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Rod-like plasmonic nanoparticles as optical building blocks: how differences in particle shape and structural geometry influence optical signal

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Rod-like plasmonic nanoparticles as optical building blocks: how differences in particle shape and structural geometry influence optical signal

by

Anthony Shawn Stender

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Major: Analytical Chemistry

Program of Study Committee:
Ning Fang, Major Professor
Pat Thiel
Robert S. Houk
Emily Smith
Clark Coffman

Iowa State University
Ames, Iowa
2013

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<tbody>
<tr>
<td>CPP</td>
<td>Crossed Polarizer Position</td>
</tr>
<tr>
<td>CTAB</td>
<td>Cetyltrimethylammonium Bromide</td>
</tr>
<tr>
<td>DDA</td>
<td>Discrete Dipole Approximation</td>
</tr>
<tr>
<td>DIC</td>
<td>Differential Interference Contrast</td>
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<tr>
<td>FWHM</td>
<td>Full-Width at Half Maximum</td>
</tr>
<tr>
<td>LCD</td>
<td>Liquid Crystal Device</td>
</tr>
<tr>
<td>LISNA</td>
<td>Laser Induced Scattering around a NanoAbsorber</td>
</tr>
<tr>
<td>LSPR</td>
<td>Localized Surface Plasmon Resonance</td>
</tr>
<tr>
<td>NA</td>
<td>Numerical Aperture</td>
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<tr>
<td>PAM</td>
<td>Photoacoustic Microscopy</td>
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<td>PAT</td>
<td>Photoacoustic Tomography</td>
</tr>
<tr>
<td>PEG</td>
<td>Poly(ethylene) Glycol</td>
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<td>PHI</td>
<td>Photothermal Heterodyne Imaging</td>
</tr>
<tr>
<td>POPAM</td>
<td>Pure Optical Photoacoustic Microscopy</td>
</tr>
<tr>
<td>QD</td>
<td>Quantum Dot(s)</td>
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<tr>
<td>QWRP</td>
<td>Quarter Wavelength Retardation Plate</td>
</tr>
<tr>
<td>SCD</td>
<td>Surface Charge Density</td>
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<tr>
<td>SMS</td>
<td>Spatial Modulation Spectroscopy</td>
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<td>TEM</td>
<td>Transmission Electron Microscopy</td>
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ABSTRACT

Gold nanoparticles, particularly those with an anisotropic shape, have become a popular optical probe for experiments involving work on the nanoscale. However, to carry out such delicate and intricate experiments, it is first necessary to understand the detailed behavior of individual nanoparticles. In this series of experiments, optical and electron microscopy were utilized for the characterization of individual nanoparticles and small assemblies of nanoparticles.

In the first experiment, gold nanorods were investigated. Single, isolated nanorods exhibit two maxima of localized surface plasmon resonance (LSPR), which are associated with the two nanorod axes. Upon the physical rotation of a nanorod at one of its LSPR wavelengths under polarized illumination, the optical behavior varies in a sinusoidal fashion. A dimer of nanorods exhibits optical behavior quite similar to a nanorod, except the LSPR maxima are shifted and broader. Under differential interference contrast (DIC) microscopy, a pair of nanorods separated by a distance below the diffraction limit can be distinguished from a single nanorod due to its optical behavior upon rotation. Dark field microscopy is unable to distinguish the two geometries.

For the second set of experiments, the optical behavior of single gold nanorods at non-plasmonic wavelengths was investigated. The same nanorod was rotated with respect to a polarized light source under DIC, dark field, and polarized light microscopy.
DIC microscopy was found to produce diffraction pattern peaks at non-plasmonic wavelengths, which could be altered by adjusting the setting of the polarizer.

In the third set of experiments, the optical behavior of a single gold dumbbell and several simple dumbbell geometries were investigated with microscopy and simulations. The single dumbbell displayed behavior quite similar to that of a nanorod, but dumbbells exhibit a shift in both LSPR wavebands. Moreover, the shape of dumbbell particles allows them to interlock with one another quite easily. The dimers that form as a result display optical behavior that differs from what has been previously reported about nanorod dimers. Simulated surface charge density patterns reveal that hybridization of LSPR modes occurs readily along the lobes of individual dumbbells in some situations. A pentamer of dumbbells also displays hybridization of modes, and “hot spots” are observed at junctions between pairings of dumbbells.

In the final set of experiments, the assembly behavior of nanoparticles in solution was observed in real time. In general, large assemblies of nanoparticles display backbone-like rigidity, but an interesting variety of movements is permitted within the larger structures.
CHAPTER 1: GENERAL INTRODUCTION

Introduction to Nanoparticles

Based on the sheer number of papers printed on the topic of nanoparticles, one might be led to believe that nanoparticle science is a relatively young field. The term nanoparticle apparently came into use during the 1970s, and in the 1990s, the term began to appear quite frequently in the literature. However, nano-sized particles have been utilized for 65 years in weather modification and for millennia in glassmaking.

The recent explosive growth in nanoparticle research has been aided by improvements on several fronts. Synthetic methods have allowed for greater control in the size and shape of manufactured nanoparticles. Advances in instrumentation and imaging have made it possible to study particles in real time and below the diffraction limit. And, of course, improvements in computers and processing speeds have created a situation where complex simulations can be performed in a matter of minutes or hours. Research is also at a point where massive amounts of data can be collected and stored quite easily, especially in comparison to twenty years ago, when nanoparticle research began to take off.

Nanoparticles are an interesting topic, because they encompass a wide range of applications and properties. For the purposes of this dissertation, the focus will be on the optical properties associated with rod-like gold nanoparticles, namely nanorods and dumbbells. Simulated and experimental data are presented and discussed as a means of explaining the theory behind the observations made. The term ‘optical building blocks’
is used in the title, because nanoparticles like to form small aggregates every time they are given the opportunity to do so. This self-assembly behavior is a curse when studying single particle behavior, but it also provided me with an opportunity to devise methods for distinguishing single particles from dimers and other assemblies of particles. In the world of single particle imaging, the scientist needs to be confident that he/she is actually looking at a single particle. Fortunately, as particles come together like building blocks, they display new optical behavior that reveals the pattern of assembly. Similarly, it is important to recognize when you are looking at a true nanorod and when you are looking at something rod-like that isn’t actually a nanorod. The chapter on dumbbells addresses this problem, and it also devotes attention to the optical differences in nanorod and dumbbell dimers.

Gold is an interesting material to work with. It is a noble metal, easily formed into stable nanoparticles, relatively non-toxic, and displays surface plasmon resonance. As will be explained in the later chapters, the plasmonic properties of gold make it an ideal candidate for use as a nano-sized imaging probe. Silver and copper are also noble metals with plasmonic properties, and they are cheaper materials to work with. However, at this time, researchers are still struggling to make reliable nanoparticles with these metals. In addition, silver is considered toxic in some biological settings, thus it is easier for most researchers to stick with gold. Alternative plasmonic materials are also in development, so it is possible that within a matter of a few years, another material will replace gold as the plasmonic material of choice for nanoparticle research. But for
the moment, gold continues to set the proverbial gold standard amongst nanoparticles as a plasmonic imaging probe.

References


(2) Vonnegut, B. *Chemical Reviews* **1949**, *44*, 277.


Overview of Dissertation

A detailed introduction to the properties of noble metal nanoparticles is given in Chapter 2. Following that discussion, an overview is given on the more popular optical techniques utilized for imaging nanoparticles.

Chapter 3 is devoted to a detailed description of gold nanorods and simple nanorod geometries. After characterizing several particles and geometries with transmission electron microscopy, the same objects were inspected with optical microscopy and modeled with a simulation. The spectral profiles and rotational behavior of each object at plasmonic wavelengths is discussed.

Chapter 4 is a continuation of the work discussed in Chapter 3. However, in this chapter, attention is paid towards the optical behavior of single gold nanorods at non-
plasmonic wavelengths with differential interference contrast (DIC) microscopy. This chapter was necessary, because DIC microscopy treats samples as phase objects. When viewing nanorods under a DIC microscope, the optical behavior at non-plasmonic wavelengths is quite different from the behavior observed with other types of optical microscopy.

In Chapter 5, gold dumbbell particles and simple dumbbell assemblies are discussed in length. To aid in this discussion, simulated data was provided by Dr. Paul Mulvaney and Dr. Xingzhan Wei at the University of Melbourne in Australia. Spectral profiles, rotation data, and surface charge density plots are examined to explain the optical behavior. Where applicable, comparisons are made to the gold nanorod behavior discussed in Chapter 3.

Chapter 6 is a brief discussion on a series of self-assembly experiments that were conducted with anisotropic nanoparticles. In these experiments, aggregated nanoparticles were observed in solution in real time as they underwent a variety of movements and interacted with one another.

