Neglecting higher order terms in this regime is also acceptable because the extinction quadrupole is proportional to $(R/\lambda)^5$ and the scattering quadrupole is proportional to $(R/\lambda)^{10}$. Thus, the dominant effect for small nanoparticles is clearly absorption.\textsuperscript{6}

Taking the first term in equation 1.1 as a dipole approximation is also often described as the quasistatic approximation. The quasistatic approximation assumes that the entire surface of the nanoparticle is experiencing a constant electric field, and thus, we retain the time-varying components of the electric field, but not the spatially varying components.\textsuperscript{6} This is a useful approximation because it allows a description of the interaction of the nanoparticle with incident light with electrostatics. In the dipole limit, we find that the extinction cross section for a nanoparticle depends on the characteristics
of the metal and the surrounding medium and scales with particle volume. From this, we learn that absorption increases linearly with volume for small particles. Additionally, from the real term ($\varepsilon_1$) and the imaginary term ($\varepsilon_2$) we may discover the origin of the plasmon resonance. The resonance will occur whenever $\varepsilon_1 = -2 \varepsilon_m$, and for gold colloid this happens at roughly 520 nm in water. The real and imaginary dielectric constants are taken from measurements on gold films by Johnson and Christie.\(^9\)

1.4 Larger particles: Quadrupole Resonances, and Broadening

We now consider nanoparticles with a size comparable to the wavelength of light. For these larger particles we must consider higher order terms to accurately describe the plasmon resonance. Because the nanoparticles are now of a size similar to the wavelength of light, the electric field can no longer be considered to be the same across the nanoparticle surface and the quasistatic approximation is no longer useful. For larger nanoparticles the dipole resonance will red shift due to phase retardation, and each side of the nanoparticle will experience a slightly different electric field. Higher energy quadrupole and other peaks will also appear.

Larger nanoparticles are also subject to broadening of the plasmon resonance linewidth due to scattering. When light couples into a nanoparticle and excites a plasmon, it may decay in a radiative or nonradiative manner.\(^10\) Nonradiative decay results in interband or intraband excitations of electron-hole pairs in the nanoparticle. These excitations cause local heating and contribute to the characteristic absorption cross section of the nanoparticles. Radiative decay is photon emission at the incident wavelength (elastic scattering) with the dominant contribution at the resonance energy.
Since energy is lost, radiative decay damps the system, and so, just as any damped oscillator has a characteristic linewidth, it broadens the plasmon resonance in metal nanoparticles. The radiative decay mechanism also explains the relatively broad linewidths of the spectral extinction cross sections of most nanoparticles that scatter brightly.

![Figure 1.3](A) Gold colloid in solution (from BBI International) has a characteristic reddish color. (B) Plot of extinction for various sizes of gold colloid. (C) Scanning electron micrograph of 100 nm gold colloid. Panel (B) is reproduced from Bohren, C.; Huffman, D. R., Absorption and Scattering of Light by Small Particles. 1983

A key result from this derivation is that for nanospheres there is little size dependence for the plasmon resonance frequency. That is, as the size of the nanoparticles increases, it affects the resonance through broadening from phase retardation, excitation of higher order terms, and from scattering. As we can see in Figure 1.3, panel B, the plasmon resonance is not very tunable with particle size for gold spheres.
Thus far, the plasmon resonance, as described, is an essentially *classical phenomenon*. Until recently it has only been of limited use because the plasmon resonance of nanoparticles was not very tunable with particle shape. Recent advances in nanoparticle synthesis$^{11-17}$ have enabled creation of highly complex nanoparticle shapes, which result in tunable plasmon resonances. The second half of this chapter presents a brief overview of some of the most interesting types of metal nanoparticles. Later, the work described in this document will focus exclusively on nanoshells and nanostars. Nanoshells and nanostars are only two of the many types of plasmon resonant nanoparticles fabricated in the past decade, but they are certainly among the most interesting.

To understand the significance of nanoshells and nanostars, I will first present background on gold nanorods, and next will consider more complex shapes such as nanocubes, bipyramids, and triangular nanoprisims. In the interest of brevity, I have limited this discussion to nanoparticles synthesized through wet chemistry, and characterized with single particle spectroscopy. I have also further narrowed the focus of this chapter to nanoparticles for which there is considerable theoretical insight, whether through exact analytical solutions (such as Mie theory), or through simulations like the discrete dipole approximation (DDA) or finite difference time domain (FDTD). Many complex and interesting particles have been created of late, but they fall beyond the scope of this chapter because they are fabricated by electron-beam lithography or are confined to a substrate.$^{18-21}$
1.5 Nanorods

Nanorods are of considerable interest due to recent advances in high-yield synthesis,\textsuperscript{22} biofunctionalization,\textsuperscript{23} and analytical formulas to understand their plasmon resonances.\textsuperscript{24} The plasmon resonances of nanorods are well described with Gans theory, an extension of Mie theory to spheroidal nanoparticles. For nanorods, the plasmon resonance splits into a longitudinal resonance along the long axis and a transverse mode along the short axis (diameter) of the nanorod. Gans' theory describes an extinction coefficient for randomly oriented ellipsoids (very similar shape to nanorods) in a surrounding dielectric medium. The theory describes three depolarization factors—one for illumination along each axis of the rod. These depolarization factors depend on the ratio of nanorod length to nanorod diameter, which is known as the aspect ratio.\textsuperscript{24} As the

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{nanorods.png}
\caption{(A) TEM image of gold nanorods with the longitudinal and transverse resonances sketched in. (B) Extinction spectra for a solution of gold nanorods in water. The longitudinal resonance is at roughly 800 nm for these rods and the transverse resonance is near 520 nm, the plasmon resonance of gold colloid. As the aspect ratio (ratio of rod length to rod diameter) increases the longitudinal resonance will shift slightly to the red and the transverse resonance may undergo a slight blue shift.}
\end{figure}
aspect ratio increases, the longitudinal mode redshifts and the transverse resonance slightly blue shifts. The transverse and longitudinal modes are illustrated with arrows in Figure 1.4. The redshift of the longitudinal mode is the dominant effect, and the intensity of the longitudinal resonance increases with aspect ratio. Nanorods are finding broad applications in biomedical fields. Recent advances have made nanorods stable for use in biological environments.\textsuperscript{23, 25} Gold nanorods are also a topic of investigation for photothermal cancer therapy and cancer cell imaging.\textsuperscript{26}

1.6 Asymmetric Nanoparticles and Single Particle Spectroscopy

For nanoparticles which are not sufficiently symmetric for analytical methods, numerical simulations provide insight. The field of plasmonics for asymmetric nanoparticles with complex structures is rapidly growing and changing. As such, this is not an attempt at a comprehensive overview, but rather an attempt to describe general trends in the field. This will serve us well in later chapters because it will provide a context for the work with gold nanostars and nanoshells described later.

An early investigation into the relationship between nanoparticle spectra and structure was conducted by Mock et al.\textsuperscript{27} They obtained the first single nanoparticle scattering spectra which were \textit{correlated} with high resolution microscopy. Mock and co-workers made an apparatus to collect single nanoparticle scattering spectra from silver nanoparticles on a transmission electron microscope (TEM) grid.\textsuperscript{27} Colloidal silver nanoparticles were immobilized on a Formvar TEM Grid, and by matching up optical dark-field scattering images with a low magnification TEM image they were able to directly correlate nanoparticle structure and spectra. The result was an elegant description
of the relationship between silver nanoparticle shape and spectra.

Mock et al found that spherical silver particles had resonances from roughly 400-500 nm. Silver pentagons were resonant from roughly 500-590 nm. Triangles appeared to show the largest size dependence and had resonances from 525 nm to past 700 nm—this effect was discussed by Schatz in 2006. The silver particles studied by Mock et al had diameters from 40 to 120 nm. In general, they found that as the particle size increased the resonances shifted to longer wavelengths. Intriguingly, they also found that particles with sharper points are resonant at longer wavelengths. Later, theoretical insight from the discrete dipole approximation (DDA) applied to single silver nanoparticles would further support this observation.

![Figure 1.5 Plot of nanoparticle size (as determined by TEM) vs. the resonance peak wavelength for various sizes and shapes of individual silver nanoparticles.](image)

*Figure 1.5 Plot of nanoparticle size (as determined by TEM) vs. the resonance peak wavelength for various sizes and shapes of individual silver nanoparticles. Figure reproduced from Shape effects in plasmon resonance of individual colloidal silver nanoparticles. By: Mock, J. J.; Barbic, M.; Smith, D. R.; Schultz, D. A.; Schultz, S.. Journal of Chemical Physics, 4/15/2002, Vol. 116 Issue 15, p6755*
DDA calculations represent a nanoparticle as a cubic array of \( N \) polarizable points. Each polarizable point represents the polarizability of a defined volume of the material, usually a metal for plasmonic nanoparticle simulations. When light is incident on the nanoparticle representation each point will develop a dipole moment in response to the local electric field. Once the polarization along the array is determined, it may be used to mathematically determine the extinction and scattering cross sections for the particle.\(^{29}\)

1.7 Triangular Nanoparticles

or Nanoprisms

The optical properties of triangular nanoprisms were first theoretically described by Mirkin and Schatz in 2001.\(^{30}\) They showed that the extinction efficiency of silver nanoprisms is related strongly to nanoprism \textit{shape}, and—in particular—nanoprism \textit{sharpness}. They used DDA to demonstrate that sharper nanoprism tips lead to redder (lower energy) plasmon

![Figure 1.6](image) Three scattering spectra from the same silver nanoparticle. Between each spectra the nanoparticles were heated to induce rounding of the corners. As the tips become less sharp the plasmon resonance blue shifts and approaches that of a sphere. Figure and caption reproduced from \textit{Shape effects in plasmon resonance of individual colloidal silver nanoparticles}. By: Mock, J. J.; Barbic, M.; Smith, D. R.; Schultz, D. A.; Schultz, S.. \textit{Journal of Chemical Physics}, 4/15/2002, Vol. 116 Issue 15, p6755
resonances. This correlation between tip sharpness and plasmon energy was used to fit the scattering spectra of single silver nanoprisms. Single nanoprism spectra were not correlated with high-resolution microscopy, but they were fit with DDA simulations by adjusting the sharpness of the nanoprism tips to match the plasmon energy. Later, single particle spectroscopy was applied to nanoprisms.\textsuperscript{28} The investigation revealed that nanoprism tip sharpness not only affected the energy of the plasmon resonance, but also the linewidth of the plasmon resonance. DDA calculations also indicated that the sharper the nanoprism tips, the broader the resonance linewidth. The correlation of sharpness to resonant wavelength was confirmed by Mock and coworkers. They heated a triangular nanoprism to dull the edges and recorded spectra after each heating. As the edges became duller, the plasmon resonance blue shifted. The figure illustrating this is reproduced in Figure 1.6.

1.8 Silver Nanocubes

Another particle of interest is the silver nanocube. Silver nanocubes (with 90 nm edges) were chemically synthesized, and it was found that their plasmon resonance dramatically shifted when the nanocubes were placed on a glass substrate.\textsuperscript{31} This substrate interaction was investigated in different dielectric media, and was found to depend very sensitively on the local index of refraction. The two resonances observed in nanocubes near a surface are thought to be due to dipole modes. The dipole mode observed in solution spectra red shifts to 550 nm when the nanocube approaches the surface, and a blue peak at 430 nm appears and strengthens as the cube approaches to the surface. It was hypothesized that the 430 nm peak is associated with electric fields away
from the surface (at the ‘top’ of the cube) and the 550 nm peak was associated with electric fields near the surface (at the bottom of the cube facing into the substrate).

1.9 Discussion

In conclusion, many new plasmonic nanoparticles have plasmon resonances which are highly tunable with particle shape and have been investigated by single particle spectroscopy. By documenting recent advances in fabrication and theoretical understanding of new plasmonic nanoparticles this section’s goal is to provide context for the work described later in this document.
Chapter 2: Single Particle Spectroscopy Background and Experimental Set-up

2.1 Single Particle Spectroscopy Background

Single particle spectroscopy is a microscopic variation on conventional optical spectroscopy. As we know, spectroscopy studies the interaction of radiation with matter to reveal information on energetic resonances and transitions in a sample. Plasmon resonances in noble metal nanoparticles are spectrally characterized with broadband optical extinction spectroscopy since the resonances occur in the visible and near IR, and have bandwidths of approximately 100 nm. For plasmon resonant nanoparticles, extinction spectra represent the sum of scattering and absorption since both remove light from the incident beam. Conventional ensemble extinction spectra of nanoparticle

![Figure 2.1](image)

**Figure 2.1** (A) Traditional spectroscopy: Light is incident on a collection of nanoparticles, usually in solution or on a substrate. Extinction or absorption spectra are obtained for the particle ensemble, averaged over all particle sizes, shapes, and orientations. (B) Single particle spectroscopy: Light is incident at high numerical aperture on a single nanoparticle on a substrate. Scattered light is collected, and spectra are obtained for an individual nanoparticle.
solutions generally include contributions from approximately $10^{10}$ nanoparticles. This means that extinction spectroscopy of nanoshells solutions is very useful when a broad, simplified understanding of the optical properties of monodisperse nanoparticle solutions is desired. However, since surface plasmon resonances are highly dependent on nanoparticle size and shape, ensemble extinction spectroscopy has significant limitations for solutions of complex, non-monodisperse nanoparticles. Single particle spectroscopy has emerged as a valuable tool for investigating the optical properties of individual nanoparticles with complex shapes that cannot be made monodisperse. As seen below, our implementation of single particle spectroscopy is based on dark field microscopy, and the light collected is due to scattering rather than extinction (scattering plus absorption). However, for nanoparticles with a diameter greater than 50 nm, the extinction spectra is generally dominated by scattering.\(^5\)

Several spectral scattering measurements of single nanoparticles have been reported in the literature.\(^{10, 27, 28, 31-34}\) One significant early report was on the plasmon resonance of single gold nanorods and nanospheres.\(^{10}\) Transmitted light, dark field spectroscopy was used to measure the plasmon resonance linewidths of these chemically synthesized nanoparticles. Linewidths of single plasmon resonant nanoparticles are interesting because incident light couples in to the plasmon resonance. The plasmon resonance may then decay in two ways, through radiative or non radiative decay. Radiative decay, as the name suggests, is energy loss by emitting (radiating) a photon. Non-radiative decay is excitation of electron-hole pairs. These pairs may be within the conduction band (intraband excitations), or between the $d$ band and the conduction band (interband excitation). From the linewidth measurements by Sönnichen et al, information
about the radiative decay rate for the particle plasmons was obtained. This work found that the resonances of individual particles were quite narrow, with linewidths as small as 150 meV, which is an extremely narrow plasmon resonance. The authors hypothesized that the narrow scattering resonances were due to weak radiative damping in the nanorods and spheres. Weak radiative damping indicates a slow dephasing of the plasmon resonance, which leads to a narrower resonance. This was a highly significant investigation, but not completely conclusive because there was no detailed microscopic correlation between nanoparticle spectra and structure.

The first single nanoparticle scattering spectra which were correlated with high resolution microscopy were reported in 2002 by Mock and co-workers. An optical microscope was modified to collect single nanoparticle scattering spectra.\textsuperscript{27} Colloidal silver nanoparticles were immobilized on a Formvar TEM Grid, and by matching up optical dark-field scattering images with extremely low magnification TEM image they were able to directly correlate nanoparticle structure and spectra. Their work provided a clear, compelling description of the relationship between silver nanoparticle shape and spectra, which showed that nanoparticle shape correlated strongly with the scattered color. Spherical silver particles were found to have plasmon resonances from roughly 400-500 nm; silver pentagons were resonant at 500-590 nm, and both spherical and pentagonal silver particles had a weak dependence of plasmon resonance on shape, which is exactly as one would expect from Mie theory. Triangular silver nanoparticles were found to have the largest size dependence and had resonances from 525 nm to past 700 nm. The shape dependence of silver triangles would later be confirmed with discrete dipole approximation (DDA) simulations by Schatz in 2006.\textsuperscript{28} The silver particles studied
by Mock and coworkers had diameters from 40 to 120 nm. In general, they found that as the particle size increased the resonances shifted to longer wavelengths, and that this effect was exaggerated for nanoparticles with sharp tips, such as the triangles. They found that particles with sharper points are almost always resonant at longer wavelengths than spherical or near-spherical nanoparticles of the same size.

2.2 Single Particle Spectrometer: Apparatus and Set-up.

The single particle spectrometer consists of an inverted optical microscope, the Zeiss Axiovert 200 MAT, coupled to an image output splitter, two Acton spectrometers, and two detectors. A quartz-tungsten-halogen (QTH) light source was used for illumination. The SpectraPro 2150i from Acton Research was used for visible light spectroscopy from 350-850 nm. A thermoelectrically cooled CCD camera, the PhotonMax512 from Roper Scientific, was used to collect spectra in the visible range. Near infra-red (NIR) spectroscopy was carried out from 850 -1800 nm with the rest of the light from the splitter with the SpectraPro 2150i spectrometer from Acton Research. NIR spectra were detected with a liquid nitrogen-cooled Princeton Instruments OMA V InGaAs array. A photo of the single particle spectrometer set-up is shown in Figure 2.2.
Two illumination geometries were employed for single particle spectroscopy. The first method is transmitted light dark-field microscopy at high numerical aperture. We use a custom oil immersion condenser (NA = 1 – 1.4) from Bernard Instruments and a 100x oil immersion objective (NA=0.7). The second illumination geometry is epi-illumination dark field. In this case the condenser (NA>0.9) is built into the objective (NA<0.9). Figure 2.3 shows diagrams (not to scale) of the light paths. Each illumination method has advantages and limitations, depending on the application. Transmitted light illumination with oil immersion offers superior signal-to-noise due to the very low background light, but sample mounting may be time-consuming because of the use of immersion oil. Transmitted light illumination also requires a considerable amount of skill, as the immersion oil is prone to bubbles, which interfere with the light path and cause bright

![Figure 2.3: (A) Transmitted light illumination. Light is incident from above at high numerical aperture (high angle). Only scattered light is collected by the objective. (B) Reflected light illumination. Epi-illumination where only scattered light is collected by the objective.](image-url)
backgrounds, or otherwise complicate imaging. Additionally, immersion oil is difficult to remove from the sample, which makes it difficult to reuse the sample. To avoid this problem, samples may also be mounted with water between the sample and the coverslip. Mounting with water contributes more background light because $n=1.33$ is a poor match for $n=1.5$, so there are reflections from the glass-water interfaces). For certain applications water is preferred because it is much easier to remove than oil, enabling reuse of samples, and it makes it possible to carry out biologically relevant reactions for sensing applications at the nanoparticle surface. Water is easier to rinse away, enables biological and chemical sensing, and even makes it possible to reuse the sample or perform SEM if desired.

The second illumination method, reflected light dark field illumination introduces higher levels of background brightness, but it is not necessary to use immersion oil, water, or cover slips for sample mounting. Nanoparticles of interest may simply be spin-coated (or drop dried) onto a microscope slide, coverslip, piece of silicon, or other substrate. No immersion oil is needed, and so reflected light illumination minimizes sample mounting time and greatly reduces the potential for sample damage. The trade-off is that reflected light illumination does not offer the excellent signal-to-noise and low background of the transmitted light geometry. Reflected light geometry is often an excellent choice for samples which scatter strongly. For samples which scatter weakly, transmitted light illumination is often the only option for single particle spectroscopy. Another consideration is that the reflected light dark field objective has a high NA and has a very short working distance, and thus it therefore cannot focus through a coverslip, making experiments in solution impossible.
2.3 Sample Preparation

Samples for single nanoparticle spectroscopy are prepared by first depositing nanoparticles on a substrate. Typical sample preparation methods for single nanoparticle spectroscopy are described here. Nanoparticles to be studied must be deposited onto a substrate (glass, silicone, or indium tin oxide) such that they are well-separated enough (approximately 1 nanoparticle per 10 square micron) to permit single particle spectroscopy. Preparation of these substrates is more complicated than the fabrication of nanoparticles films because the nanoparticles must be well-separated and at very low density (greater than 10 micron separation). A drop of nanoparticles in solution may be drop-dried on a glass slide or coverslip, but it is often necessary to dilute the nanoparticle stock solution, which often has an absorbance of 1.5 at the plasmon resonance.

A more controlled deposition may be accomplished if nanoparticles are put into ethanol or methanol and then spin-coated onto the substrate. Resuspending nanoparticles in ethanol or methanol requires centrifuging the nanoparticle solution to remove most of the excess water, and then resuspending with ethanol or methanol. Some nanoparticles—like nanostars or nanorods need special chemical modification if they are to be centrifuged and resuspended. These techniques are described in detail in later chapters. Following sample preparation, if reflected light illumination will be used, the prepared sample is simply loaded into the microscope. For transmitted light illumination, a drop of immersion oil or water (with a volume of approximately 10 μL) is placed on the substrate, followed by a coverslip. The sample is then loaded into the microscope.
2.4 Single Particle Spectroscopy Collection and Analysis

Once the sample is mounted the procedure for collecting scattered spectra will be the same for both illumination geometries. The single particle spectrometer can take both images and spectra by rotating a turret in the Acton spectrometers to put either a mirror or a grating in the light path of a Czerny-Turner spectrograph. Step 1: Focus on the substrate with the spectrometer in the mirror position and identify the particle you want to collect spectra for. Next, select the particle of interest in the silt while still in the mirror position. The third step is to switch to the grating position and collect an image of the particle. In the grating position the spectra of the particle has been spread out into wavelength components vertically along the CCD. This image is saved as a 16-bit tiff file for maximum resolution (65536 levels as opposed to the standard tiff 256). Following collection of the tiff files, the next step is analysis of the spectra with MATLAB.

For a basic visible-light scattering spectra of a nanoparticle, data is saved as a 512 x 672 16-bit tiff which is analyzed in MATLAB. The code used for analysis is

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Figure 2.4: (1) In the mirror position individual nanoparticles will be visible as points of light. (2) The nanoparticle of interest is centered in the slit while still in the mirror position. (3) Switch to the grating position. Light from the nanoparticle will be spread out by wavelength.
qualitatively described here. First, the scattered spectra from the nanoparticle are summed along the $y$ direction. The scattered light has a width of a few elements of the CCD—this width is due to chromatic aberration in the microscope optics. Each wavelength of light has a slightly different focal distance. This is accounted for by summing element intensities along the $x$ direction, which helps account for spectral aberrations and increases the signal-to-noise. Background is collected 20 pixels over from the position of the center of the spectra and is then subtracted from the spectra. Next, the background is summed over the same number of pixels in the $x$ direction as were summed over for the spectra. The background is then subtracted from the intensity.

At this point the spectrum still has variations which are a result of the spectral properties of the grating, varying intensity of the light source, and varying sensitivity of the detector. These variations are removed by dividing by the spectrum of a white calibration standard obtained from Edmund Industrial Optics. In general, this is thought of as $(\text{Intensity} - \text{Background})/\text{White} = \text{Spectra}$. The light source is a 12 V, 100 W

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**Figure 2.5:** (A) In the grating position light from the nanoparticle will be spread out by wavelength. MATLAB sums the spectra, subtracts the background, corrects for intensity variations in the illuminating light and grating, and gives (B) The scattering spectra of an individual nanoparticle. Visible data is plotted with the gray squares and data from the near infra-red is plotted with the black circles.
halogen lamp which is standard on the Zeiss Axiovert 200 or the Axiovert 200M inverted microscope. Spectra in the near infra-red (NIR) are obtained from the OMA V InGaAs array. The OMA V is one dimensional, so the analysis method is slightly different; collecting background spectra requires a separate measurement. Simultaneous measurement of visible and NIR gives excellent overlap and allows measurement of nanoparticle spectra out to 2 microns.

Many applications of single particle spectroscopy, such as localized surface plasmon resonance sensing, require knowledge of the exact position of a peak center. The center wavelength of a peak is determined with a non-subjective, easily automated mathematical approach, which is ideal for noisy or low-signal spectra. In MATLAB the final nanoparticle spectra are fit with a Lorentzian lineshape, which yields the peak center. This automated method also makes it possible to do time-dependent peak tracking by successively fitting the peaks for hundreds of spectra.

2.5 Correlated Microscopy and Spectroscopy

To further extend the potential of single particle spectroscopy, I have developed a method which uses alignment marks to directly compare nanoparticle spectra and structure. For this procedure nanoparticle structures were determined by atomic force microscopy (AFM) or scanning electron microscopy (SEM). For AFM characterization glass slides or coverslips may be used as substrates. If SEM characterization is desired, a conductive substrate is needed. Silicon is suitable for reflected light, but for transmitted light, indium tin oxide (ITO) coated slides are preferable because ITO is both transparent and conductive. ITO slides were obtained from Delta Technologies Limited, $R_s = 6 \pm 2 \ \Omega$. 
Once the optimal substrate is chosen, TEM grids (part number 79021C from Ted Pella) are carefully attached to the substrate with a small amount of carbon tape. The sample(s) are then placed in a thermal evaporator (custom-made in the Hafner lab), and a thin (20 nm) adhesion layer of chromium is evaporated onto the substrate. A thick layer of Au (300 nm) is then evaporated onto the sample.

After the sample is unloaded from the evaporator the SEM grid is removed, leaving behind an enumerated ‘shadow’ which is used to locate the same nanoparticles in the SEM and in the optical microscope. As figure 2.6 illustrates, the TEM grid is at a scale which overlaps well with the SEM and optical microscope magnification scales.
Wavelength calibration is performed in order to assign specific wavelengths to the pixel spectral data from the CCD array. The calibration is carried out with CCD exposures of a white sample which is illuminated through narrow bandpass filters from Edmund Optics are used to create a series of wavelength calibration images at specific wavelengths which are used to calibrate the single particle spectrometer.

2.6 Time-lapse Single Particle Spectroscopy

A further extension of single particle spectroscopy I have developed is time-lapse single particle spectroscopy. For time-lapse single particle spectroscopy, design choices have been made which make the optical/SEM correlation described above impossible. However, it should be possible to reengineer the sample holder to enable this in the future. The key problem to overcome in time-lapse single particle spectroscopy is sample drift. On the timescale of 10's of minutes to hours, mechanical drift allows the nanoparticle under investigation to drift with the substrate out of the focal plane, or laterally within the focal plane, or both. The result is a defocused nanoparticle image and/or a change of the image position on the CCD. To account for this defocusing we simply sum over a larger number of lateral (horizontal) pixels in the CCD image that contains the spectrum.

Lateral drift is more problematic because it makes the peak position appear to change, which, unfortunately mimics the spectral shifts of interest. Lateral drift can lead to false-positives for single particle LSPR sensing work. To help account for this, a sharp cutoff filter—which excludes all light below 610 nm—has been added to the microscopes optical path. This filter also has the highly useful side effect of substantially decreasing
the background levels; much of the background light is bluish, and most of the spectral region of interest is reddish, so the cutoff-filter also improves contrast.

Once the cutoff filter has been inserted the spectral shape will change (see Figure 2.7, panel B) and introduce a sharp edge at 610 nm. This edge may be fit with an error function, which returns the edge position. This edge position may be used for two purposes throughout the experiment. The first and simplest uses the edge position to recalibrate the wavelength position as needed—MATLAB constantly sets the edge position back to 610 nm and offset the spectra to account for it. The other benefit of the edge filter is that drift of the edge position yields information about drift in sample focus, and so by subtracting peak position(s) from the edge position focal drift along the y direction may also be accounted for, and so the effects of spectral shifts induced by

![Figure 2.7: Illustration of how an edge filter may be used for spectra correction (A.) Single nanoparticle spectra without cutoff filter. (B.) Single nanoparticle spectra with cutoff filter. (C.) Plot of peak center and edge position vs. time. (D.) Difference between peak center and edge position vs. time. Note that variation in the peak center due to drift has been almost completely eliminated through use of the cutoff filter.](image-url)
defocusing are also mitigated.