The final chapter, Chapter 7, provides brief examples of further research that can be conducted with nanorods, dumbbells, and similarly-shaped nanoparticles.
CHAPTER 2: ON THE OPTICAL IMAGING OF NOBLE METAL NANOPARTICLES

Excerpted from “Single Cell Optical Imaging and Spectroscopy”

Anthony S. Stender, Kyle Marchuk, Chang Liu, Suzanne Sander, Matthew W. Meyer, Emily A. Smith, Bhanu Neupane, Gufeng Wang, Junjie Li, Ji-Xin Cheng, Bo Huang, and Ning Fang

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Introduction

Before focusing on the specific research problems addressed in this dissertation, it is important to present an extensive overview of nanoparticles and the more common optical techniques that are used to study them. The following sections were excerpted from a review paper and have a focus on single cell imaging. Nanoparticles are frequently used to analyze biological environments, but because of their optical properties, they can also be utilized in many other applications, even though they are not discussed in this section. Finally, a discussion on the usage of nanoparticles in complex environments such as cells is a proper means of comparing imaging techniques and the properties of nanoparticles.
Noble Metal Nanoparticles

Broadly speaking, the term nanoparticle encompasses any and all types of particles (single phase, hybrid, solid, core-shell, metallic, silica, diamond, etc.) with a diameter on the nanoscale. However, for the purposes of non-fluorescent imaging, noble metal nanoparticles stand apart as the most popular group of probes. The noble metals have retained their preeminence amongst researchers due to their stability, ease of synthesis, and optical properties. Silver and gold are the two most popular options, and they can be easily tailored to provide a strong optical signal in the visible or near-IR range.

The ideal nanoparticle for single-cell imaging would be composed of nominal mass in order to avoid any interruption of the processes within the cell and also provide a sufficiently intense signal for optical detection. At the current stage of research, nanoparticles on the scale of 10 – 100 nm have been found to work quite well for single-cell research. Particles below 10 nm in diameter are not as well-understood, because they are impacted by the quantum behavior of the conduction electrons, and they offer a much weaker signal. However, researchers are beginning to look at quantum-sized plasmonic nanoparticles more carefully, and particles within that size regime could prove valuable for single-cell imaging in the near future.

The optical properties of noble metal nanoparticles arise from a phenomenon known as localized surface plasmon resonance (LSPR). Briefly stated, when a nanoparticle is irradiated by light within a specific, narrow band of wavelengths, the vibrating electrical field around the nanoparticle displaces the electrons in the
nanoparticle’s conduction band. Coulombic forces within the nanoparticle act to restore
the electrons to their former positions, but as long as the illumination continues, the
electrons in the particle will oscillate in phase and produce a signal that can be detected
with the optical techniques discussed below. For a nanoparticle to produce a LSPR
signal, it must be much smaller than the wavelength of light that it is being irradiated by.

In order to image noble metal nanoparticles, it is thus necessary to locate the
band of wavelengths where the LSPR behavior will appear. Fortunately, the position of
the LSPR is determined by several factors, the most important of which are the size and
shape and composition of the nanoparticle. The surrounding dielectric environment
(substrate and medium) can also produce a shift in the LSPR position.

The optical spectra of nanospheres can be determined by the application of Mie
Theory, which is an exact solution of Maxwell’s equations for spherical particles.\(^3,4\)
Spheres much smaller than the wavelength of light can be described even more simply
by use of the quasi-static approximation to Mie Theory. Under this approximation, only
dipolar contributions need to be considered when determining the cross sections for the
sphere. The scattering (\(\sigma_{sc}\)) and absorption (\(\sigma_{Abs}\)) cross sections are thus given by:\(^4,5\)

\[
\sigma_{sc} = \frac{128\pi^5}{3\lambda^4} a^6 \left| \frac{m^2 - 1}{m^2 + 1} \right|^2 \tag{1}
\]

\[
\sigma_{Abs} = \frac{8\pi^2}{\lambda} a^3 \text{Im} \left( \frac{m^2 - 1}{m^2 + 1} \right) \tag{2}
\]
where $\lambda$ is the illumination wavelength, $a$ is particle radius, and $m$ is the relative refractive index (the particle’s refractive index divided by the medium’s). The extinction cross section is merely the sum of the scattering and absorption cross sections. At small diameters, the extinction spectrum is dominated by absorption, but scattering grows in importance as the particle diameter increases.\textsuperscript{5,6} Because spheres are isotropic, they produce a single dipolar LSPR, but as sphere diameter increases, the LSPR red-shifts and undergoes broadening.\textsuperscript{4,7}

The spectrum of a rod-shaped particle can be calculated exactly with the theory developed by Gans.\textsuperscript{2,8} According to Gans’ Theory, the absorption cross section is:\textsuperscript{8-10}

$$
\sigma_{\text{Abs}} = \frac{2\pi V}{3\lambda} \varepsilon_m^{3/2} \sum_{i} \frac{(1/P_i^2)\varepsilon_2}{(\varepsilon_1 + (1 - P_i)\varepsilon_m / P_i)^2 + \varepsilon_2^2} \tag{3}
$$

where $V$ is the rod’s volume, $\varepsilon_1$ and $\varepsilon_2$ are the particle’s complex dielectric constants, $\varepsilon_m$ is the medium’s dielectric constant, and the polarization factors, $P_i$, and the factor, $e$, are defined as:

$$
P_1 = \left( \frac{1 - e^2}{e^2} \right) \left( \frac{1}{2e} \ln \left( \frac{1 + e}{1 - e} \right) - 1 \right) \tag{4}
$$

$$
P_2 = P_3 = \frac{1 - P_1}{2} \tag{5}
$$
where L and T are the lengths of the particle’s longitudinal and transverse axes.

Nanorods have two LSPR, one associated with each axis. The directionally dependent behavior of nanorods makes them a highly regarded probe for single-cell imaging. The position of the maximum longitudinal LSPR (in nm) can also be determined with:

$$\lambda_{\text{max}} = \left( 53.71 \frac{L}{T} - 42.29 \right) \varepsilon_m + 495.14$$

Although other nanoparticle shapes have been synthesized, they cannot be solved with exact methods as is the case for spheres or rods. Instead, their spectra can only be deduced by way of discrete dipole approximations (DDA) or other calculation-intensive methods. Another concern with imaging nanoparticles is being able to distinguish single, isolated nanoparticles from assorted aggregated conformations. While aggregates can display more complex behavior than single particles, they can be distinguished from single, isolated particles.

More recently, researchers have turned an eye towards hybrid nanoparticles, which consist of a noble metal and a non-noble material or a combination of noble metals. Core-shell and multi-layered silver-gold nanoparticles have complicated spectra, instead of spectra that are linear combinations of the components. Magnetic metals
can be added to a nanoparticle in order to provide for manipulation of the particle. However, magnetic materials alter and typically dampen the optical behavior of nanoparticles.\textsuperscript{4,21-23} Noble metal nanoparticles combined with semiconductors are capable of quenching or enhancing the fluorescence of the particle and could possibly serve as a sensor.\textsuperscript{21,24,25} The hybrid options are seemingly endless, but much research is still needed in that arena. For a more detailed description of alternative nanoparticle options, please refer to the recent extensive review by Cortie and McDonagh.\textsuperscript{21}

A final topic that deserves consideration when selecting a nanoparticle probe for cell imaging is nanoparticle toxicity. Nanoparticles may present themselves as toxic due to the metal or semi-conductor material with which they are made. Alternatively, nanoparticles may be toxic as a result of their surface coatings. One reason for gold’s popularity is that gold itself is typically viewed as being non-toxic to cells. In comparison, QDs are considered to be quite toxic, regardless of surface coating. The collection of other nanoparticles that exist (e.g. silver, copper, mesoporous silica, carbon, iron oxides, etc.) are highly variable in their individual toxicities, thus toxicity should not be overlooked when designing a study. Nevertheless, further research into the toxicology of nanoparticles is certainly warranted, especially for such particles that are being designed for \textit{in vivo} applications. Several detailed reviews and research papers have been written recently on this topic, and interested readers are encouraged to consider these other papers for more information on the subject.\textsuperscript{26-30}
Optical Imaging Techniques

Rayleigh Scattering-Based Microscopy

Dark field microscopy is the most commonly utilized mode of microscopy for non-fluorescent nanoparticle detection. This technique relies on the collection of Rayleigh scattered light for image generation (see Figure 1A). Under conventional dark field microscopy, a specialized condenser is relied upon that prevents any light rays from passing directly through the condenser to the sample plane (i.e. the zeroth order light rays). As a result, only oblique rays of light are permitted through the condenser and interact with the sample. Because the numerical aperture (NA) of the objective is set to a lower value than that of the condenser, the oblique rays will fail to enter the objective in the absence of a sample, and the imaging field will appear dark. Light that is scattered by the sample can enter the objective, and it will appear as a white or colored feature against the dark background. However, cells are complex structures, and many of the surfaces within the cell can scatter light, thus creating a problem with large background noise and making nanoparticle detection a difficult challenge in some experiments.