Time-lapse single particle spectroscopy is carried out in custom-made flow cells which consist of a flat, rectangular capillary tube (part number W510, Vitro Tubes) with silicone tubing attached to the ends with epoxy. The tubes have an inner thickness of 0.1 mm with a width of 2 mm, a wall thickness of 0.1 mm, and a length of 100 mm. It is essential to use rectangular tubes because the flat inner surface ensures that all nanoparticles are in the same optical plane. It is also essential to choose very flat and thin capillary tubes due to the very small working distance of the transmitted light oil immersion 100x objective used. The silicone tubing was obtained from McMaster-Carr, and was attached with waterproof epoxy. Flow of reagents was accomplished with a simple gravity feed system, consisting of an open syringe attached to one end of the tubing, with a beaker at the other side to collect reagents which have flowed through the flow cell. Nanostars (or other nanoparticles of interest) are deposited inside the tubes by filling the tubes with a nanoparticle solution, which may be conveniently accomplished by capillary action, and allowing the nanoparticles to physisorb to the walls of the tube. Now that the experimental techniques employed in this work have been described, we may turn our attention to the experimental results obtained through single particle spectroscopy.
3: Nanoshells and the Linewidth of the Plasmon Resonance

3.1 Nanoshell Background

Nanoshells are versatile nanophotonic particles which consist of a spherical dielectric core surrounded by a metal shell.\textsuperscript{17,35,36} By changing the ratio of the diameter of the core to the thickness of the shell the plasmon resonance of the nanoshells may be tuned from the visible out into the near infra red. The first report of a shell of gold grown on a dielectric core was by Zhou et al in 1994.\textsuperscript{37} A thin shell of Au was grown onto a core of Au\textsubscript{2}S. As the shell grew, they observed a redshift of the peak wavelength. This shift

![Diagram of nanoshell resonances](image)

**Figure 3.1.** Plot of extinction vs. wavelength for nanoshells with different core:shell ratios. For nanoshells with a 60 nm inner core radius and a 20 nm thick shell the dipole resonance will be in the red at 720 nm. As the shell thickness decreases the dipole resonance shifts to the near infra-red.
was attributed to quantum confinement by these authors. Later work on Au/Au$_2$S nanoshells in the Halas lab at Rice correctly described this redshift as a classical electromagnetic effect, not a result of quantum confinement.\textsuperscript{38} Currently, nanoshells are chemically synthesized to consist of a silica core and a gold shell.\textsuperscript{17} This growth method results in solutions of nanoshells which are more monodisperse than Au/Au$_2$S nanoshells. Nanoshells are usually made with a silica core and a gold shell, but they may also be made with a silica core and a silver shell.

Gold nanoshells with silica cores are chemically synthesized in solution.\textsuperscript{17} First, small colloidal gold particles (diameter 2-5 nm) are chemically attached to a silica colloid made by the Stober process or obtained commercially.\textsuperscript{17} These small gold particles will serve as nucleation points for reduction of gold from solution. Gold is then reduced from solution onto the seed particles until the core is completely encased in a shell of gold. The tunability inherent in nanoshell geometery makes them fascinating nanoparticles with a rich array of plasmonic properties. Unfortunately, the objectives of this chapter do not permit that it be a comprehensive review of nanoshells. The interested reader is referred to the many excellent review articles available on nanoshells and other nanoparticles and their applications.\textsuperscript{3, 39, 40}

3.2 \textit{Theoretical Description of Nanoshells}

Gold/silica nanoshells are monodisperse and their optical properties are well described by theory. Because nanoshells are spherically symmetric, it is possible to calculate their spectral extinction analytically with a variation of Mie scattering theory for concentric spheres.\textsuperscript{35} Standard Mie scattering theory considers a plane electromagnetic
wave incident on a sphere of radius \( r \) made up of any material, given its complex dielectric function. Aden and Kerker extended this analysis by altering the boundary conditions to allow two different materials for the core and shell.

Aden and Kerker's theoretical description has been applied in a computer program written by the Halas group which calculates the wavelength-dependent scattering, absorption, and extinction efficiencies and cross sections for a nanoshell with a given core size, shell thickness, and shell material (usually gold or silver). This program also accounts for the local dielectric environment and may return the angular distribution of the scattered light. Figure 3.2 shows an experimental extinction spectrum of Au/SiO\(_2\) nanoshells, plotted with circles. The solid line is the calculated extinction spectra where the dotted line is the scattering component, and the dot-dashed line is absorption component. Note that there is good agreement between experiment and theory, except at low energies in the NIR. The lack of agreement in the NIR is due

![Figure 3.2](image-url)
to fused nanoshells or lumps of gold which yield contributions to the extinction spectra in this region and are not accounted for in the calculation.

Now that we are familiar with a current incarnation of Mie theory for nanoshells, we can consider the history of Mie scattering theory as applied to nanoshells. When Aden and Kerker's version of Mie theory for concentric spheres was initially used to fit the extinction spectra of a nanoshell solution, it was found that the Mie theory calculation of the LSPR was significantly narrower than the extinction spectra of the nanoshell solution.\textsuperscript{17, 41} Two major hypotheses were posited, the first was that broadening was due to a size distribution of the nanoshells. The second hypothesis was that electron surface scattering within the gold shell contributed to the linewidth broadening.\textsuperscript{41} A size distribution was ruled out as a cause, following intensive structural characterization of the variation of silica core and nanoshell diameters with TEM. Calculated ensemble spectra which corresponded to this measured variation still did not have a sufficiently broad linewidth. Once it was shown that a size distribution was not responsible, the remaining factor was electron surface scattering, which we now discuss in detail.

3.3 Electron surface scattering in metal nanoparticles

The Mie theory expression for the spectral extinction (equation 3.1) can be simplified by only considering the first term (dipole), as in equation 3.2.

\begin{equation}
\sigma_{ext} = \frac{2\pi}{|k|^2} \sum_{L=1}^{\infty} (2L+1) \text{Re}(a_L + b_L)
\end{equation}

(3.1)

\begin{equation}
\sigma_{ext}(\omega) = \frac{9\omega}{c} \epsilon_m^{3/2} V \frac{\epsilon_2(\omega)}{[\epsilon_1(\omega) + 2\epsilon_m]^2 + \epsilon_2(\omega)^2}
\end{equation}

(3.2)
This approximation is justified for particles much smaller than the wavelength since the electron excitation is in phase across the particle (no higher order multipoles) and since small particles have negligible radiation relative to scattering. From this equation, one would predict that the plasmon linewidth would be independent of nanoparticle diameter. However, studies of plasmon resonances in ensembles of nanoparticles typically showed linewidth broadening to be inversely proportional to nanoparticle diameter for diameters less than 20 nm, as shown in Figure 3.3.42

The Mie scattering calculations for gold nanoshells are generally carried out with the complex dielectric function measured from a gold film, so it is assumed that the electron dephasing rate is equal to the bulk scattering rate for gold. However, for nanoscale gold structures, it was thought possible that the scattering rate would increase due to the small size of the particles.6 Once the particle size (in at least one dimension) approaches that of the MFP of gold (42 nm), electrons are thought to interact (by scattering) with the edges of the nanoparticle, leading to an increased scattering rate. We can understand this conceptually with the aid of Figure 3.4. On the left is a representation
of a macroscopic metal. In macroscopic metals the electrons are free to scatter with little interference from the surface because all dimensions of the metal are considerably larger than the mean free path. For nanoscale metal particles (illustrated on the right), as soon as one dimension becomes smaller than the mean free path electrons are thought to scatter from the interfaces more frequently. This is mathematically accounted for in Mie theory by replacing the bulk scattering rate with a modified (increased) scattering rate, thus effectively increasing the bulk scattering rate of the particles.

The cross sections calculated from Mie scattering theory are dependent on the dielectric function, as discussed in Chapter 1. The dielectric function may be thought of as the sum of the interband (bound) and the Drude (free) electron contributions, as shown in equation 3.3. The Drude contribution includes the free electron scattering rate. In equation 3.3 the frequency is $\omega$, the plasmon frequency is $\omega_p$, and the bulk scattering rate is $\gamma_{bulk}$.5,6

\[
\varepsilon(\omega) = \varepsilon(\omega)_{\text{interband}} + \left( 1 - \frac{\omega_p^2}{\omega^2 + i \omega \gamma_{\text{bulk}}} \right)_{\text{Drude}}
\]
Once at least one dimension of the nanoparticle becomes comparable to the mean free path of the electrons, the scattering rate is thought to increase, and this increase is mathematically accounted for by replacing the bulk scattering rate $\gamma_{\text{bulk}}$ with the modified scattering rate, $\Gamma$, as shown in equation 3.4.

$$\Gamma = \gamma_{\text{bulk}} + A\nu_F / d$$

In equation 3.4 the Fermi velocity $\nu_F$, the reduced electron mean free path length is $d$, and $A$ is a parameter which depends on the details of the specific surface scattering process. $^{42-44}$ For clarity, $A$ will be referred to in this work as the surface scattering parameter. A surface scattering parameter of $A=1$ corresponds to complete dephasing of the plasmon excitation upon surface scattering, and a parameter of $A=0$ corresponds to no dephasing upon surface scattering, and therefore no broadening of the plasmon resonance.

For spherical particles, $d$, the reduced electron MFP, may be simply related to the nanoparticle diameter. This modification of the bulk scattering rate reproduces the observed inverse relation between linewidth and nanoparticle size for small nanoparticles. The nanoshell extinction calculation shown in Figure 3.2 requires a surface scattering parameter of 1 to achieve a reasonable linewidth fit to the bulk nanoshell extinction measurements. This is consistent with previous results on extinction spectra for nanoshell solutions.$^{41}$ Though these nanoshells have an outer diameter larger than the electron mean free path in gold, it is the shell thickness which limits the electron path
length and determines the amount of surface scattering. Therefore, in equation 3.4 \( d \) is set to the shell thickness for nanoshell calculations.\(^{38}\)

### 3.4 Brief Review of Surface Scattering Literature

Elsewhere in the literature, the surface scattering parameter is generally considered to be a factor on the order of 1, but the range of reported values is quite large. This section’s objective is to provide some brief background on the surface scattering parameter as it relates to Mie scattering theory for gold nanoshells. Initial work by the Halas lab set the value of the surface scattering parameter at 2 or 3 for ensemble measurements on gold nanoshells.\(^{41}\) These values allowed for good agreement between the extinction spectra of nanoshell solutions and the calculated extinction cross section obtained from Mie scattering theory for concentric spheres.

In 2004 Raschke et al. reported a measurement of the scattering linewidth of single Au/Au\(_2\)S nanoshells.\(^{45}\) Single particle measurements completely remove ensemble effects, and thus are more likely to reveal the true value of the surface scattering parameter, and therefore the nature of the role of electron surface scattering in nanoshells. Their work found the value of the surface scattering parameter to be 0.5. They hypothesized that the previously reported larger values of this parameter were caused by nanoshells with rough and possibly incomplete gold shells. Additional surface roughness would provide more opportunities for the electrons to scatter at domain boundaries, thus contributing to linewidth broadening and leading to an inflated value of the surface scattering parameter.
Work by Berciaud et al. later measured the *absorption* spectra of gold nanoparticles with diameters of 35 nm or smaller. The technique employed was the photothermal heterodyne imaging method which allowed careful measurement of absorption spectra (rather than scattering), but which did not permit microscopy of the nanoparticles under investigation.

They found that the linewidths of the absorption resonances of the nanoparticles were best fit with a surface scattering parameter of $A = 0.25$. Interestingly enough, the Berciaud paper has some data points (reproduced in Figure 3.5) which are best fit by values of $A$ which are *less than zero*. This corresponds to LSPR resonances that are narrower than one would predict based on the bulk dielectric function. The researchers did perform TEM on nanoparticles prepared under identical conditions to account for

![Figure 3.5](image_url)

*Figure 3.5.* Size dependence of the resonant energy, $E_R$ (a) and the full-width at half-max, $\Gamma_{1/2}$ (b). Experimental data (circles with standard deviations) are compared with Mie theory for $A = 0$ (dotted line) and $A = 0.25$ (gray area). The gray area accounts for the experimental uncertainties on the bulk dielectric function of gold. Figure and caption reproduced from "Observation of Intrinsic Size Effects in the Optical Response of Individual Gold Nanoparticles" Berciaud, S.; Cognet, L.; Tamarat, P.; Lounis, B. Nano Lett.; (Letter); 2005; 5(3); 515-518.
structural variations. Nanoparticle ellipticity (mean aspect ratio) was measured to be 1.1 +/- 0.1. This ellipticity only partially accounted for the reported values.

Another recent measurement of the value of the surface scattering parameter was carried out with gold nanorods by Novo et al. Gold nanorods are a potentially difficult system because they are not analytically described by Mie theory and thus the value of the surface scattering parameter may not be easily extracted. This investigation used an effective mean free path for nanorods from work by Coronado and Schatz. Use of an effective mean free path makes it difficult to separate the different contributions to the electron surface scattering rate. Novo et al found the value of the surface scattering parameter to be 0.30. The close agreement between the Novo result and the Berciaud result lends credence to the Novo result, but further work on theoretically well-described nanoparticles which correlates spectra with nanoparticle structure would be ideal.