Dark field microscopes are often coupled with additional components to aid with spectroscopy and nanoparticle detection. Since the sample is illuminated with white light, a monochromator or grating is often placed in front of the detector in order to provide a high resolution spectral profile of metal noble nanoparticles.\textsuperscript{31-33} In some experiments, filters (tunable or fixed) are preferred.\textsuperscript{14,34,35} When orientation information is needed from the nanoparticles, additional components can be added to the light path to provide the desired polarization setting.\textsuperscript{14,36} When a spectrometer is utilized, an
additional option is to replace the spectrometer’s entrance slit with a liquid crystal device (LCD). This modification allows each pixel to act as an individual shutter with fast response, and as many as 20 isolated particles can be investigated at once using this technique.\textsuperscript{33,37}

As an alternative to dark field microscopy, Louit et al., developed a confocal microscope that also collects Rayleigh scattered light.\textsuperscript{38} The system uses a tunable laser and filters to provide homogeneous illumination across the visible spectrum (see Figure 1B). Furthermore, the instrument provides sufficient time resolution for monitoring plasmon shifts as nanoparticles interact with cellular features. The technique also allows for the tracking of nanoparticles during endocytosis and exocytosis events.

**Absorption-Based Microscopy**

As shown above, the scattering signal of a nanoparticle drops off more quickly than the absorption signal with decreasing radius. Therefore, some researchers choose to work with absorption-based spectroscopy. The simplest absorption technique is bright field microscopy. For this method, light is transmitted directly through the condenser and the sample plane. As a result, nanoparticles and other features that absorb light appear to be dark upon a bright background. When video-enhancement became available in the 1980s, researchers were finally able to track small gold nanoparticles (≥15 nm) to research on endocytosis and lateral protein motions on cell exteriors.\textsuperscript{39} Bright field is still used by researchers today for cell and nanoparticle imaging, but it is typically used to complement other imaging techniques.\textsuperscript{40,41}
A second absorption-based microscopy is spatial modulation spectroscopy (SMS). This technique measures nanoparticle absorption by moving the particle in and out of the focal plane. Although this technique can detect particles as small as 5 nm, the nanoparticles must be fixed to a surface. As such, it has not been utilized for biological studies to date.

A third method, photothermal heterodyne imaging (PHI), utilizes a dual laser beam configuration to detect a nanoparticle by way of a thermal lens that is created by the pump laser (see Figure 1C). Nanoparticles absorb energy from a time-modulated excitation laser and convert the energy into heat. Subsequently, as the heat is dissipated from the nanoparticle, the surrounding medium undergoes a time-modulated variation in its refractive index. The non-resonant probe beam interacts with the modulating medium and produces a scattered field. By using the probe beam’s beatnote to monitor the scattered field, gold nanoparticles as small as 1.4 nm can been detected. However, the technique’s sensitivity is closely linked to the fluid surrounding the nanoparticle. Additionally, PHI can be used to detect noble metal or semiconductor nanoparticles, and it can detect the orientation of a nanoparticle when polarized light is utilized.

When PHI is coupled to a piezoscanner stage for raster scanning of the sample, the technique is referred to as Laser Induced Scattering around a NanoAbsorber (LISNA). LISNA has been used with live cell imaging at video rate for several minutes at a time, but because it is a raster scanning technique, it is somewhat limited to slower biological processes. As such, few PHI experiments with this design have been conducted. Instead, recent PHI experiments have tracked dynamic nanoparticles by
focusing multiple laser beams at fixed locations within the cell to detect time fluctuations in the photothermal signal. \(^\text{49}\)

**Photoacoustic Methods**

Photoacoustic tomography (PAT) is a specialized absorption technique that effectively combines optical and ultrasonic imaging, thereby providing a means of noninvasively imaging tissues (see Figure 1D).\(^\text{50-54}\) As a result, it provides deeper imaging than other optical techniques while avoiding radiation and the high costs associated with diagnostic, molecular imaging.\(^\text{54}\) Moreover, a wide variety of nanoparticles can be used as functionalized probes for use with PAT, including gold, copper, magnetic materials, and QDs.\(^\text{51,53-56}\) By functionalizing the nanoparticles, it is possible to target and detect specific types of cells within tissue, such as cancerous cells.

PAT produces a high-contrast image of tissues and molecular contrast agents (e.g. nanoparticle probes) that absorb sufficient radiation.\(^\text{51}\) To produce the image, a short-pulse laser beam first irradiates the sample, typically in the near-infrared range. As tissues absorb the incoming radiation, they undergo thermoelastic expansion and re-radiate the energy in the form of photoacoustic waves. A transducer is responsible for detecting the photoacoustic signals.

PAT can be split into two distinct modes based on how the transducer collects the signal.\(^\text{57}\) If the transducer raster scans the sample, the mode is referred to as focused scanning tomography. This mode includes photoacoustic microscopy (PAM) and confocal dark field PAM. The time to complete a scan is dependent on the laser pulse
rate, the step size of the scan, and the size of the area being scanned. Alternatively, an array of transducers can be used in parallel for detection, in which case the mode is referred to as photoacoustic computed tomography. Under this mode, frame rates as high as ~50 MHz can be achieved.

The maximum penetration depth attainable with PAT is ~30 mm in biological tissue, but the actual depth is dependent upon the ultrasonic frequency used by the transducer. Furthermore, as penetration depth is increased, axial and transverse resolution is drastically reduced. At a depth of ~3 mm with the transducer set to 50 MHz, PAT provides an axial and transverse resolution of 15 and 45 μm, respectively.

More recently, efforts have been made to match PAM’s lateral resolution to that of optical methods. In one such case, the sample is doubly illuminated from the top and bottom, simultaneously. However, the penetration depth is limited to ~2 mm in tissue at a wavelength of 532 nm. In another variation known as pure optical PAM (POPAM), weak photoacoustic signals are detected by means of a focused excitation beam and a specialized micro-ring resonator with a broad bandwidth for signal detection. This technique has been capable of providing a lateral and axial resolution of 5 and 8 μm, respectively.

**Interference-Based Microscopy**

An alternative method for detecting nanoparticles is interferometry. This method is capable of distinguishing nanoparticles from its background, because it exploits the
interference of the background reflection with the nanoparticle-induced scatter. Thus, the normalized cross-section for the images seen under this method is described by:

$$\sigma_{\text{int}} = \frac{I_m - I_r}{I_r},$$  \hspace{1cm} (8)

where

$$I_m = |E_r + E_s|^2 = |E_i|^2 \left(r^2 + s^2 - 2|r||s|\sin \phi\right),$$  \hspace{1cm} (9)

and $I_m$ is the measured intensity of a particle at the center of the focus as a function of the particle diameter ($d$), $I_r$ is the average measured intensity of the reflected light in the absence of the particle, $E_i$ is the electric field at the particle’s location, $E_r$ is the electric field of the background illumination, $E_s$ is the electric field of light scattered by the particle, $r$ is field reflectivity, $s$ is the complex scattering amplitude, and $\phi$ is the phase of the scattering. The three terms on the right-side of Equation 9 represent the background intensity, the purely scattered intensity that scales as $d^6$, and the cross-term that scales as $d^3$. For very small particles, the second term becomes smaller than the noise observed with the first term, while the third term becomes the dominant factor.

A standard homodyne instrument setup utilizes a low powered (≤10 mW) laser for illumination on a commercial microscope. Either white or monochromatic light can be used to illuminate the sample. A piezo stage is utilized for scanning the sample area. The reflected signal is collected by a photomultiplier with a low-noise current-voltage amplifier. Because of the destructive interference between scattered and reflected light,
small particles appear to be dark against the background. Using this instrument design, 5
and 10 nm gold nanoparticles at a water-glass interface were detected at an integration
time of 2 and 1 μs, respectively under confocal microscopy. However, a limitation of
this design is that the phase must be known in order to elucidate the electric field
scattered by the particle.62

More recently, heterodyne and phase-shifting interferometers have been
designed, and they are capable of independently measuring the amplitude and phase of
the scattered field.62–64 Under heterodyne interferometry, the signal is allowed to
interfere with a frequency-shifted reference beam, thereby producing a beat frequency.
The signal is demodulated at the beat frequency, resulting in a decoupled phase and
amplitude. Simple dual-phase interferometers can even be scaled down to a portable unit
for forensic and biodefense applications.62 When heterodyne interferometry is combined
with dark field microscopy, the background noise is reduced, and ~48 nm diameter
viruses and bacteriophages have been detected label-free in solution in real-time.63
Under phase-shifting interferometry, known phase shifts are introduced.62 By taking
independent measurements of the signal intensity in sequence or simultaneously, it is
possible to determine the scattering by the particle. With this design, immobilized 25 nm
gold particles have been detected at 1 ms in water.62

Heterodyne interferometry can also be combined with cross-polarization
microscopy (see Figure 1E).65 In such a design, linear polarized light is sent through a
beamsplitter to create a signal and a reference beam that are x- and y-polarized,
respectively. A high NA objective along the signal beam induces a partial conversion of
x polarized light into y-polarization. An objective collects any y-polarized scattering that occurs by objects in the signal beam path. By then overlapping the two beams, only y-polarized components of the electric fields will interfere, and as a result, the background is shot-noise limited. Particles thus appear as a cross-polarized object with white and black spots upon a black or dark gray background. This design has been able to detect gold nanospheres on a glass slide as small as 5 nm at an excitation power of ~1 μW.