3.5 Discussion of the Surface Scattering Parameter

By now it should be clear to the reader that measurements of the linewidth and the value of the surface scattering parameter which are based on ensemble extinction measurements in solution are problematic because the true linewidth may be masked by size variations. Unfortunately, the few existing measurements of the surface scattering parameter from single particle spectra may also be problematic, as discussed in the previous section. To avoid these problems I have performed scattering spectroscopy on single gold nanoshells with correlated electron microscopy. As we know, the surface scattering parameter has a direct, measurable effect on the scattering linewidth, and so by measuring the scattering spectra of individual nanoshells I may determine the surface
scattering parameter without interference from ensemble effects. From the scattering spectra the linewidth may be extracted. The value of the surface scattering parameter is obtained in the following experiment by comparing the experimental linewidth to scattering spectra calculated from Mie theory. In general, it was found that the experimental linewidths were best fit by Mie theory with the value of the surface scattering term set to zero, essentially without any use of the surface scattering term.

As mentioned before, previous single particle measurements were, unfortunately, not correlated with microscopy, so their results—while compelling—cannot be considered conclusive. The method described here combines high resolution microscopy and optical spectroscopy to determine the scattering spectra, as well as the linewidth and the value of the surface scattering parameter. This technique allows measurement of the surface scattering parameter without any need to consider sample inhomogeneity and with structural information about the actual nanoparticle under investigation.

3.6 Sample Preparation

The details of sample preparation and set-up follow. Nanoshells were obtained from the Halas lab. The nanoshells had previously been determined by electron microscopy to have a core diameter of 60 nm and a total diameter of 80 nm and this was confirmed with Mie theory fits to ensemble extinction spectra. Nanoshells were centrifuged and resuspended in methanol to simplify nanoshell deposition. The nanoshells were deposited on substrates by simple drop-drying. Substrates were either glass slides or indium tin oxide (ITO) coated slides. Both types of substrate had thermally evaporated alignment marks, which were made as described in Chapter 2. The optimal
density of nanoshells was less than 1 nanoshell per 100 square microns. Higher particle densities made it prohibitively difficult to optically distinguish between adjacent particles. Spectra were collected either on ITO in water, or on glass surrounded by immersion oil ($n=1.515$).

Correlated electron microscopy and optical spectroscopy were critical to this study and necessitated the use of alignment marks. Previous correlated spectroscopy and high-resolution microscopy investigations had been performed with alignment marks which were fabricated by electron-beam deposition or by simply matching up particle positions between low-magnification TEM and optical pictures.\textsuperscript{27} For this investigation I have developed a simpler method of aligning microscopy and spectroscopy which was described in detail in Chapter 2. Following nanoshell deposition, optical alignment pictures are taken in air in the microscope. These alignment pictures clearly show the

![Figure 3.6. Optical (A) and SEM (B) images of nanoshells on indium tin oxide (ITO) relative to an alignment mark. (C)-(E) are high-resolution SEM images of three representative nanoshells.](image)
outlines of the alignment marks, as well as the positions of nanoshells, and are shown in Figure 3.6. The alignment pictures are taken before spectra are collected or SEM is performed because they make the SEM process easier by offering an approximate particle position. As you recall, nanoshells are deposited at a very low surface density, and unfortunately, this low surface density of nanoparticles makes it difficult to find individual nanoshells with SEM or AFM, so optical alignment marks are necessary. When AFM was used to obtain microscopic structural information the same method was used, and for AFM it was even more critical to take optical alignment marks first because of the slow image acquisition times. Following acquisition of alignment pictures, SEM and AFM were carried out on the well-separated nanoshells. Once good candidates (isolated, single, complete) for single particle spectroscopy were identified, the sample was loaded into the single particle spectrometer and spectra were taken as described in Chapter 2.

3.7 Results from Single Nanoshell Spectroscopy

Single nanoshell scattering spectra were collected under two conditions. Scattering spectra were measured in oil on glass and in water on indium tin oxide (ITO). The nanoshells on glass were characterized with AFM. Two methods were used because, despite the superior structural information of SEM, there were concerns about the non-uniform dielectric environment (water on ITO), as well as the possibility of interactions between the ITO and the nanoshells. By also performing this experiment with nanoshells on glass, characterized by AFM, we could work in a uniform dielectric environment. The single nanoshell scattering spectra matched well to Mie theory calculations in terms of
their peak wavelength and linewidth. Before discussing the single nanoshell results, we will first consider a simpler result: fused nanoshell spectra.

Spectra from fused nanoshells were observed. SEM of two fused nanoshells and their scattering spectra are plotted in Figure 3.7. Spectroscopy of these particles revealed that the spectra have some substantial broadening in the NIR, as expected. This broadening also helps explain the broadening seen in the extinction spectra of solutions of nanoshells. Ensemble nanoshell extinction spectra in solution feature a NIR ‘shoulder,’ which is thought to be due to fused nanoparticles. Correlated electron microscopy enabled us to attribute this to hybridization or a longitudinal resonance between the two nanoshells, and not to a nanoshell with a broader spectrum.

Now we return to the real topic of interest: scattering spectra from single gold nanoshells. Fits to Mie theory for the single nanoshells were first carried out with the
values of \( r_1 \) and \( r_2 \) (the core and total radii for the nanoshells) from SEM and the fit to the bulk extinction spectra. In many of these cases, we found that \( r_1=60 \) and \( r_2=80 \) returned scattering spectra which were not resonant at the same energy as the single nanoshells. In these cases, we adjusted \( r_1 \) and \( r_2 \) (with help from the SEM structural data) to match the peak energies of the spectra and the Mie theory prediction. Once the inner and outer radii were determined, the surface scattering parameter was adjusted to fit the linewidth of the single nanoshell scattering spectra.

We discovered that scattering spectra for single gold nanoshells were best fit by Mie scattering theory with no surface scattering. The single nanoshell scattering spectra we observed had a significantly narrower linewidth than had been previously observed for ensemble gold nanoshell extinction measurements in solution.\(^{41}\) The full width at half-maximum (fwhm) of the scattering portion of the spectrum in Figure 3.8 is 760 meV.

**Figure 3.8.** (A)-(C) Scattering spectra for single nanoshells in water on indium tin oxide substrates. Measured spectra are plotted with circles and Mie theory calculations are plotted with lines. Parameters used for the Mie theory calculations are given in the inset boxes.
while the single nanoshell spectra in Figures 3.8 have an average fwhm of 545 meV. The single nanoshell spectra in Figures 3.8 and 3.9 fit Mie theory calculations well with no surface scattering (A=0).

In other words, it was found that the scattering spectra were not only best fit with a surface scattering parameter of zero, but that they were often narrower than predicted by Mie scattering theory, even for fits with no surface scattering, as shown in Figures 3.8 and 3.9. This phenomenon has been noted in other single particle studies.\textsuperscript{49, 50, 46} We believe that this deviation is real, since the vertical error bars are similar to the symbol sizes and the band-pass of the spectrometer is approximately 20 meV. This result, while strange, was not entirely unexpected. In their work on the resonant linewidths of small gold particles, Berciaud and coworkers also observed some linewidths best fit with no surface scattering, and even a few linewidths which would be best fit with a surface scattering term less than 0, as shown in Figure 3.5.\textsuperscript{51}
The particle-to-particle spectral variation in linewidth suggested that it was related to surface roughness or defects in the shell. Close observation of Figures 3.8 and 3.9, reveals that many of the scattering spectra are significantly narrower than Mie theory, and that many of them also exhibit unique shapes. Panel (b) in Figure 3.9 shows the appearance of apparent quadrupole and octapole resonances. Usually unique spectral shapes like these are thought to be due to variations in the nanoparticle shape, but these nanoshells, have been verified to be spherical by scanning electron microscopy. This means that any structural variations of the nanoshell had to be \textit{internal}. The most likely scenario is that the inner core was slightly offset from the expected position, and thus we refer to this as the offset core hypothesis.

My experimental results indicate that the bulk nanoshell extinction spectra are \textit{not}\textit{ broadened due to interfacial electron scattering, but due to variations in particle size and shape}. A full quantum description of nanoshell plasmon resonances using with time-dependent local density approximation (TDLDA) also supports this view.\textsuperscript{52} This calculation, which does not require the bulk dielectric function of gold as an input parameter, accurately predicts the dependence of the nanoshell plasmon resonant energy on the nanoshell structure, and yet shows no dependence of the linewidth on the total diameter. Additionally, a report on single Au/Au\textsubscript{2}S nanoshells observed even narrower linewidths, down to 180 meV, by studying smaller nanoshells (80 nm diameter) to reduce phase retardation effects which also broaden the spectrum. When compared with Mie theory, the scattering parameter that best fit the data was found to be $\Lambda = 0.5$, which was still smaller than values typically required to fit ensemble measurements.\textsuperscript{45,41}
3.8 Conclusion and Discussion of Results

We now return to the offset core hypothesis. As discussed earlier, this hypothesis was inspired by the single nanoshell scattering spectra work described in this chapter. The hypothesis was supported by initial calculations from the Nordlander group at Rice, and building on this observation, the Halas lab systematically fabricated nanoshells with offset cores. Single particle spectroscopy of these nanoshells revealed unique spectra which were very similar to the spectra I observed for some single nanoshells. We know that the plasmon resonances of nanoshells are due to hybridization between the sphere and cavity plasmons.\textsuperscript{53} By offsetting the core slightly, it became possible for the dipole elements of the plasmon resonance to mix into all plasmon modes of the particle. The result, confirmed theoretically and experimentally, was a multi-peaked spectrum which is dependent on the degree of the core offset.

In conclusion, the single nanoshell scattering spectra result is consistent with results from investigations on single Au/Au\textsubscript{2}S nanoshells which featured linewidths which were fit with a surface scattering parameter of 0.5.\textsuperscript{45} Work by Cognet and coworkers on small gold nanoparticles indicated a surface scattering parameter value of 0.25. These investigations into the value of $A$ are critical because they offer information about inhomogeneous broadening in nanoparticles. The factors which control electron surface scattering are of tremendous import because they directly affect the linewidth. Narrow linewidths are highly desirable in LSPR sensing applications, and thus are the topic of intensive investigation. Nanoparticles with narrow linewidths may also help enable multicolor bioimaging. Until we have a comprehensive understanding of the degree to which electron surface scattering affects the linewidth of individual
nanoparticles we will have difficulty creating nanoparticles with the desired narrow linewidths. Further investigations into the true role of electron surface scattering in the linewidths of metal nanoparticles will enable the rational creation of nanoparticles with the desired optical properties.
Chapter 4. Nanostar Synthesis and Structure

4.1 Anisotropic Gold Nanoparticle Synthesis

Gold nanostars are produced by seed mediated, surfactant-directed synthesis. Small spherical gold nanoparticles (seeds) are placed in a solution of \( \text{Au}^{1+} \) ions under condition which cause them to reduce to \( \text{Au}^{0} \) on the seed surface. Through this reduction the seeds grow into larger nanoparticles. Due to the symmetric face-centered cubic lattice of metallic gold, the formation of anisotropic structures requires a selective capping agent. For example, the room-temperature synthesis of gold nanorods with 97% yield has recently been demonstrated by seed-mediated growth directed by the surfactant cetyltrimethyl-ammonium bromide (CTAB). This synthesis is routinely carried out in the Hafner lab to make gold nanorods for biological applications, and to

Figure 4.1 Scanning electron micrograph of nanostars. Note the high degree of structural complexity and heterogeneity.
study the growth mechanism. The initial seed nanoparticles are synthesized through the reduction of gold chloride by ice cold sodium borohydride, which is tedious and sensitive to the exact temperature history of the reagents. In an attempt to simplify the nanorod synthesis, we found that replacing the surfactant-stabilized seed with commercially available colloid (10 nm diameter gold colloid from Ted Pella, part number 15703) results in growth of ca. 100 nm star-shaped gold nanoparticles. These nanostars are formed under the exact growth conditions which normally produce gold nanorods. The procedure for this synthesis method and an alternate synthesis method are described in detail in the next section. These nanostars are distinct from those previously reported due to their relatively small size and their complex three-dimensional structure.\textsuperscript{13,57-59}

4.2. Growth Procedures

A gold nanoparticle growth solution was prepared by adding the following reagents to a plastic tube in the order listed and then gently mixing: 4.75 mL of 100 mM CTAB, 0.2 mL of 10 mM HAuCl₄ (3H₂O), 0.03 mL of 10 mM AgNO₃. Next, 0.032 mL of 100 mM ascorbic acid was added, which changed the solution from brown-yellow to colorless, indicating the reduction of Au\textsuperscript{3+} to Au\textsuperscript{1+}. To initiate nanoparticle growth, 10 µL of undiluted 10 nm gold colloid (Ted Pella number 15703) was added to the growth solution, mixed gently, and left still for 3 h. This resulted in growth of multi-branched nanoparticles, which we call nanostars.

We found the gold nanostar synthesis method with commercial seed to be quite reproducible, having been successful with colloid from three separate shipments. However, the unknown surface chemistry of commercial nanoparticles makes it difficult
to unravel the growth mechanism and guarantee future reproducibility. We have therefore developed an alternate gold nanostar synthesis protocol from standard reagents.

The alternate synthesis method for gold nanostars does not rely on commercial seed particles, but it uses similar reagents with an altered order of mixing and the critical addition of NaOH to raise the pH. A gold nanoparticle seed solution for nanostar growth was synthesized by combining 0.250 mL of 10mM HAuCl₄ with 7.5 mL of 0.1 M CTAB. To this we added 0.600 mL of ice cold NaBH₄ resulting in a pale brown seed solution. A growth solution was prepared by adding the following reagents to a plastic tube in the order listed and then gently mixing: 4.75 mL of 0.1 M CTAB, 0.2 mL of 10 mM HAuCl₄, and 0.03 mL of 10 mM AgNO₃. The solution should be yellow-brown after addition of HAuCl₄. To this growth solution we added 10 uL of seed, followed by 0.032 mL of 100 mM ascorbic acid. For successful nanostar growth, the solution should be clear after addition of ascorbic acid, indicating the complete reduction of Au³⁺ to Au¹⁺. This clear color is critical for successful nanostar growth. Finally, 0.025 mL of 0.1 M NaOH was added, followed by gentle mixing. Within 15 minutes the solution was faintly blue-purple, and color deepened over the next 3 hours.