**Differential Interference Contrast Microscopy**

Differential interference contrast (DIC) microscopy is a specialized variation of interferometry. Nomarski DIC is the primary microscope design for imaging in the visible and near-IR range, and it is also commercially available. Other variations of DIC microscopy do exist, but they are either used for X-ray imaging, or they have not proven as popular as Nomarski DIC. Nomarski DIC’s popularity largely stems from its reliance on a large condenser aperture, which provides a higher lateral resolution and a shallower depth of field than either dark field or bright field microscopy. As a result, DIC provides sharper cell images than dark field or bright field, because it doesn’t suffer from out-of-plane scattering or absorption.

In addition to a condenser and objective, the main components along the light path of a Nomarski DIC microscope consist of two birefringent Nomarski prisms, a polarizer, and an analyzer (see Figure 1F). Non-polarized white light passes through the polarizer and then undergoes shearing into two orthogonal beams at the first prism. Resultantly, two coherent beams with a lateral shear of ~100 – 200 nm pass
through the condenser and illuminate the sample plane. Both beams are also at a 45° angle relative to the polarizer. After being gathered by the objective, the two orthogonal beams are recombined by the second prism before passing through the analyzer on its way to the detector. Filters are usually placed in the light path to collect images at specified wavelengths, since it isn’t trivial to introduce a spectrophotometer to the light path due to DIC’s reliance on the principles of interferometry. To perform rotational studies, a rotating stage is also preferred.14

Nanoparticles and other features that introduce a phase shift to either of the two sheared beams will appear as a shadow-cast object on a gray background in a DIC image.14,71-73 At the LSPR wavelength(s), the shadow-cast appearance of an isotropic nanoparticle will not change with rotation of the object in relation to the polarizer, because the particle remains equally aligned with both of the sheared beams at all orientations. However, an anisotropic nanoparticle can appear as predominantly white, predominantly black, or as shadow-cast, depending on the orientation of the LSPR axis with the two orthogonally sheared beams. In fact, for gold nanorods with a LSPR in the visible range, the relative intensities of the black and white signal oscillate according to a \( \cos^4 \) and \( \sin^4 \) relationship, respectively.72 By monitoring the changes to the two signal components, it is possible to discern the 2D and 3D orientation of a nanorod, even if it is in motion.

Image quality and nanoparticle contrast can be adjusted by introducing an intentional phase shift to the wave train. To introduce a phase shift, traditional Nomarski DIC microscopes rely on translating the condenser-side prism while the objective-side
prism remains fixed. Modern de Sénarmont-Nomarski DIC microscopes employ a birefringent quarter wavelength retardation plate (QWRP) in the light path before the condenser. While the fast axis of the QWRP is fixed at a 90° angle to the analyzer, the polarizer is rotated to adjust the phase shift between the two beams. Researchers must take care in selecting an intentional phase shift, however, particularly when working with a gold nanorod sample that displays lots of polydispersity in the LSPR position. Adjusting the phase shift alters the optical behavior of gold nanorods at non-plasmonic wavelengths.  

Nomarski DIC has already been proven to be effective at cell research. For example, DIC has been used to observe nanorods rotating on a live cell membrane at 200 frames/s,  and elucidate the rotational dynamics of cargos at pauses during axonal transport in neurons at 500 frames/s. Because DIC is non-intrusive and does not require staining, as fluorescence microscopy does, DIC can be used for monitoring cells and nanoparticles over extended periods of time. As such, DIC has been utilized to track nanoparticles undergoing endocytosis and transport through cells.

Concluding Comments

In summary, noble metal nanoparticles are a highly popular probe for the purposes of non-fluorescent imaging. They provide a photostable signal that does not bleach. The signal strength varies between techniques and is dependent on the component of the signal that is actually detected (i.e. scatter, absorption, interference effects, etc.). Particle size ranges from several nm to hundreds of nm, and the optimal
detection wavelength is dependent on the shape, size, and surroundings of the particle. Nanoparticle toxicity is a function of the particle’s metal constituents and surface coating, as well as particle concentration in the cell. Gold nanoparticles are frequently used with cell research, because they are considered non-toxic, particularly in comparison to silver.

Of all the probe options that are available, the gold nanorod has become the tried and true workhorse that investigators rely upon for cell imaging experiments. Gold nanorods are easily synthesized, and can be designed to provide a strong signal in the UV, visible, or near-IR range of the spectrum. Because of the shape anisotropy associated with nanorods, they produce a LSPR signal that is orientation dependent under polarized illumination. Furthermore, gold nanorods can be readily and easily delivered into cells with no significant disruption of the normal activities of the cell. For these reasons, the gold nanorod and its close relative, the gold dumbbell, are the focus of this dissertation.

References


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Figure 1. Optical imaging techniques for non-fluorescent nanoparticles: (A) Dark field microscopy. (B) Louit, et al.’s light scattering confocal microscope. Reprinted with permission from ref 38. Copyright 2009 American Chemical Society. (C) Photothermal. Reprinted with permission from ref 48. Copyright 2005 American Chemical Society. (D) Photoacoustic. Reprinted with permission from ref 54. Copyright 2010 American Chemical Society. (E) Interferometric cross-polarization microscopy. Reprinted with permission from ref 65. Copyright 2011 American Chemical Society. (F) Differential interference contrast (DIC) microscopy.
CHAPTER 3. INFLUENCE OF GOLD NANOROD GEOMETRY ON OPTICAL RESPONSE

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Abstract

As noble metal nanoparticles are deployed into increasingly sophisticated environments, it is necessary to fully develop our understanding of nanoparticle behavior and the corresponding instrument responses. In this paper, we report on the optical response of three important gold nanorod configurations under dark field and differential interference contrast (DIC) microscopy after first establishing their absolute geometries with transmission electron microscopy (TEM). The observed longitudinal plasmon wavelengths of single nanorods are located at wavelengths consistent with previously developed theory. A dimer is shown exhibiting a multipole plasmon at wavelengths that are consistent with the dipole plasmon of single nanorods in the sample. DIC can also distinguish a single nanorod from a pair of uncoupled nanorods with an interparticle distance below the diffraction limit. The experimental observations are consistent with simulated DIC images using a DIC point spread function. The findings herein are a
critical step towards being able to characterize nanorods in dynamic environments without the use of electron microscopy.

**Introduction**

Noble metal nanoparticles have quickly developed into an attractive probe for use in theoretical and analytical research. Due to their plasmonic properties, nanoparticles can be readily detected with a variety of imaging techniques.\(^1\)\(^-\)\(^3\) By simply adjusting the size, shape, or composition of a nanoparticle,\(^4\)\(^-\)\(^6\) it is possible to design a non-fluorescent probe with a well-defined surface plasmon resonance (SPR) in the visible or near-infrared region of the spectrum. Furthermore, nanoparticles do not suffer from blinking or photobleaching,\(^7\) and they can be imaged with a high temporal and spatial resolution.\(^2\)\(^,\)\(^3\) Because of the aforementioned traits, nanoparticles can be utilized for observing either short-lived events or lengthy processes.

An important aspect of noble metal nanoparticles is that their optical response can be manipulated by environmental factors. For example, the position of the SPR can be influenced by the dielectric constants of the surrounding medium or the substrate.\(^8\)\(^-\)\(^11\) The sensitivity to a dielectric substrate has been shown to be dependent on the level of contact between the particle and the substrate.\(^12\) A single particle’s SPR can also be affected by the presence of other nearby particles due to interparticle coupling of the plasmons. Coupling between two or more nanoparticles is highly dependent on the interparticle distance and the geometry of the interacting particles.\(^13\)\(^-\)\(^19\)
The technique required for observing the optical response is often dependent on the application. Dark field microscopy is a popular method with high temporal resolution, and it is considered a benchmark for other modes of optical microscopy in imaging nanomaterials. Dark field images of nanoparticles resemble bright dots of light on a black background, because the microscope only collects the light that is scattered by the sample. In order to prevent any illuminating light from reaching the detector, the numerical aperture (NA) of the objective must be set smaller than the NA of the condenser. Furthermore, dark field has decreased sensitivity in complex systems, such as cells, because it must contend with scattering and distortions that arise from features along the optical path.

Differential interference contrast (DIC) microscopy is a commercially-available alternative to dark field microscopy. In the past, DIC has been used primarily for biological imaging, but it is gaining popularity as a tool in single particle research. DIC utilizes the principle of interferometry to produce high contrast images on a grey background. Because DIC utilizes a full NA at the objective, it supplies a higher lateral resolution and a shallower depth of field than dark field microscopy. As a result, DIC is capable of monitoring nanoparticles in complex environments, such as cells, for long periods of time with less interference from out-of-focus features. However, the optical response of nanoparticles under DIC microscopy has not been correlated to the absolute configuration of the nanoparticle(s).

To address the aforementioned concern about DIC microscopy, we studied the optical response of three types of gold nanorod configurations under DIC and dark field
microscopy. The optical responses were compared against observations collected with transmission electron microscopy (TEM). In the present study, isolated nanorods are defined as single rods separated by a distance greater than the diffraction limit, and they act independently of one another. Proximate neighbors is a new term used here to describe the case of two uncoupled nanorods with an interparticle distance below the diffraction limit. The proximate neighbors act independently, but the microscope detects a single point of light. Dimers consist of two interacting nanorods that are touching or nearly touching, and they have received much attention in recent papers. Depending on the sizes and relative orientations of the individual nanorods in a given dimer, it is possible for the individual longitudinal plasmons to couple and shift through electric field interactions. In some instances, as the dipole plasmon red shifts, multipole peaks will emerge in the dimer’s scattering spectrum. Using a previously-developed DIC point spread function, a simulation was also employed to model the appearance of the DIC image. The simulation is designed for isolated nanoparticles and nanoparticles separated by a distance greater than 20 nm.

**Results and Discussion**

A UV-VIS absorption spectrum was initially collected from the original gold colloid with a Varian Cary 300 UV-Visible Spectrophotometer. Between 400 nm and 900 nm, two absorption peaks appeared, with centers at 521 nm and 627 nm (Figure S1 in the Supporting Information). The peak at 627 nm is associated with the longitudinal axis, whilst the peak at 521 nm arises from the transverse axis. These values are
reasonable for nanorods with an aspect ratio of ~2. At larger aspect ratios, the two SPR peaks are quite distinct in size, with the peak from the longitudinal axis dominating the spectrum. As the aspect ratio approaches unity, the two peaks become similar in size and eventually merge. However, the presence of particles of other shapes may also contribute to the 521 nm peak. Sample polydispersity is also known to affect the actual peak positions, widths, and heights under UV-VIS absorption. As a result, electron microscopy must be relied upon for a more detailed characterization of the nanorods.

After applying an aliquot of the gold colloid to a holey carbon substrate, a distinct group of four nanorod features was located and characterized with a Philips CM-30 transmission electron microscope. It was quite fortuitous to locate these four features in one small area. TEM, DIC, and dark field images of the four features are shown in Figure 1, while the TEM-determined sizes of these features are reported in Table 1. Features N1 and N2 are single, isolated nanorods. Feature D is a non-overlapping dimer with a total length of 141 nm. Feature P is a pair of proximate nanorods with a center-to-center interparticle distance of 227 nm (180 nm tip-to-tip). At wavelengths near the expected longitudinal SPR (~660 nm) of feature P, the calculated diffraction limit \((\lambda/2NA)\) is 236 nm, a value that is greater than the distance between P’s two individual nanorods.

The expected dipolar longitudinal SPR wavelengths of the four features were calculated using the following equation:

\[
\lambda_{\max} = (53.71 \times R - 42.29) \varepsilon_m + 495.14,
\]

(1)
where \( \lambda_{\text{max}} \) is the longitudinal SPR wavelength; \( R \) is the aspect ratio; \( \varepsilon_m \) is 2.30, the dielectric constant of the Immersol 518F immersion oil from Zeiss that served as the surrounding medium. The calculated values are presented in Table 1. For the dimer, a range is given for the dipolar SPR wavelength, in order to reflect the different lengths of the two transverse axes involved.

By relying on the pattern recognition of permanent landmarks on the substrate, the same four nanorod features were located and inspected under dark field and DIC microscopy using a Nikon Eclipse 80i upright microscope. Once the features were located, the microscope’s stage was rotated to find the angles that excited the longitudinal SPR of each feature. Under dark field microscopy with polarized illumination, an isolated nanorod produces its brightest intensity at the longitudinal SPR when its longitudinal axis is aligned with the polarizer; its weakest intensity occurs when the longitudinal axis is oriented perpendicular to the polarizer.\(^{13, 14, 25, 34} \)

In comparison, DIC microscopy relies on a set of polarizers and Nomarski prisms. Before reaching the sample plane, the incoming light is split into two orthogonal wave fronts (Figure S2 in the Supporting Information).\(^{35-38} \)

When a nanorod’s longitudinal axis is aligned with the bright wave front, the nanorod appears entirely white. The nanorod is completely black in appearance when the nanorod is aligned with the dark wave front. At angles in between the two wave fronts, nanorods take on a shadow-cast appearance.
Single, Isolated Nanorod

For the case of a single, isolated nanorod, images were collected over the range 500 to 780 nm using a Photometrics CoolSnap ES CCD camera. Under dark field conditions, the spectra of nanorod N1 were collected at the angles that excited the highest and lowest intensity at the longitudinal SPR wavelength, in following with the prior work of other researchers.\textsuperscript{13, 14, 25, 34} Intensity data were normalized in relation to the nanorod’s highest observed intensity. Nanorod N1 has a longitudinal SPR peak at 660 nm under dark field conditions (Figure 2A). This peak appears at both particle orientations, and its intensity is greater when the longitudinal axis is aligned with the polarizer.

A perfect dipole should exhibit no longitudinal SPR peak when the nanorod is aligned perpendicular to the polarizer.\textsuperscript{31, 39} However, the coupling between a particle and the substrate can lead to a SPR that is predominantly, but not entirely, dipolar in nature.\textsuperscript{10, 26} In particular, as the aspect ratio of the nanorod decreases, the depolarization becomes more pronounced.\textsuperscript{26}

Because of the shadow-cast appearance of nanorods at certain angles under DIC microscopy, it is possible to measure the intensity for both the bright and the dark portions of each particle as a function of the nanorod’s angle under the DIC microscope. The intensity spectra for nanorod N1 were collected with the longitudinal axis aligned along both the bright and the dark wave fronts. The resultant spectra are presented in Figure 2B as they are related to the mean background intensity, which was assigned a value of one. When the longitudinal axis is aligned with the bright wave front, the
nanorod’s dark side intensity is close to the background intensity at all wavelengths, while a maximum in the bright side intensity appears near 645 nm, as predicted in Table 1. Likewise, when the nanorod is aligned with the dark wave front, the bright side intensity is close to the background intensity, and the dark side intensity is significant at the longitudinal SPR. However, due to the working principle of DIC microscopy, the depolarized component is not involved in the image formation, and thus is not observed.\(^{38}\)

Nanorod N1 was also observed at 640 nm, the longitudinal SPR wavelength, during a 180° rotation of the microscope’s stage in 5° steps. The intensity profiles under rotation are shown in Figure 2C. At the angle 0°, the longitudinal axis is aligned with the polarizer; at 45°, the axis is parallel to the dark wave front; at 135°, the axis is parallel to the bright wave front. Both the bright and the dark side intensities exhibit a sinusoidal pattern. As expected, the nanorod’s darkest and brightest intensities are centered at 45° (the dark wave front) and 135° (the bright wave front), respectively.

Figure 2D compares the observed and simulated DIC images that appear at 45°, 75°, 105°, and 135° for the nanorod rotation in Figure 2C. When a nanorod is aligned with the dark wave front, the simulation produces a particle that is entirely black in appearance. As nanorods are rotated away from the dark wave front, they take on a slowly increasing bright side component at the expense of the dark side. Once aligned with the bright wave front, the dark side disappears completely, and the particle’s appearance is completely white. These simulated results agree well with the observed behavior of the single nanorod, N1.
The behavior of nanorod N2 (Figure S3 in the Supporting Information) was similar, but not identical, to that of N1, as expected.\textsuperscript{41} As predicted in Table 1, DIC microscopy found that the longitudinal SPR for N2 was red shifted in comparison to the SPR for N1.

**Proximate Nanorods**

Feature P is a pair of nanorods with an interparticle distance of 227 nm, and the angle between the two longitudinal axes is \(\sim120^\circ\). Images of feature P were collected from 500 to 780 nm using the Photometrics CoolSnap ES CCD camera. Based on the sizes of the nanorods and their interparticle distance, the effects of interparticle coupling were expected to be minimal.\textsuperscript{25,42} However, because the interparticle distance is less than the diffraction limit, optical microscopy detects a single, convoluted particle image.

Using dark field microscopy, the spectra were collected with the interparticle axis aligned either parallel or perpendicular to the polarizer. The spectra presented a maximum in intensity at 660 nm for both orientations (Figure 3A). The intensity was greater in value when the interparticle axis was aligned parallel to the polarizer, because the longitudinal axes of both nanorods are well-aligned with the polarizer at this angle, as demonstrated in the Figure 3A inset. When the interparticle axis was rotated by 90\(^\circ\), a longitudinal component of each nanorod was still aligned with the two wave fronts, and a small peak was observed in the spectrum. The dark field spectra of the proximate nanorods thus resembled those of a single nanorod.
In the DIC mode, spectra were collected while the interparticle axis was aligned either parallel or perpendicular to the polarizer. At these angles, the two nanorods are well-aligned with the two DIC wave fronts (see Figure 3B inset), and the DIC image has a significant bright side and dark side component (Figure 3B). A closer inspection of the spectra reveals that each pair of bright and dark intensity profiles has slightly offset peak positions. For example, when the interparticle axis is aligned perpendicular to the polarizer (the solid curves in Figure 3B), we find a bright-side intensity maximum at 660 nm and a dark-side intensity maximum at 680 nm, clearly demonstrating the contributions from the two individual nanorods. When the interparticle axis is aligned parallel to the polarizer, the dark-side maximum is now at 660 nm, and the bright-side maximum is at 680 nm. At both angles, the peak at 660 nm is considerably stronger than the peak at 680 nm, thus demonstrating a difference in scattering abilities.