4.3 Yield Comparison

We have evaluated the yield of nanostar synthesis through SEM analysis of hundreds of particles. For each particle the number of tips was counted, such that zero tips correspond

![Figure 4.2 Nanostars were tilted in the SEM from 0° to 20° to confirm that the bright points on the particles were nanostar tips.](image-url)
to a spherical nanoparticle and one, two, three, four, five, or six tips correspond to increasingly complex nanostars. If we compare the nanostar growth yield for both synthesis methods we find that the method with commercial seed particles has a yield of well-formed nanostars with three or more tips of approximately 14%. With seed made from standard chemicals, we find a nanostar yield of 9%. A detailed yield histogram is shown in Figure 4.3. The bright points on the nanostars in the SEM image are tips oriented normal to the plane of the figure as confirmed by sample tilting and illustrated in Figure 4.2.

During this analysis we found a significant fraction of nanostars with small or poorly formed tips. We suspect these particles will not have the optical properties of interest, so we counted them separately and refer to them simply as ‘nanoparticles.’ Histograms of the yield analysis for a typical preparation with representative images are given in Figure 4.3. Note that the analysis undercounts the number of tips on the nanostars since not all tips will be visible in the SEM images.

Figure 4.3. Star-shaped gold nanocrystal yield. SEM images were analyzed to determine yield in terms of the number of points per particle. Separate data are given for (A) the surfactant-stabilized seed and (B) the commercial colloid seed. All scale bars are 100 nm.
4.4 Structure

A transmission electron microscopy (TEM) image of a well-formed nanostar is presented in Figure 4.4. Selected area electron diffraction (SAED) of that individual nanoparticle demonstrates that it consists of multiple crystal domains, but that it is not highly polycrystalline like an evaporated gold film. Careful examination of Figure 4.4 and Figure 4.5 reveal twin defects along the nanostar tips. These defects are similar to those found in gold nanorods, where poor CTAB binding to twin defects is thought to be a source of growth anisotropy. 60, 61

4.5 Gold Nanostar Growth Mechanism

We can understand the

Figure 4.4 A TEM image of a well-formed nanostar. Note the twin defects visible along the tips. Electron diffraction (inset) demonstrates that the nanostars have defects and consist of multiple crystal domains.

Figure 4.5 A TEM image of a well-formed nanostars. Note the twin defects visible along the tips.
growth of gold nanostars by first understanding general factors controlling growth of gold nanoparticles in solution. In solution, nanoparticle growth occurs by diffusion of a monomer—for gold nanostars and nanorods, the monomer is Au$^{1+}$ ions. Diffusion of the Au$^{1+}$ ions to the nanoparticle surface and reaction of those monomers with the surface are the mechanisms which control the growth rate of gold nanoparticles. The current model of nanorod growth is that the rate of Au ion reduction is controlled by the CTAB (cetyltrimethylammonium bromide) bilayer on the nanorod surface. Conventional wisdom holds that the CTAB bilayer regulates the reaction rate by controlling the reduction of Au onto the surface. If the CTAB bilayer is selectively adsorbed to different facets of the nanoparticle (as in gold nanorods) then Au ions will reduce onto different faces of the nanoparticle at different rates, and this mechanism is believed to be the source of the elongated shapes of gold nanorods. Use of surfactants like CTAB for nanorod growth has been shown to improve the yields and monodispersity of nanorods.$^{23}$

Growth of nanostars and nanorods both rely on CTAB. As we know, nanorod growth is thought to be due to increased reduction of Au ions at regions with poor CTAB binding; nanostar growth is likely due to a similar mechanism. The nucleation of growth anisotropy at multiple sites on the nanostars could also be due to a high defect density caused by the use of commercial gold colloid as seed particles. The commercial seed particles used to grow nanostars may have multiple crystalline defects or patchy surface chemistry due to the (proprietary) stabilization method used on the particles.$^{55, 62}$ In the growth method which uses standard chemicals, increasing the pH by adding NaOH we increased the rate of Au$^{1+}$ reduction and likely increased the density of defects in CTAB
1. CTAB-capped gold seed particles grow by reduction of Au+ on their surface while surrounded by a surfactant bilayer. The bilayer regulates the growth rate.

2. A defect forms on the nanoparticle with poor surfactant binding to create anisotropic growth.

3. Anisotropic growth produces elongated gold nanorods in high yield.

2. Rapid growth induced by the addition of NaOH yields multiple defect sites with anisotropic growth.

Figure 4.6 Illustration of the growth mechanism for gold nanostars.

binding. Increased CTAB defects, led to more sites which nanostar tips may grow from. The addition of NaOH to the nanorod synthesis method probably also contributed to more rapid growth due to the forced reduction of gold ions. It is quite probable that the increased density of CTAB binding defects is the source of the nanostars unique structure.
Chapter 5 Nanostars: The relationship between spectra and structure

5.1 Ensemble Nanostar Spectra in Solution

As we know from previous sections, nanostars are approximately 100 nm from tip to tip, have multiple points, and may manifest in a wide variety of shapes. Many other anisotropic gold nanoparticle shapes have been reported recently,\textsuperscript{13,57,58,16} most of which were fabricated through surfactant-directed synthesis. The gold nanostars described here are unique because of their relatively small size—most particles with similar shapes are significantly larger—and their complex structure. As discussed in the previous chapter, high-resolution TEM of nanostars revealed twin defects along the axis of each nanostar tip which are very similar to those documented in gold nanorods, which was not surprising, given the similar growth mechanisms.

Though nanostars exhibit marked structural heterogeneity, ensemble spectral measurements reveal two broad, well-defined extinction peaks, as seen in Figure 5.1. We can understand this ensemble spectrum by considering two theoretically and experimentally well-established properties of gold nanoparticles.

![Figure 5.1. An extinction spectrum of the nanostar solution exhibits broad visible and NIR peaks.](image)
First, spherical gold nanoparticles have a plasmon resonance at 520 nm that red shifts towards 600 nm as the nanoparticle diameter grows due to phase retardation and radiative damping. Secondly, elongated gold nanoparticles feature a longitudinal plasmon resonance that redshifts from 520 nm (the value for spherical gold nanoparticles) by an amount proportional to their aspect ratio.\textsuperscript{24, 63} For example, the gold nanorods on which gold nanostar synthesis is based have a longitudinal plasmon resonance at around 700 – 800 nm and an aspect ratio of 3 – 4.

Given these trends, we can gain some insight into the ensemble nanostar extinction spectrum shown in Figure 5.1. The 586 nm peak can be thought of as due to the nanostar core, which acts like a spherical gold nanoparticle, or it could be thought of as a transverse plasmon resonance of the nanostar tips, a feature also found in nanorods (described in Chapter 1). However, the transverse plasmon resonance in gold nanorods is usually much weaker than the longitudinal, and here the 586 nm peak is quite strong. The 586 nm peak could also have a considerable contribution from the presence of spherical or nearly spherical colloids in the nanostar solution. Recall from Chapter 4 that there is a significant yield of spherical and poorly formed nanostars in solution that would likely exhibit a peak between 520 nm and 600 nm. The longer wavelength peak near 773 nm can be thought of as due to the longitudinal resonances along the length of the nanostar tips. For comparison, gold nanorods with an aspect ratio of 3.3 (50 nm length by 15 nm diameter) have a plasmon resonance at 755 nm in water.\textsuperscript{23} Since the tips have a similar size and shape to those nanorods, this assignment is likely correct. The 773 nm peak is much broader than that observed for gold nanorods, which is almost certainly due to inhomogeneous broadening by the various shapes and aspect ratios of nanostar tips.
5.2 Correlated Single Nanostar Spectroscopy and Microscopy

Detailed characterization of the optical properties of complex, heterogeneous nanoparticles such as gold nanostars is best accomplished by single particle spectroscopy, the technique for which is described in Chapters 2 and 3 and background on which is given in Chapter 1. The measurements presented here were carried out as previously described in Chapters 2 and 3 with the addition of an analyzer for some of the data. Note that the single particle spectra recorded in this investigation represent only the scattering spectrum, rather than scattering plus absorption as in extinction measurements. Before nanostars could be deposited on a substrate it was necessary to remove excess CTAB present in the nanostar growth solution. One might naively think that the CTAB could be removed by centrifugation, but that is not the case. If gold nanostars are removed from CTAB they aggregate and become approximately spherical lumps of gold. This is readily visible if nanostars are centrifuged and resuspended in water—the solution is initially blueish purple, but it rapidly becomes reddish, indicating the restructuring of the nanostars. The same phenomenon is also observed when solutions of CTAB-stabilized nanorods are centrifuged and resuspended in water.

For nanorods this problem may be solved by coating the nanorods with poly-ethelyne glycol with an attached thiol group (PEG-SH). PEG-SH displaces the CTAB by replacing it with PEG. This makes it possible for nanorods to maintain their structure even outside of a solution containing CTAB. After stabilization with PEG, nanostars could be resuspended in water and drop-dried onto substrates. As with the single nanoshell work, electron microscopy of nanostars was correlated with nanostar spectra.
The same procedure described in Chapters 2 and 3 was used for the nanostars. Indium tin oxide (ITO) coated glass slides with thermally evaporated alignment marks were used for single nanostar spectroscopy.

Parts A and B of Figure 5.2 demonstrate alignment between SEM and optical imaging conditions. Figure 5.2 panel D presents the scattering spectrum for the gold nanostar shown in panel C. The spectrum consists of three sharp peaks at 647, 700, and 783 nm. Their positions and relative strengths suggest that the broad NIR peak in the extinction spectrum of the nanostar solution is a sum of several sharper peaks, as one would expect from a heterogeneous sample. The spectrum for a spherical 100 nm gold colloid (resonant at 520 nm) recorded under the exact same conditions is plotted as a reference to demonstrate that the small peaks are not noise or an artifact of the measurement. Note that despite the similar sizes of the gold colloid and the nanostar, the gold colloid has a much larger full width at half max (fwhm), and that the nanostar resonances are significantly narrower and are resonant at longer wavelengths.
Single nanostar spectroscopy revealed that complex nanostar shapes have very complex scattering spectra. We attempted to determine if the sharpness of the nanostar tips determined the wavelengths and/or the linewidths of the resonances. Experimental work by Mock et al\textsuperscript{27} and theoretical insight from the Schatz group\textsuperscript{28} have indicated a correlation between tip sharpness and resonance wavelength. Sharper triangular nanoprisms are predicted to have broader linewidths by Schatz’s DDA calculations. In the system he simulated, the relationship between tip sharpness and the broadness of the resonant linewidths was attributed to the “lightning rod” effect.\textsuperscript{28} This effect and its possible role in affecting nanostar spectra will be discussed in detail in Chapter 6. I checked for the effect of sharpness on spectra by plotting the radius of curvature of the tips (as determined by SEM) vs. the wavelength of the resonances and vs. the fwhm of the resonances. This did not reveal any correlation between the sharpness and resonant wavelength or between sharpness and linewidth.

5.3 Polarized Single Nanostar Spectroscopy

We also checked to see if each spectral peak would correspond to a tip on the nanostar. This was tested by collecting polarized scattering spectra from single nanostars characterized by electron microscopy. If each resonance is a longitudinal resonance along the nanostar tip, then each resonance will be polarized in a different direction. To collect polarization data an analyzer was inserted into the microscope between the light source and the sample. Polarization spectra were collected with dark field illumination. In dark-field polarization light is incident at a high angle and there will always be a component of the light perpendicular to the substrate. In this case the
component of light perpendicular to the substrate has little effect on the spectra because this component does not significantly excite any resonances which would radiate toward the objective and be collected. As the analyzer rotates, the intensity of different resonances will grow and diminish, as illustrated in Figure 5.3. Analysis of these resonances was carried out in MATLAB. The resonances were fit with a sum of two Lorentzians, which yielded the center wavelength of each resonance. These values were tracked for each polarization angle and the intensity of each resonance was plotted against the polarization angle. The result was the polar plot shown in panel B of Figure 5.3. Careful examination of SEM pictures and optical alignment marks enabled me to match the orientation of the polarizer with the orientation of the nanostars. This clearly revealed a correlation between tip orientation and plasmon resonances.

The peak heights were found to follow \( \cos \Theta \) dependences with analyzer angle (Figure 5.3 and 5.4), demonstrating that the scattered light is linearly polarized at a distinct angle for each peak and is in good agreement with the polarization dependence of
the longitudinal resonance of gold nanorods. The polarization dependent spectra for four more nanostars, along with high-resolution structure are presented in Figure 5.4, revealing similar multispectral polarized light scattering. Lines have been added to the SEM images that represent the polarization angle in order to assign spectral peaks to

**Figure 5.4 (A)-(D)** High-resolution structure and polarization-dependent scattering spectra for four gold nanostars. The radial axis is the scattered intensity (arbitrary units). The scale bars are 100 nm. The data in (C) were only recorded over 180° and are simply repeated to complete the plot.
specific tips. All tips for each nanostar are not represented in the spectrum due to several factors. First, if multiple peaks are at a similar wavelength, their angular separation is difficult to resolve due to significant spectral overlap. Secondly, some peaks may be beyond the range of the detector, or beyond the effective range of our polarizer. Third, some of these tips are pointing slightly out of the plane of the substrate, which greatly diminishes the ability of the single particle spectrometer to effectively capture their scattered radiation.

The results of the polarization analysis of nanostar scattering spectra are shown in figure 5.4 which shows polarization plots for four different nanostars. The white lines on the SEM images show which tips of the star the resonances were assigned to. It was found that the angle of the resonances often aligned more closely with the *edge* of a tip, rather than a *bisector* to the tip. This could be due to a slight angular experimental offset, perhaps from the SEM. For the nanostar shown in panel D, the scattering spectrum was so complex (with at least three resonances) that it was not possible to clearly resolve all the resonances into dipoles. The 900 nm peak plotted in panel D of figure 5.4 was at the edge of the polarizer's effectiveness, and so it could not be resolved into a dipole. Despite these issues, the polarization data clearly suggest that the spectral peaks can be assigned to nanostar tips. Further evidence of the correlation between nanostar structure and nanostar plasmon resonances was provided by FDTD analysis and plasmon hybridization. Both methods are described in detail in the next chapter.
Chapter 6 Theoretical Description of Nanostars

6.1 Introduction to Theoretical Methods

Further evidence of the correlation between the structure and plasmon resonances of gold nanostars was provided through theoretical simulations in collaboration with Peter Nordlander’s group. A numerical simulation of nanostar optical properties by FDTD, (finite difference time-domain) analysis showed that the plasmons of a nanostar result from hybridization between the plasmons of the nanostar core and the nanostar tips. Before I describe the theoretical results I will provide a brief description and review of FDTD and plasmon hybridization as applied to plasmon resonances in gold nanoparticles. 

Finite-difference, time-domain (FDTD)

FDTD is a brute-force method which solves Maxwell’s equations at multiple points along a spatial and temporal grid. This gives detailed information about the time evolution of electric and magnetic fields. FDTD simulates the electric field intensities along the surface of a nanoparticle in response to an applied sharp electric field pulse containing many frequencies. In this work a nanostar was simulated with FDTD to calculate the near- and far-field properties. A nanostar was modeled as a solid core with protruding prolate tips. The shape of the nanostar model was such that it agreed qualitatively with the shape of the nanostar determined by SEM, as shown in panels A

![Figure 6.1 Structure of the gold nanostar investigated in this chapter. Panel (a) shows an experimental scanning electron micrograph. The scale bar is 100 nm. Panel (b) shows the theoretical model, consisting of a truncated spherical core and tips, consisting of truncated prolate spheroids.](image-url)
and B of Figure 6.1, and as discussed later. FDTD also produced results which are well-understood through plasmon hybridization, which is reviewed next.

**Plasmon Hybridization**

We can understand the optical spectra of nanostars through plasmon hybridization. Plasmon hybridization is essentially an electromagnetic version of molecular orbital theory.\(^{53}\) By understanding the elementary plasmon modes of simple nanostructures, one can look at their interactions (also known as their “hybridization”) to understand the plasmon modes of complex nanostructures. This concept is illustrated for nanoshells in Figure 6.2. Plasmon hybridization was initially applied to a nanoshell by looking at the interaction of the plasmon modes of a nanosphere and a cavity and considering the hybridization of their simple, fundamental plasmons, as in Figure 6.2. Plasmon hybridization offers

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**Figure 6.2.** An energy level diagram depicting the plasmon hybridization in metal nanoshells resulting from the interaction between the sphere and cavity plasmons. The two nanoshell plasmons are an antisymmetrically coupled (antibonding) \(\omega_s\) plasmon mode and a symmetrically coupled (bonding) \(\omega_c\) plasmon mode. Figure and caption reproduced from E. Prodan, C. Radel, N. J. Halas, P. Nordlander “Hybridization Model for the Plasmon Response of Complex Nanostructures,” Science 302, 419 (2003).
an intuitive way to think about plasmon modes of complex nanostructures, and it accurately predicts and explains the spectra of nanoshells. Plasmon hybridization has also been successfully applied to nanoshells, multilayer nanoshells, nanorice and—most recently—nanostars. \textsuperscript{53, 54, 64}

6.2 \textit{Theoretical}

\textbf{Polarized Single Star Spectra}

It was found that the extinction spectra calculated by FDTD for the model nanostar agreed quite well with the measured scattering spectra for multiple polarization angles. For ease of analysis, calculated extinction spectra,

\textbf{Figure 6.3} Experimental scattering (a) and theoretical extinction (b) spectra of the nanostar shown in Figure 5.3 for different polarizations of the incident light. The polarization angle $\theta$ varies from 0° 30° 60° to 90° and is indicated by the arrows in the panels. The bottom panels show the polarization dependence of the intensity for the two low-energy peaks indicated by the red and the blue arrows in panels (a) and (b).
rather than scattering, were used for comparison. The extinction spectra for nanostars are
dominated by scattering, and so comparing extinction and scattering spectra is still quite
informative. By comparing the experimental and simulated spectra for a variety of
polarization angles (Figure 6.3, top panels), we discovered excellent agreement between
the experimental and theoretical spectra. We found that the simulated extinction spectra
exhibited the same polarization-dependent changes in the intensity of the plasmon
resonances, and that changes in the polarization angle caused very similar changes in the
relative intensities of the resonances. The major features of the experimental spectra are
two distinct peaks near 800 nm and 720 nm with weaker features (possibly resonances)
visible at 650, 600, and near 500 nm. The spectra from FDTD feature two distinct
resonances at 804 nm and 717 nm, with a weak feature at 600 nm and many weak
features near 500 nm. The theoretical model for the nanostar was developed with
knowledge of the experimental spectra, and so the nanostar shape was optimized to have
resonances at 800 nm and 720 nm.

There is excellent agreement between the experimental polar plots of peak
intensity vs. angle and the theoretically generated polar plots, as shown in the bottom
portion of Figure 6.3. In the lower panels of Figure 6.3, two polar plots display the
intensity of the 717 nm and the 804 nm peaks plotted as a function of polarization angle.
We find good agreement between the theoretical and experimental data. Due to the
asymmetric nanostar tip orientation, many different plasmons could be observed for
different polarizations. The probability of a plasmon mode being excited is proportional
to the square of the mode's dipole moment along the direction of the incident electric
field.
Interestingly enough, there is a slight offset between the theoretical and experimental polarization spectra. It is possible that this offset was an experimental artifact from the SEM. When the SEM used for this study (JEOL 6500) zooms in to high magnifications, there is an occasional slight (few degrees) rotation of the filed view of view. This rotation effect helps explain why comparison of experimental polar plots with nanostar SEM had the dipole resonances more closely aligned with the edges of the nanostar tips rather than with the bisectors to the nanostar tips, as would be found by comparing SEM of the nanostar to the theoretical polarized spectra.

6.3 Calculated Electric Field Enhancements from FDTD

Figure 6.4 shows the calculated electric field enhancements for nanostar spectra collected at three different nanostar polarizations. These FDTD simulations offered strong evidence that the plasmon resonances are spatially localized to different regions of the nanostar. Three polarizations are shown, 0°, 60°, and 90°. The top panel for 0° has an extinction spectrum with a primary resonance at 804 nm, and so the top panel shows the electric field enhancements (surface charges) plotted for a wavelength of 804 nm. Note that the large electric field enhancement is localized to the long horizontal tip. The 804 nm resonance is the longest wavelength plasmon resonance, and it occurs on the longest 'spire' of the nanostar. This makes sense qualitatively — for nanorods it has been shown that as the aspect ratio (the ratio of nanorod length to diameter) increases, the plasmons shift to longer wavelengths,24 and a similar phenomenon appears to be relevant for nanostars. The field enhancements on the downward and upward pointing tips are very small, and so we find that at 804 nm only very small surface charges are induced.
The middle panel of Figure 6.4 shows the electric field enhancements for a polarization angle of $60^\circ$. The spectra at $60^\circ$ are dominated by the plasmon at 717 nm, and so we plot the field enhancements for a wavelength of 717 nm. This plot shows that the largest field enhancements are localized on the bottom tip with slight contributions from the upper tips. This means that at this polarization angle both the 804 nm and the 717 nm resonances may be excited. The field enhancements at 804 nm (not plotted here) with a polarization of $60^\circ$ look similar to those shown in panel B. The $0^\circ$ and

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure6.4}
\caption{Color contour plots of the electric field enhancements for excitation of the (a) 804 nm, (b) 717 nm, and (c) 594 nm nanostar plasmon resonances. The polarization angles are indicated by the insets to the right of the panels. The maximum field enhancements are indicated on top of each panel.}
\end{figure}
60° scattering spectra in Figure 6.3 show that both resonances are excited at both polarization angles.

For the plot in the lower panel, at 90° polarization, the field enhancements are localized on the tip at the top and the tip in the upper-left quadrant of the star. Extinction spectra showed two resonances—one at 717 nm and a weak resonance at 594 nm. In the lower panel we plot the field enhancements at 594 nm. The resonances at 717 nm (not plotted here) would have showed a plot which looked very similar to that seen in the bottom panel of Figure 6.4.

The FDTD results presented clearly show that the nanostar structure (tips) strongly affect the nanostar spectra. This has been also verified by examining a model of a nanostar with a single tip. Calculated spectra from nanostars with single tips only feature the plasmon resonance associated with the single. We now consider the nanostar spectra within the framework of plasmon hybridization to gain physical insight into the origin of the plasmon resonances of nanostars.

6.4 Plasmon Hybridization in Nanostars

The complex structures of nanostars make them an interesting system for plasmon hybridization. Figure 6.5 shows the calculated extinction spectra for the nanostar core (left), the nanostar tips only (right), and for the entire nanostar (center). The spectra are plotted as a function of energy. Spectra at three polarizations are plotted here, as indicated by the arrow on the graphs at the right. The inset boxes on all the graphs show the FDTD calculated electric field enhancements for the nanostars at specific wavelengths. The panels on the left which show the extinction spectra for the nanostar
core have plasmon resonances at 2.5 and 2.6 eV, with only a very minor polarization dependence due to the slight lack of core symmetry. The resonance at 2.5 eV is dominant at 90°, indicating that it is polarized parallel to the cut of the sphere. The resonance at 2.6 eV is dominant at 0°, indicating that it is polarized perpendicular to the cut on the sphere.

On the right the panels show the calculated extinction spectra for the nanostar tips. These spectra feature the strongest

Figure 6.5 Microscopic origin of the three lowest energy nanostar plasmons. The left panels show the extinction spectra of the individual core. The right panels show the spectra of the tips. The middle panels show the extinction spectra of the interacting system. The dotted lines indicate interactions that result in the bonding and antibonding nanostar plasmons. The contour plots show the electric field enhancements for resonant excitation of the different plasmon modes at the different angles. To make the extinction spectra more visible, the spectra for the core and tips have been multiplied by factors of 2 and 4 respectively. The same color scale bar is used in all the insets.
dependence on the polarization of the incident light.

Figure 6.5 also shows that there is a weak interaction between the plasmons of the nanostar tips. This is visible in the nanostar tip spectra as bonding and antibonding plasmon modes. For the purposes of this section, we will only consider the lower energy bonding tip modes which are localized on the tips. At 0° the main feature of the spectrum is a resonance near 1.9 eV. This resonance is a plasmon due to the long (horizontal) tip, as we see in the inset. At 60° we find two resonances—the 1.9 eV resonance is still excited but is weaker—as well as another resonance at 2.1 eV. As discussed earlier, the 2.1 eV resonance is localized on the bottom tip. At 90° the 2.1 eV resonance is still visible as well as a weaker feature at 2.5 eV which is due to the top two tips.

Now that we understand the plasmon resonances present in the core and the tips we may consider their hybridization in the nanostar. Theoretical spectra for the complete nanostar at three polarizations are shown in the middle panels. Dotted black lines indicate the hybridization which results in the different modes of the nanostar. At 0° the core resonance hybridizes to form a bonding nanostar plasmon at 1.5 eV and an antibonding plasmon at 2.5 eV. The red-shift of the bonding mode is much, much larger than the blue shift of the antibonding mode because the core has more conduction electrons than the tip. At 0° the 1.7 eV bonding plasmon is formed by hybridization of the longitudinal core plasmon and the upper tip plasmons. For this polarization the 1.5 eV bonding plasmon associated with the long tip is also excited.
6.5 Discussion of Theoretical Results

The way the plasmon resonances of this nanostar result from hybridization of plasmons associated with the core and the plasmons associated with the tips is illustrated in Figure 6.6, panels A, B, and C. The lower energy bonding nanostar plasmons are dominated by tip plasmons, but also mix with the core plasmon modes. The nanostar tip plasmons are at lower energy (longer wavelength) due to the length and sharpness of the nanostar tips. The nanostar core plasmons are at a higher energy because they are closer to the resonance of an Au nanosphere, which is resonant at roughly 520 nm. Mixing of the tip modes with the core plasmon mode increases the excitation cross section and results in very large local electric field enhancements at the ends of the nanostar tips. These enhancements are theoretically demonstrated to be much, much larger than the electric field enhancements provided by the tips alone.

This theoretical work illustrates how the hybridization of core and tip plasmons substantially increases the excitation cross section. The extinction cross sections for individual nanostar tip plasmons are roughly four times smaller than for the bonding nanostar plasmons. Physically, we can understand this phenomenon as a mixing of the core plasmons into the nanostar tip modes. Because the plasmon resonances associated
with the nanostar core have much larger frequencies than the tip plasmons the conduction electrons of the core can follow the lower-frequency tip plasmon oscillations. Through this coupling the effective dipole moment of the tip plasmons is increased. As an analogy we can think of the core as an antenna, which couples more energy into the plasmons on the nanostar tips. This antenna-like coupling increases the excitation cross sections and the electric field enhancements of the bonding nanostar plasmon modes.

The dependence of nanoparticle extinction, absorption, and scattering cross sections on nanoparticle shape are of tremendous interest. Nanostars are extremely interesting nanoparticles for three reasons. First, they have very large scattering cross sections for their size. Secondly, they have extremely narrow scattering resonances for nanoparticles which scatter so brightly. Third, nanostars have very large LSPR shifts in response to changes in the local dielectric medium. The third attribute (large LSPR shifts) is a topic discussed extensively in the following chapter. For now, this discussion is limited to the extraordinarily bright and narrow plasmon resonances of nanostars. To understand why it is so uncommon to find these two attributes together, we must first review radiative damping in plasmonic nanoparticles.