Feature P was observed during a 360° rotation under DIC microscopy at 680 nm, and it was found that the intensity profiles were dependent on the orientation of the interparticle axis (Figure 3C). However, the DIC rotational profile of the proximate nanorods is more complex than the profile observed with N1. Maxima in the contrast (the difference in intensity between the bright and the dark sides) appear near 180° and 270°, with minor maxima appearing near 0° and 90°. These four angles coincide with the interparticle axis being aligned parallel (0° and 180°) or perpendicular (90° and 270°) to the polarizer. The profile does not have the sinusoidal shape that was found with N1.
Based on these observations, it is possible to conjecture as to how the convoluted contrast would change as the relative orientation of two identical nanorods in a proximate configuration is altered. If two proximate nanorods were aligned tip-to-tip or side-by-side, the individual plasmons would exhibit equivalent optical behavior as they were rotated on the stage. At the other extreme, if the two nanorods were aligned at 90° angles to each other, a total of four distinct maxima should appear in the bright side (and the dark side) intensity profile during a full rotation of the feature. With the latter orientation, the maximum bright side and dark side intensities would always appear at the same angles. As the nanorods go from a parallel to a perpendicular orientation, the intensity maxima should gradually broaden and eventually split into four peaks. Moreover, at these intermediate interparticle angles, the feature is always partially aligned with both DIC wave fronts, regardless of the feature’s orientation angle.

Because feature P includes two nanorods at different aspect ratios and with different scattering abilities, its rotational behavior is further complicated, yet its optical behavior remains quite distinct from that of the single nanorod, N1.

The DIC simulation was utilized to model the proximate nanorods, and the simulated images were compared to the actual DIC images (Figure 3D). At most angles, the dark and bright sides remain apparent simultaneously in both sets of images. As the interparticle axis comes into alignment with either of the wave fronts (45°, 135°), the simulated image takes on an appearance that is almost entirely white or black, as with the actual image.
Nanorod Dimer

To explain the observed behavior of feature D, the dimer, it is first necessary to review some of the other recent work in this area. As two individual nanoparticles are brought together, their plasmons interact and hybridize, resulting in a red shift in the longitudinal SPR wavelength. The plasmon shift remains negligible for a pair of parallel particles that are separated by distances greater than 2.5 times the length of the nanorod’s transverse axis.\textsuperscript{42} For other nanorod orientations, the reach of the plasmon interaction is highly variable.\textsuperscript{25}

When a pair of nanorods interact and couple at relatively long interparticle distances (d), their plasmons behave as classical dipoles and shift according to 1/d\textsuperscript{3}, while at relatively short distances, the plasmon shift depends on 1/d.\textsuperscript{16, 43} More recently, dimer plasmons have been studied at extremely short interparticle distances, where the separation to rod length ratios are below 0.09.\textsuperscript{14, 27, 44} In this near-contact regime, shifting of the dipole plasmon does not follow the aforementioned theory. Instead, the geometry of the junction between the two particles becomes critical in the extent of the plasmon shift.\textsuperscript{19}

As two particles approach each other towards a single point of contact within the near-contact regime, the dipole peak becomes narrower and undergoes enhanced red shifting.\textsuperscript{19, 27, 45} To compensate for the decreased scattering by the dipole, additional peaks emerge at shorter wavelengths through the interactions of multipoles. As the interparticle distance is further decreased, the dimer’s multipole peaks will also red shift and gradually decrease in size, while the dipole peak can disappear completely.\textsuperscript{27}
According to the calculation used in Table 1, the dimer was expected to have a longitudinal SPR located between 880 and 940 nm. In the TEM image (Figure 4A inset), the surfaces of the two nanorods appear to be within 1 nm of each other. The presence of surfactant molecules at the surface of the two nanorods could easily prevent the two gold surfaces from making actual contact. Assuming the nanorods are not touching, the dimer fits in the near-contact region, because it has a separation to rod length ratio below 0.015.

Feature D was initially examined from 500 to 780 nm with the Photometrics CoolSnap ES CCD camera, because of its high resolution. At longer wavelengths, the data was collected with a Photometrics Evolve CCD camera, since the Evolve has higher quantum efficiency in this region than the CoolSnap ES. Dark field spectra were collected with the interparticle axis aligned both parallel to and perpendicular to the polarizer. Dark field microscopy detected a strong SPR peak between 640 and 720 nm, as shown in Figure 4A. The peak here was broader than that of the single nanorods, and the intensity at shorter wavelengths (540 to 620 nm) was also increased. At wavelengths longer than 750 nm, the observed intensity was weak at both particle orientations.

The full DIC spectra collected for the dimer are presented in Figure 4B. Broad regions of resonance occur between 620 and 720 nm, with a maximum at 680 nm. The expected dipole longitudinal SPR near 900 nm was not observed. Based on the prior work of other research groups, we believe that the dipole SPR was either red shifted beyond 900 nm, or more likely, it disappeared as a result of the near-contact coupling. The peak at 680 nm should be from a multipolar resonance.
Figure 4B reveals that the dimer is entirely white in appearance from 620 to 740 nm when the dimer is aligned with the bright wave front, and it is completely black over the same region when aligned with the dark wave front. Figure 4C displays the DIC rotational profiles collected for the dimer’s multipole at 680 nm. The data reveal that the darkest and brightest intensities are centered around 45° and 135°. The rotational profile exhibits a pattern similar to that of the single nanorod, N1.

Concluding Remarks

To summarize, we have reported on the optical response of three gold nanorod configurations under DIC microscopy after obtaining a precise characterization of the nanorods with TEM. Such fundamental work is of importance to the basic understanding of nanorod behavior, particularly for single-particle tracking experiments and other dynamic environments where electron microscopy cannot be applied. Because of the presence of two wave fronts in DIC microscopy, DIC provides an added dimension to the observation of gold nanorods in comparison to dark field microscopy. This added dimension is especially influential when looking at the dimer and the proximate nanorod configurations.

The results herein also suggest that single particles cannot always be distinguished from other configurations by simply inspecting the sample at a single wavelength or at a single observation angle. Multipoles from dimers can prove problematic, because they can appear at or near the wavelengths where a single nanorod’s dipole SPR is expected. Proximate nanorods can be likewise troublesome.
DIC’s added dimensionality was able to reveal the presence of two nanorods for the proximate configuration encountered here, and DIC microscopy should be able to distinguish most proximate nanorod configurations from a single nanorod, unlike dark field microscopy. The results here also stress the importance of investigating additional nanorod configurations with DIC microscopy.

Materials and Methods

Materials and Sample Preparation

The hemispherically-capped gold nanoparticles used in this study were purchased from Nanopartz as a colloidal suspension (Salt Lake City, UT). Before applying gold nanoparticles to the substrate, 50 µL of the gold colloid were centrifuged at 5500 rpm for 10 minutes, re-suspended in 15 µL of milliQ water, and sonicated for 20 minutes. This process removed excess surfactant from the solution. The substrate was a TEM grid made of holey carbon and backed by a 200-mesh copper grid (SPI Supplies, West Chester, PA). This substrate was selected, because holey carbon is known for its stability under an electron beam. Holey carbon is known to be dielectric, but the exact dielectric constant of the substrates used in this study is unknown. The circular substrate had an outer diameter of 3.05 mm, and each grid square was 97 µm x 97 µm. After setting the substrate on Whatman filter paper, 5 µL of the gold colloid were applied to the substrate and allowed to dry.

After the sample was first analyzed by TEM, the substrate was prepared for optical microscopy. The substrate was placed on 25×75×1 mm precleaned microscope
slide from Fisher Scientific (Pittsburgh, PA). Next, the substrate was suspended in Carl Zeiss Immersol 518F immersion oil (Thornwood, NY), which has a refractive index of 1.518. The sample was immediately covered with a No. 1 cover slip from Corning (Corning, NY). Single-sided and double-sided tapes were utilized to hold the cover slip in place.

**Transmission Electron Microscopy**

A Philips CM-30 transmission electron microscope operating at 200 kV was used for collecting the TEM data. Images were collected with a Gatan Orius SC 1000 CCD camera at an 11 Megapixel (4008 x 2672) resolution using Gatan DigitalMicrograph. ImageJ was used to determine the size and orientation of the nanorods observed. All measurements were collected multiple times, and the mean values were reported herein.

**Optical Microscopy**

All optical microscopy was completed with a Nikon Eclipse 80i upright microscope equipped with a 12V-100W halogen lamp. A Photometrics CoolSnap ES CCD camera (1392 x 1040 pixel imaging array) was utilized for images collected between 500 and 780 nm due to its high resolution capabilities. Above 780 nm, a Photometrics Evolve CCD camera (512 x 512 pixel imaging array) was employed due to its greater quantum efficiency at longer wavelengths. At intermediate wavelengths (600 to 800 nm), the two cameras yielded similar particle spectra. In both the dark field and
DIC modes, the microscope’s zoom knob was set to 1.6×, and a 100× objective was used.

To collect data at specified wavelengths, a set of bandpass filters from Thorlabs, Inc. (Newton, NJ) were employed. Each filter had a central wavelength in the range of 500 to 900 nm and a full width at half maximum (FWHM) bandpass region equivalent to 10 nm. Filters were placed between the condenser and the sample slide. The sample slide was supported by a rotating stage, and the actual orientation of the slide was determined by focusing the microscope on the copper grid. The nanorods of interest were readily found after each rotation of the stage through the use of permanent and easily identifiable landmarks on the substrate. All data were analyzed with ImageJ.

In dark field mode, the microscope utilized a Nikon Plan Fluor 100× 0.5-1.3 oil iris objective with its numerical aperture (NA) set to 0.7 and a Nikon dark field condenser with a 1.43-1.20 NA in oil. A polarizer was placed between the condenser and the bandpass filter. To excite the brightest intensity of a single nanorod, the longitudinal axis was aligned parallel to the polarizer. The darkest intensity was examined by orienting the longitudinal axis perpendicular to the polarizer. For the dimer and for spaced nanorods, the two modes were studied by aligning the interparticle axis either parallel or perpendicular to the polarizer. In image analysis, a circular area immediately adjacent to each feature was chosen for the background readings for both DIC and dark field microscopies.

The differential interference contrast (DIC) mode required a Nomarski prism and polarizer on either side of the sample plane, as well as a Nikon 100× 1.40 NA Plan Apo
VC oil immersion objective and a 1.40 NA Nikon oil immersion condenser.\textsuperscript{35-37} Because the prism is oriented at a 45° angle to the polarizer, the two wave fronts are oriented at 45° and 135° to the polarizer. The bright and dark modes of a nanorod are examined by aligning the nanorod’s longitudinal axis along one of the wave fronts, not the polarizer. After the wave fronts pass through the sample plane, they are recombined by the second Nomarski prism (oriented at 135° to the polarizer) before exiting through the analyzer (oriented perpendicular to the polarizer). By combining the two wave fronts, an interference pattern is generated, and objects such as nanoparticles take on a 3-dimensional shadow appearance with a bright side and a dark side. Thus, DIC can be used to collect either intensity or contrast data.

**DIC Simulation**

A home-written C++ computer program using an established DIC point spread function\textsuperscript{28} was utilized to simulate a single nanoparticle or a pair of nanoparticles separated by at least 20 nm. The simulation represents the expected 2D DIC image at the longitudinal SPR wavelength. The shape, size, location, and orientation of the nanoparticle(s) were read into the program before running each simulation. DIC images of the nanoparticle(s) were output as a 1 \( \mu m \times 1 \mu m \) matrix with grid spacing of 10 nm.

The DIC point spread function is a function of: the shear distance, the phase bias applied on the two illumination beams, and the point spread function for the transmitted light optics under coherent illumination of the microscope.\textsuperscript{28} In the simulation, the shear distance was assumed to be 100 nm. The phase bias was assumed to be 90°. The phase
delays of the ordinary and the extraordinary illumination beams caused by the nanorod were assumed to be $0^\circ$ and $30^\circ$, respectively. A 2D Gaussian approximation was utilized to model the point spread function of the transmission microscope. With our microscope, single sub-diffraction limit particles have an apparent diameter of ~600 nm at a wavelength of 660 nm. Assuming the particle image represents the 99% confidence limits for the true central position of the particle, the image has a width of $6\sigma$. As a result, $\sigma$ is approximately 100 nm. This value agreed well with the equation for the paraxial point spread function of a wide field fluorescence microscope that imposes peak matching, $\sigma = 0.21\lambda/\text{NA}$.\textsuperscript{46} For the case of $\lambda = 660$ nm and $\text{NA} = 1.40$, $\sigma$ is equivalent to 99 nm.

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Table 1. Characteristics of the four features studied. For the dimer and proximate nanorods, “l” and “r” refer to the individual nanorod that appears to the left or right of the feature’s center point in the TEM images. The full length of the dimer was measured to be 141 nm. The center-to-center distance of the proximate nanorods was 227 nm (180 nm tip-to-tip). The expected position of the longitudinal SPR is based on a formula from reference 25: \( \lambda_{\text{max}} = (53.71 \cdot R - 42.29) \cdot \epsilon_m + 495.14 \), where \( R \) is the aspect ratio given in the table, and \( \epsilon_m \), the dielectric constant for the medium, is 2.3. An averaged aspect ratio was used for the dimer, because the nanorods are not aligned tip-to-tip.
Figure 1. TEM, DIC, and dark field images of the four nanorod features investigated in this study. P: proximate nanorods; D: dimer; N1 and N2: single, isolated nanorods.
Figure 2. Profiles for isolated nanorod, S1: A) Normalized dark field spectra with the longitudinal axis aligned parallel (solid line) and perpendicular (dashed) to the polarizer. Inset: TEM image of nanorod S1. B) DIC intensity spectra at the bright and dark wave fronts. The mean background has a value of one. BWF and DWF stands for bright and dark wave fronts, respectively. Dark and Bright refer to the intensity on the dark and bright sides of the nanorod. C) DIC intensity profiles of the dark (solid) and bright (dashed) sides during 180° rotation in 5° increments at a wavelength of 640 nm. At 0°, 45°, and 135°, respectively, the nanorod is aligned with the polarizer, dark wave front, and bright wave front. D) DIC images at 45°, 75°, 105°, and 135° (above), and DIC simulated images at the same angles (below).
Figure 3. Profiles for proximate nanorods, P. (A) Normalized dark field spectra with the interparticle axis aligned parallel (solid) and perpendicular (dashed) to the polarizer. Insets: TEM image of P, and schematic of optimal nanorod alignment with the polarizer (arrow). (B) DIC intensity spectra with the interparticle axis aligned perpendicular (90°, \( \perp \)) and parallel (180°, \( // \)) to the polarizer. Inset: Schematic of optimal nanorod alignment with the wave fronts under DIC microscopy. (C) DIC intensity profiles under a full 360° rotation at 680 nm. (D) DIC images (above) and simulations (below) with the interparticle axis at angles of 15°, 45°, 135°, and 165°.
Figure 4. Profiles for the dimer, D: A) Normalized dark field spectra with the interparticle axis aligned parallel and perpendicular to the polarizer. Inset: TEM image of feature D. B) DIC intensity spectra with the interparticle axis aligned parallel to the bright and dark wave fronts. C) DIC intensity profiles from 180° rotation in 5° increments at 680 nm.
Appendix of Supporting Information

Figure S1. UV-visible-near IR absorption spectrum of the original gold colloid. The two absorption peaks have maxima at 521 and 627 nm.
Figure S2. (A) In dark field rotational studies of nanorods, a nanorod appears at its brightest when aligned parallel to the incoming polarized light. Traditionally, one spectrum is collected when the longitudinal axis is aligned parallel to the polarizer (arrow), and a second spectrum is collected with the longitudinal axis aligned perpendicular to the polarizer. (B) In DIC microscopy, a nanorod appears completely white or black when the longitudinal axis is aligned with the respective wave front (bold arrows). The wave fronts are offset from both the polarizer (solid line) and the analyzer (dashed) by $45^\circ$. 
Figure S3. A) Dark field and B) DIC intensity spectra for the single, isolated nanorod, N2. Inset: TEM image of nanorod N2. It is noteworthy that the observed longitudinal SPR of N2 was red-shifted in comparison to N1, as predicted by the formula used in Table 1, but the shift is more apparent under DIC microscopy than under dark field microscopy.
CHAPTER 4. THE INFLUENCE OF POLARIZATION SETTING ON GOLD NANOROD SIGNAL AT NON-PLASMONIC WAVELENGTHS UNDER DIFFERENTIAL INTERFERENCE CONTRAST MICROSCOPY

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Abstract

Researchers rely on a variety of microscopic techniques for observing and tracking anisotropic nanoparticles in real time experiments. This technical note focuses on the optical behavior exhibited by gold nanorods at non-plasmonic wavelengths under differential interference contrast microscopy (DIC). Intense diffraction patterns appear at non-plasmonic wavelengths, and the behavior of these patterns can be altered by adjusting the surrounding medium or the polarizer setting. Such patterns are absent when linear and crossed polarizations are utilized. Making polarization adjustments is important in DIC microscopy, because it affects bias retardation and image contrast. The non-plasmonic diffraction bands that were observed could potentially be exploited for rotational tracking, but more importantly, researchers should exhibit care in selecting a nanorod sample and the polarization setting when working with DIC microscopy.
Introduction

After a decade of strong interest in nanoparticles, researchers are still flocking to this class of probes for use as a tool in chemistry, physics, biology, and medicine. Noble metal nanoparticles garner attention due to the well-described phenomenon known as localized surface plasmon resonance (LSPR).¹ Nanoparticles with an anisotropic shape (e.g. nanorods) are especially popular by researchers who desire to elucidate probe orientation as part of their experiments. In many cases, such experiments can be conducted with dark field microscopy under polarized illumination. When the nanoparticle’s surrounding medium is complex, researchers prefer instruments that provide better lateral resolution, shallower depth of field, and full numerical aperture from both the objective and condenser, such as differential interference contrast microscopy (DIC). However, it is important to note that DIC and dark field rely on different components and principles for image generation.