In Chapter 1 we discussed what happens to the plasmons which are excited by light incident on a metal nanoparticle. To review, the plasmons in a nanoparticle may decay in a radiative or nonradiative manner. Nonradiative decay results in interband or intraband excitations of electron-hole pairs in the nanoparticle, which produce heat and contribute to the absorption cross section. The second mechanism for plasmon decay is radiative decay which is photon emission at the incident wavelength (elastic scattering) with the dominant contribution at the resonance energy. From this it is apparent that the
scattering cross section for a nanoparticle is directly related to the radiative decay rate. However, because radiative decay is a loss of energy and therefore a form of damping on the system, the plasmon resonance in metal nanoparticles will broaden—just like the resonance of any damped harmonic oscillator. The damping from radiative decay explains why nanoparticles that exhibit strong scattering have resonances with inherently broad linewidths.

The bright scattering and sharp resonances of nanostars point toward a major frontier for engineered nanoparticles. For imaging applications we want nanoparticles which are relatively small, scatter brightly, and have narrow linewidths. For LSPR sensing applications we also want the nanoparticles to have a strong LSPR shift in response to changes in the local index of refraction. Unfortunately, to scatter strongly particles usually must be large. Nanoparticles which scatter light strongly also must, by definition, have a relatively large amount of radiative damping. Since radiative damping damps the plasmon resonance this seems to indicate that nanoparticles which scatter strongly must have broad plasmon resonances. This is why nanostars are so unique: nanostars have very narrow linewidths and large scattering cross sections.

The physical reason for these special properties is the special antenna-like properties conferred by the nanostar core. Coupling of incident light to the core of the nanostar sets up a resonance which effectively ‘drives’ the resonances of the tip plasmons. The plasmon resonances are constantly undergoing radiative decay, but they are also being ‘driven’ by the larger-frequency core plasmons. Thus nanostars manage an incredibly rare feat for a nanoparticle: they have narrow resonances which scatter brightly.
Chapter 7: Biosensing with Single Gold Nanostars

7.1 Plasmon Resonant Nanoparticles for Biosensing

As we know from the previous chapters, the electrons in noble metal nanoparticles exhibit a collective oscillation which is known as the localized surface plasmon resonance. The plasmon resonances (LSPR) of noble metal nanoparticles are highly sensitive to the local dielectric environment. Small changes in the index of refraction of the surrounding medium result in measurable changes in the resonant wavelength of the extinction or scattering spectra of these particles. Thus by monitoring the LSPR spectra of noble metal nanoparticles, one can monitor the local dielectric environment. This is the basis of LSPR sensing.\(^\text{65}\)

To understand how LSPR can be used for biosensing we will first review how other biosensors work. Typically, a biosensor will consist of an element which is sensitive to the biological molecules of interest, a transducer which converts the binding event into an optical or electronic signal, and a detector element. For LSPR biosensing with plasmonic nanoparticles, the element which is sensitive to the biological environment will be the bare or biofunctionalized metal of a plasmonic nanoparticle. The transducer element is the nanoparticle. Changes in the local dielectric environment-- like those caused by a molecule binding to the nanoparticle surface-- result in a change in the scattering or extinction spectra of the particle. Finally, the detector element will be a spectrometer which monitors the resonant wavelength of the plasmonic nanoparticle. While many proteins and other molecules will strongly bind to bare gold, specificity is desired for nanoparticle-based LSPR sensors. This specificity only exists if the binding is
made specific through surface functionalization with antibodies or other specific binding agents. LSPR sensors have already been applied to the detection of biomarkers in clinical samples relevant to the diagnosis and understanding of Alzheimer’s disease.\(^{66}\) This experiment was carried out with a pattern of nanometer-scale triangles of silver which are created by a process called nanosphere lithography. Currently other particles, including nanorods, nanoshells, and nanocubes, are being studied for this purpose. LSPR biosensors have the potential to be a powerful, inexpensive, label-free medical diagnostic technology, and so are of tremendous current interest.\(^{67}\)

7.2 \textit{Localized Surface Plasmon Resonance and the Surface Plasmon Resonance}

LSPR sensors are similar in principle to surface plasmon resonance (SPR) sensors, a well developed technology which has found wide application in biochemical laboratories.\(^{68}\) However, the terminology used to describe these technologies can be somewhat confusing because many similar terms have different meanings. Nanoparticles are described as possessing a \textit{localized} surface plasmon resonance because the plasmon resonance of a nanoparticle is localized to the nanoparticle. For SPR, the plasmon is only localized to the surface of the metal film and is not confined to any particular location on the surface. SPR sensors monitor the effect of molecular binding on surface plasmon resonances which are delocalized in a thin gold film. SPR sensors are much more sensitive to the dielectric environment, but they have a large sensing volume which includes the entire evanescent field over the 2D gold film. LSPR sensors are inherently less sensitive to the dielectric environment, but their active area is localized to a region near the nanoparticle surface, and so they may be used to probe smaller volumes.
Additionally, LSPR sensors may offer a significant advantage over SPR when limited quantities of analyte are available. Both LSPR and SPR ultimately provide a very similar signal upon binding of an analyte molecule, but the high dielectric sensitivity of SPR makes it more vulnerable to temperature effects and thus the usefulness of SPR sensing is inherently limited.\textsuperscript{68} LSPR sensors are robust to temperature changes and are capable of returning highly specific, label free analyte detection in a very small region.

7.3 The Role of Nanoparticle Shape in LSPR Sensing

The sensitivity of LSPR sensors is, in fact, even more localized than just to the of the nanoparticle location. Shifts in the plasmon resonance are mainly influenced by the area on the nanoparticle which has the highest electric field enhancement. The degree of LSPR sensitivity localization can be readily controlled through changing the shape and optical properties of the nanoparticles. A recent investigation into the LSPR sensitivity of single triangular silver nanoprisms found a clear correlation between tip sharpness and LSPR shift.\textsuperscript{28} The investigation with single silver nanoprisms was an indirect investigation—sharpness was not microscopically measured but was determined from the resonant linewidth of the nanoparticles, but the results were still very compelling. Sharper nanoprisms were found to have a larger shift upon a change in the local index of refraction than nanoprisms with duller tips.

Nanoparticles with sharper features experience larger LSPR shifts because the electric near-field is largest near sharp tips. This is the familiar “lightning rod effect.” This relationship between LSPR sensitivity and particle sharpness has also been demonstrated systematically for substrates with silver triangles fabricated by nanosphere
lithography.\textsuperscript{63} The silver nanotriangles were annealed to dull the tips and it was found that the sharpness of the nanotriangles strongly affected the LSPR sensitivity, with sharper tips giving far larger LSPR shifts than dull tips.\textsuperscript{63} In fact, DDA calculations showed that less than 10% of the surface area of the triangles contributed to the majority of the LSPR peak shift.\textsuperscript{63}

7.4 The Figure of Merit (FOM)

To compare the sensitivity of nanoparticles we need to quantify their LSPR shifts. The sensitivity of a nanoparticle’s LSPR shifts is quantified with several numerical parameters. The peak wavelength shift per refractive index unit, often reported as nm/RIU, ranges from 90 to 800 nm/RIU for different nanoparticles.\textsuperscript{31, 34, 45, 54, 64, 65, 69-72} This range is somewhat exaggerated because some of the nanoparticles have very low energy resonances. If one considers the shift in photon energy, eV/RIU, the range of sensitivities is reduced by a factor of two: 330 to 1410 meV/RIU. Another consideration is the linewidth of the resonance, which will affect the minimum wavelength shift that can be resolved. A unitless figure of merit (FOM) has been proposed in which the sensitivity is divided by the linewidth.\textsuperscript{31} The sensitivity is measured as the resonance shift in electron volts (eV) per change in the local environment as measured in refractive index units (RIU) divided by the full width at half max.

\begin{equation}
\text{FOM} = \frac{(eV \cdot RIU^{-1})}{\text{fwhm(eV)}}
\end{equation}
7.5 Review of Reported Figures of Merit

Analysis of recent literature reports put this figure of merit from approximately 1 to 5. These LSPR shifts, usually reported in eV/RIU (shift in photon energy per change in refractive index unit) help quantify their applicability as biological and chemical LSPR sensors, and are given in Table 7.1. LSPR shifts have been determined for gold nanospheres, silver triangles fabricated by nanosphere lithography, single silver nanoparticles, and gold nanoshells with values ranging from 500 to 800 meV/RIU.

<table>
<thead>
<tr>
<th>Ref</th>
<th>Particle</th>
<th>Single/Ensemble</th>
<th>$\lambda_{\text{peak}}$ nm</th>
<th>$\Delta \lambda$ nm</th>
<th>shift/RIU nm</th>
<th>FOM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tam 2004</td>
<td>Au/SiO$_2$ shell</td>
<td>Ensemble</td>
<td>770</td>
<td>1.61</td>
<td>350</td>
<td>732</td>
</tr>
<tr>
<td>Sun 2002</td>
<td>Au/AuS shell</td>
<td>Ensemble</td>
<td>700</td>
<td>1.77</td>
<td>400</td>
<td>1012</td>
</tr>
<tr>
<td>Wang 2006</td>
<td>Au nanorice</td>
<td>Ensemble</td>
<td>1600</td>
<td>0.775</td>
<td>600</td>
<td>291</td>
</tr>
<tr>
<td>Underwood 1994</td>
<td>Au sphere</td>
<td>Ensemble</td>
<td>530</td>
<td>2.34</td>
<td>060</td>
<td>265</td>
</tr>
<tr>
<td>Raschke 2004</td>
<td>Au/AuS shell</td>
<td>Single</td>
<td>660</td>
<td>1.88</td>
<td>077</td>
<td>220</td>
</tr>
<tr>
<td>Sherry 2005</td>
<td>Ag cube</td>
<td>Single</td>
<td>510</td>
<td>2.43</td>
<td>091</td>
<td>433</td>
</tr>
<tr>
<td>Malinsky 2001</td>
<td>Ag NSL</td>
<td>Ensemble</td>
<td>564</td>
<td>2.20</td>
<td>104</td>
<td>405</td>
</tr>
<tr>
<td>Nehl 2006</td>
<td>Au star</td>
<td>Single</td>
<td>675</td>
<td>1.84</td>
<td>125</td>
<td>340</td>
</tr>
<tr>
<td>Mock 2003</td>
<td>Ag sphere</td>
<td>Single</td>
<td>520</td>
<td>2.38</td>
<td>073</td>
<td>335</td>
</tr>
<tr>
<td>Sherry 2006</td>
<td>Ag Nanoprism</td>
<td>Single</td>
<td>630</td>
<td>1.97</td>
<td>080</td>
<td>246</td>
</tr>
<tr>
<td>Sherry 2006</td>
<td>Ag Nanoprism</td>
<td>Single</td>
<td>635</td>
<td>1.95</td>
<td>063</td>
<td>195</td>
</tr>
<tr>
<td>Sherry 2006</td>
<td>Ag Nanoprism</td>
<td>Single</td>
<td>631</td>
<td>1.97</td>
<td>053</td>
<td>166</td>
</tr>
<tr>
<td>MacFarland 2003</td>
<td>Ag particle</td>
<td>Single</td>
<td>585</td>
<td>2.12</td>
<td>49</td>
<td>178</td>
</tr>
<tr>
<td>Mock 2003</td>
<td>Ag triangle</td>
<td>Single</td>
<td>760</td>
<td>1.63</td>
<td>080</td>
<td>172</td>
</tr>
<tr>
<td>Sherry 2005</td>
<td>Ag cube-sub</td>
<td>Single</td>
<td>430</td>
<td>2.88</td>
<td>022</td>
<td>146</td>
</tr>
<tr>
<td>Nehl 2006</td>
<td>Au star</td>
<td>Single</td>
<td>770</td>
<td>1.61</td>
<td>124</td>
<td>260</td>
</tr>
<tr>
<td>Sherry 2006</td>
<td>Ag Nanoprism</td>
<td>Single</td>
<td>635</td>
<td>1.95</td>
<td>063</td>
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<tr>
<td>Sherry 2006</td>
<td>Ag Nanoprism</td>
<td>Single</td>
<td>631</td>
<td>1.97</td>
<td>053</td>
<td>166</td>
</tr>
</tbody>
</table>

Table 7.1. LSPR properties reported for several gold and silver nanostructures. To compare the figures of merit, results are only shown for reports which provide either the resonance linewidth or LSPR spectrum.
Each nanoparticle investigated for LSPR sensing brings specific assets and potential limitations to sensor applications. Silver nanotriangles fabricated by nanosphere lithography have been highly successful, but may not be amenable to large scale, large area production. Gold nanorods have tunable resonances with a narrow linewidth,\textsuperscript{10} but the resonances observed for nanorod films are significantly broadened due to heterogeneity.\textsuperscript{23} Nanoshells may be reliably and reproducibly fabricated and may be tuned over a large range by changing the core radius and shell thickness.\textsuperscript{17} Their geometry leads to very high nm/RIU sensitivity,\textsuperscript{71} but their relatively large size also results in large resonant linewidths.

As indicated in the table, LSPR sensing has also been demonstrated for single nanoparticles. Single nanoparticles are especially interesting LSPR sensors because they are free from the problems of broadening due to sample heterogeneity. Additionally, single nanoparticles are tiny sensors which would only require very small quantities of analyte and could be easily incorporated into multiplexed biosensors.

Previous chapters described the synthesis and optical properties of gold nanostars. Their scattering exhibits multiple spectral peaks which can be assigned to the nanostar tips.\textsuperscript{54} Here we employ LSPR analysis on single gold nanostars to demonstrate that they exhibit a behavior consistent with ensemble LSPR and SPR sensors. The following sections of this chapter describe the characterization of the LSPR shift and the figure of merit for nanostars, as well as initial biodetection data and a roadmap for future biosensing work in the Hafner lab.
7.6 LSPR Sensitivity of Gold Nanostars

We have investigated the sensitivity of the gold nanostar resonances to the dielectric environment by measuring their scattering spectra in water (n=1.33), sucrose (n=1.38), and immersion oil (n=1.515). In this investigation, gold nanostars were first deposited on a glass slide. A drop of water (10 μL) was then placed on the sample, followed by a coverslip. The sample was then loaded into the microscope and spectra were collected as described in Chapter 2. Spectra were first collected in water. Following collection of spectra in water, the sample was unloaded from the microscope. The coverslip was gently removed, taking care not to disturb the region of interest on the sample. The sample was then gently dried with compressed air. Next, a drop of immersion oil (n=1.515, a 10 μL drop was used) was placed on the sample. A cover slip was added and spectra were collected as described in Chapter 2.

Figure 7.1 Single gold nanostar scattering spectra in water (left peak) and oil (right peak). The gray curve displays the Lorentzian curve fit. The nanostar in (A) has an LSPR shift of 649 meV/RIU and in (B) 1410 meV/RIU.
The results from this investigation are shown in Figure 7.1. Scattering spectra from single nanostars collected in immersion oil and in water are shown, as well as the LSPR shifts for two nanostars when the medium was changed from water to oil. The Lorentzian fits in Figure 7.1 indicate dielectric sensitivities of 649 meV/RIU and 1410 meV/RIU for the individual gold nanostars. As mentioned before, the spectral line width must be considered when evaluating nanoparticles for LSPR sensing since it will affect the minimum detectable shift.

These large LSPR shifts for nanostars are encouraging, but LSPR shift measurements on gold nanostars may be complicated by their three-dimensional structure and multiple scattering peaks, and so rotation of the nanoparticle on the surface is an important factor we must consider. If the nanostar were to rotate upon wetting or drying due to meniscus forces, the observed shift could be due to the alignment of a different tip with the microscope objective, rather than a true LSPR shift due to the altered dielectric environment. We checked for any rotation effect for the nanostars shown in Figure 7.1. Neither of these nanostars translated in the field of view, strongly suggesting no rotation. Evidence for a lack of rotation is also found in the consistent spectral shape of the LSPR in both media.

7.7 Orientation-dependent Spectra from Single Nanostars

While we are confident that the nanostars shown in Figure 7.1 did not rotate, we have also attempted to quantify how dramatically nanostar rotating could affect the LSPR spectra. This was done by confirming the LSPR shift and monitoring the position of the nanoparticle upon several changes of solution. Figure 7.2 shows LSPR spectra and
images for four nanostars in a field in water, then sucrose, then water, then sucrose. The substrate was dried between each measurement, coverslips were removed, and samples were remounted as described for the water/oil shifts. The three nanostars that show no translation (C, E, H) have highly reproducible spectra. However, the nanostar that does translate, and therefore likely rotates, has nonreproducible spectra with no consistent shift. We believe that the change in scattering spectra for the nanostar which translated was due to a change in the nanostar orientation on the substrate.

By now it should be clear that nanostars have scattering spectra which depend very strongly on their orientation. This may enable nanostars to be used as highly sophisticated probes in particle tracking experiments in cells or intracellularly. Additionally, nanostars could be used in bioimaging experiments. Other shapes of gold

![Figure 7.2 Gold nanostar dielectric shifts in water and sucrose. The top panels show dark field microscopy images of a field of nanostars which were taken from water, to sucrose, to water, and back again to sucrose. The bottom panel shows spectra of each particle in all four experiments. Note that the spectra of the three nanostars that do not translate are reproducible (C, E, and H), while the spectra of one that does translate, and presumably rotates (star D), is not reproducible.](image_url)
nanoparticles have been used as labels in a variety of systems, the advantage being that they are very bright and do not photobleach or blink like fluorophores and quantum dots. An early example of bioimaging with nanoparticles is the work of Yguerabide in 1998. Epithelial buccal cells were labeled and imaged with 94 nm gold particles. Additionally, by labeling colloidal gold with antibodies, it was demonstrated that the gold colloid can selectively bind to CD4 lymphocytes in blood.\textsuperscript{75} More specialized work by Sokolov et al.\textsuperscript{76} conjugated gold colloids to antibodies for EGFR (epidermal growth factor receptor) for use as scattering contrast agents. Beyond simple gold spheres, nanoparticles with engineered structures have been employed for bioimaging. Nanoshells, which consist of a silica core surrounded by a shell of gold or silver, have been used as imaging contrast agents for dark field microscopy and optical coherence tomography.\textsuperscript{77-79}

Due to the large size of noble metal nanoparticles, biomedical applications have largely focused on particle tracking rather than simple structural labeling. An example of this used two-photon photoluminescence from gold nanorods for \textit{in vivo} tracking of blood flow in the ear of a mouse.\textsuperscript{80} Magnetic resonance imaging (MRI) has also benefited from nanoparticle contrast agents. Recently, iron oxide nanoparticles were conjugated to the cancer-targeting antibody Hercpetin. It was shown that these nanoparticles enabled \textit{in vivo} MRI imaging of cancers as small as 50 mg.\textsuperscript{81} It is entirely possible that nanostars could contribute greatly to this area in the future because their scattering spectra depends strongly on the orientation of the nanostar. This would provide the same tracking information possible from plasmonic nanoparticles, but it would offer an additional level of information by telling us the orientation of the nanostar being tracked.
7.8 Biosensing with Single Gold Nanostars

Due to their high figure of merit we are using gold nanostars to explore the limits of LSPR sensing. As a first step, we exposed nanostars to mercaptohexadecanoic acid (MHA), an alkanethiol which forms self assembled monolayers on gold, and monitored the LSPR peak resonances of two tips as a function of time. Recall from the work presented in chapters 5 and 6 that each plasmon resonances of a nanostar corresponds to an individual nanostar tip, so we may say that each resonance is due to an individual tip. Functionalization with MHA has also been used to demonstrate LSPR sensing with single Au$_2$S/Au nanoshells, and so MHA is a good test case for nanostars. As seen in Figure 7.3, the peaks red-shifted by 16 and 6 meV due to the formation of the self-assembled monolayer. The shift for MHA coating a single Au$_2$S/Au nanoshell has been previously reported to be 19 meV for the single resonance of the Au$_2$S/Au nanoshell.

We next observed the binding of bovine serum albumin (BSA) to the nanostars. BSA is well-characterized and is known to strongly bind to gold surfaces. BSA at 1.5 µM was introduced into the flow cell and found to cause redshifts of 14 meV and 13 meV for two resonant peaks on the gold nanostar. The spectral shifts for the MUA and BSA were converted to wavelength shifts and plotted in Figures 7.3 (MHA) and 7.4 (BSA). The curves were fit to a simple bimolecular interaction model (equation 7.2) used to analyze SPR data.

\[ R = R_0 \left( 1 - e^{-k_{off}} \right) \]  

(7.2)
Where \( R \) corresponds to the spectral response (shift in peak wavelength), \( R_{eq} \) is the equilibrium response, and \( k_{obs} \) is the observed rate constant. For both MHA and BSA, the \( R_{eq} \) values were different for each tip on the nanostar, which is reasonable since different tips will have different structures, and therefore they may have different nm/RIU sensitivities. The LSPR sensitivity depends on the structure of the nanoparticle, as mentioned in the introduction to this chapter. However, in each case the observed rate constants for the two nanostar tips were very similar, which is expected since the nanostar tips were exposed to the same concentration of reactant. Indeed, these values for \( k_{obs} \) are similar to those found in SPR experiments at similar concentrations.\(^{83}\)

Figure 7.3 A single gold nanostar is functionalized with MHA. (A) The scattering spectra for this nanostar—note the two resonance peaks. The middle and bottom figures plot the peak wavelength for each resonance vs. time. (B) The high energy peak and (C) the low energy peak both redshift upon analyte binding.
Figure 7.4 Peak shift in nanometers for the same star peaks shown in Figure 4. Data is here plotted as shift (nm) vs. time. We fit this data with a one phase exponential association equation: \( R = R_{\text{max}} (1 - \exp(-k_{\text{obs}}t)) \). The fits are the solid lines plotted with the data shown above.

The equilibrium value \( R_{eq} \) is due to the formation of a self-assembled monolayer of MHA or a layer of BSA bound to the gold nanostar surface. In SPR experiments, the contribution to the observed shift from this bound layer must be distinguished from contributions due to solvent changes in the larger sensing volume. Therefore, although BSA has a refractive index of 1.57, about 0.24 RIU greater than that of the solvent, it will cause a shift of only 0.003 RIU in SPR measurements.\(^6\) Given the 3 – 5 nm shifts observed here for BSA (see Figure 6) and a typical gold nanostar sensitivity of 400 nm/RIU, the formation of a BSA layer on the nanostar corresponds to a shift of 0.010 RIU. The larger LSPR index shift reflects the smaller sensing volume of the localized nanoparticle sensors. However, LSPR measurements are clearly still affected by the solvent index since a pure protein shift of 0.24 RIU is not observed. Assuming that the measured LSPR \( R_{eq} \) corresponds to a near complete monolayer of BSA on the nanostar tips, one can estimate the number of molecules which contribute to the shifts in Figure 7.4. From the literature, we know that BSA adsorption onto gold yields a density of
about $2 \times 10^{-7}$ g/cm$^2$.\textsuperscript{68} Approximating the tip as a right circular cone of height 30 nm and base radius 10 nm, we find that the total area available for BSA binding is 1320 nm$^2$. This corresponds to $2.6 \times 10^{-18}$ grams, or about 24 BSA molecules per nanostar tip. As a check, we have overlaid the crystal structure of BSA over a TEM of a nanostar in Figure 7.5. We see that it is entirely reasonable that 24 BSA molecules could pack onto a nanostar tip.

7.9 Toward Single Molecule Detection

Thus far, we have learned that gold nanostars are highly sensitive nanoparticles for LSPR sensing. Their complex structure and spectra allow the assignment of a spectral peak to a single tip on the nanostar, and they exhibit high-sensitivity to their dielectric environment. Measurements of the shift in the peak resonant wavelengths of a single nanostar upon absorption of MHA and BSA are consistent with results from similar studies with SPR sensors. We have found similar observed rate constants for each nanostar tip, and the LSPR shifts seen are consistent with a monolayer coverage of 10’s of molecules at the tip for BSA.

The next step is to attempt to demonstrate the ultimate limit of LSPR biosensing: single molecule detection. We believe that we are already detecting tens of

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{image}
\caption{TEM image of a single gold nanostar. A simulated BSA molecule has been added to this illustration for reference.}
\end{figure}
molecules, so detection of a single molecule would only require improving our method by a factor of ten. Detection of the binding of a single biomolecule would be ideal, but it is difficult for many reasons. First, molecules may adsorb rapidly to nanoparticles, and the resolution of the camera makes detection of a single binding event prohibitively difficult. Additionally, looking for a single binding event could take a very long time because we would have to work at extremely low (nanomolar or lower) concentrations.

![Diagram](image)

**Figure 7.6** Resonance wavelength for the scattering spectra from a single gold nanostar is plotted vs. time. BSA was added at $t=30$ sec, PBS buffer was added at $t=180$. Anti-BSA was added at $t=700$, and PBS buffer was added at $t=1150$. Note the discrete jump (circled) in the resonance wavelength before $t=1400$.

To solve this problem, rather than look at single molecule binding we will look for single molecule unbinding.

The experimental data shown in Figure 7.6 track the resonant wavelength of a nanostar for approximately 30 minutes during several changes of solution. Single nanostars were deposited onto the inside of a flat capillary tube. It was necessary to use capillary tubes because molecular binding experiments require flow of reagents. We began with single nanostars in water. BSA was flowed in ($t=30$ s) at a concentration of 1.5 mg/mL. A redshift was observed upon binding of the BSA to the nanostar. Next, a PBS (phosphate buffered saline) rinse ($t=200$ s) was used to remove any excess, unbound
BSA from the tube. Next, Anti-BSA (1.5 mg/mL) was flowed in (t=700 s) and a further redshift was observed upon the binding of anti-BSA to the BSA. Due to the poorly defined surface chemistry of the nanostar, it is likely that much of the anti-BSA/BSA binding was nonspecific. The nanostar was next rinsed with buffer (t=1100 s) to remove any excess, nonspecifically bound anti-BSA, resulting in the blue shift seen right before 1200 seconds. Now we believe that we are left with a nanostar with only BSA and specifically bound anti-BSA on its surface. The unbinding rate for anti-BSA/BSA is such that we would expect to see unbinding events between hundreds and thousands of seconds. We expect that a single molecule unbinding event would show up as a discrete jump in the resonant wavelength. Approximately 250 seconds after the non-specifically anti-BSA was rinsed away, a discrete jump was seen. We believe that this result is a promising—though not conclusive—indication of a single molecule unbinding event. Proof of single molecule binding will require many such measurements that give an average unbinding time of a few hundred seconds. Unfortunately, the crude surface chemistry applied here prohibits frequently experimental success.

Future experiments will work towards selective and biomedically relevant experiments with antibody-conjugated nanostars and other nanoparticles. Recent work on nanorod substrates demonstrated that use of rational, well-understood chemistry to biofunctionalize nanorods dramatically improved results. Researchers in the Hafner lab have coated glass slides with bare nanorods, and have coated those nanorods with a self-assembled monolayer (SAM.) They next chemically bioconjugated BSA to the SAM by using N-hydroxysuccinimide (NHS) and 1-ethyl-[3-dimethylaminopropyl]carbodiimide (EDC) chemistry. Through this improved chemistry they were able to measure binding
rates for anti-BSA/BSA which were consistent with those reported in the literature. It is anticipated that in the future NHS/EDC bioconjugation chemistry will be applied to single gold nanostars for higher throughput single molecule biosensing. In conclusion, gold nanostars and gold nanoshells have compelling plasmonic properties which should enable advanced biosensing applications in the future.
Bibliography