The ideal nanoparticle colloid is composed of particles that are uniform in particle shape and size. Minor discrepancies in particle shape or size can be critical to single particle experiments, especially when electron microscopy is not a viable option. Another common but rarely discussed concern is the selection of the proper wavelength for imaging nanoparticles. Ideally, spectroscopists want to use the wavelength that corresponds to the LSPR, but they may be limited to specific wavelengths due to instrument design or filter availability. Moreover, because of heterogeneity in nanoparticle morphology, single particle tracking experiments are oftentimes carried out at non-ideal wavelengths.
As our understanding of nanoparticles continues to grow, it is also important to understand the theory and the limitations of the instruments we use for experimental studies of nanoparticles. In many cases, it is simply a matter of revisiting theory that was developed prior to the widespread development of nanoparticles, but in others, it may mean the development of new theory. The purpose of the current article is to investigate the influence of polarization on the observed optical behavior of an isolated gold nanorod affixed to a substrate. Linear, crossed, elliptical, and circular polarizations are discussed. This technical note also provides practical advice for imaging nanorods under DIC microscopy, which has been previously demonstrated to be a highly capable, but more complex tool for single particle tracking.2-8

Either elliptical or circular polarization can be produced with a DIC microscope, but some introductory background information is necessary to explain how this is accomplished. DIC microscopy is a technique that relies on interferometry for producing high-contrast images of nanoparticles against a low-contrast background. Older DIC microscopes were designed such that the polarizer and analyzer were fixed in a crossed position. To adjust image contrast, one Nomarski prism was translated back and forth while the second prism remained fixed. In modern de Sénarmont-type Nomarski DIC microscopes, contrast is adjusted by rotating the polarizer, which is mounted on a housing that also holds a quarter-wavelength retardation plate (QWRP). Figure S1 provides a detailed description of the QWRP-polarizer housing. The two types of DIC microscopy are related by the following relationship.9
\[ \psi_b = \frac{2\pi}{\lambda} \Delta_b \]  

where \( \psi_b \) and \( \Delta_b \) are bias retardation in units of degrees (or radians) and units of length, respectively, while \( \lambda \) is the wavelength of light. For the remainder of the discussion, the focus will be on de Sénarmont Nomarski DIC, since it is widely and commercially available, but the theory presented herein applies to either variant of DIC.

Bias retardation is one of several key variables relevant to image formation and polarization in DIC microscopy. Before reaching the condenser, light passes through a birefringent Nomarski prism, where it is split into two orthogonal (and independent)\(^{10}\) wavefronts that are rotated 45° relative to the polarizer.\(^{11}\) These two coherent beams of light undergo a lateral shear that is less than the diffraction limit and is \(~100 – 200\) nm in length.\(^{9,10,12}\) Thus, the illumination areas of the two beams are slightly offset as they pass through the sample plane. Here, the lateral shear is also larger than the nanorod. Behind the objective, a second Nomarski prism recombines the two wavefronts, causing them to interfere and form a shadow-cast image that is dependent on the amplitude and phase difference of the two independent beams.\(^{10,11}\) Assuming no bias is intentionally introduced, the two beams will have identical path lengths.\(^{10}\)

To intentionally introduce bias and adjust image contrast, a birefringent QWRP is placed in the light path after the polarizer. The fast axis of the QWRP is fixed at a 90° angle to the analyzer’s transmission axis. When the polarizer’s transmission axis is also aligned at 90° to the analyzer, the polarizers are crossed, no bias is introduced, and the background is at maximum extinction.
Upon rotating the polarizer, bias retardation is introduced as a phase shift between the two wavefronts passing through the microscope. The light that emerges from the QWRP is no longer linear, and one wavefront is phase retarded relative to the other. If the polarizer is rotated 45° (i.e. a full quarter-wavelength), the light becomes circularly polarized; however, DIC is rarely used in this setting due to the poor sample contrast encountered there. At intermediate polarizer angles, the light is elliptically polarized. The polarization can be either right or left-handed, and the shear can be positive or negative, depending on which direction the polarizer is turned relative to the QWRP. The polarizer setting is not typically reported in the literature.

When transmitted light interacts with an object in the light path, the difference in the optical path lengths of the two beams is described by: \(^9\)

\[
\Delta = \Delta_0 + s \frac{d\delta}{dx}
\]

(2)

\[
\frac{d\delta}{dx} = (n_m - n)dt
\]

(3)

where \(\Delta\) is the difference in optical path length of the two beams, \(s\) is lateral shear, \(n\) and \(n_m\) are the refractive indices for the object and surrounding medium, and \(dt\) is the change in object thickness corresponding to a change in lateral distance of \(dx\). The differential, \(d\delta/dx\), reveals that a change in refractive index or particle thickness can introduce a phase shift. Samples that introduce a phase shift are known as phase objects.\(^{12}\)
Previous studies of gold nanorods under DIC microscopy have focused on LSPR wavelengths and nanorod angles that run parallel to the two wavefronts.\textsuperscript{3,6} As a nanorod is rotated at the LSPR wavelength, the amplitude of the bright and dark components of the DIC image varies sinusoidally as the LSPR axis aligns with either of the wavefronts. At the midpoint between the wavefronts, nanorod images appear equally white and black. Isotropic particles appear equally white and black at all rotation angles.

**Experimental Procedures**

**Materials and Sample Preparation**

Colloidal gold nanorods with hemispherical caps were purchased from Nanopartz (Loveland, CO). To prepare the nanorods, 0.1 mL of colloidal gold underwent centrifugation for 10 minutes at 5500 rpm, followed by removal of the liquid layer and resuspension in 0.1 mL of 18.2 MΩ Milli-Q water. The nanorods were then functionalized with 1 µL of 20 mM NHS-PEG-thiol in dimethyl sulfoxide. The final solution was mixed on a rotating mixer for more than three hours before being applied to the substrate.

The substrate was a 25 x 25 x 1 mm\textsuperscript{3} microscope slide from Fisher Scientific. Registration marks were added to the slide to serve as landmarks for locating the same nanorods under the various microscope settings and modes. The slide was functionalized with poly-L-lysine for capture of the nanorods. A 7 µL aliquot of thiolated gold nanorods was drop cast onto the functionalized slide and immediately covered with a No. 1 coverslip from Corning. To prevent
evaporation of the medium, the edges of the cover slip were coated with nail polish. After collecting data of the nanorods in water, the cover slip was removed, and the water was allowed to evaporate. The slide was then coated with one drop of Carl Zeiss Immersol 518F immersion oil. A clean cover slip was placed on the slide, and the edges were coated with nail polish. Immersol 518F was also used as the lens immersion oil.

**Instrumentation**

Optical microscopy was conducted on a Nikon Eclipse 80i upright microscope connected to a 12V-100W halogen lamp. A magnification of 160X was used in all modes. Images were collected with a Hamamatsu C11440-10C, Orca-Flash 2.8 CMOS camera with a 1920 x 1440 pixel array (43.6 x 32.3 µm²). HCImage software from Hamamatsu was employed for operating the camera. A gain setting of 64 (2x, actual gain) was used for DIC mode, whilst a gain setting of 255 (8x, actual) was used for the dark field mode. ImageJ and Matlab version 7.11.0 (R2010b) were utilized for image analysis.

For the DIC mode, the lenses were a 1.40 NA Nikon oil immersion condenser and a Nikon 100X 1.40 NA Plan Apo VC oil immersion objective. The two Nomarski prisms, quarter-wavelength retardation plate, analyzer, and polarizer used for this mode were standard components from Nikon. Methods for the linear and crossed polarization modes are located in the Supporting Information (SI).

For all modes, the slide was set upon a Rotating Stage Z150 from Märzhäuser Wetzlar (Wetzlar, Germany). The rotating stage was turned in 7.5° or 10° increments, as noted, to collect spectra at multiple orientations.
To perform spectroscopy, bandpass filters from Thorlabs, Inc. were placed in the light path between the condenser and the polarizer. The filters ranged from 500 to 760 nm, and each had a full width at half-maximum (FWHM) of 10 nm.

The original gold colloid was tested with a Varian Cary 300 UV-Visible Spectrophotometer over a range of 300 – 900 nm. An aliquot was also inspected with a Philips CM-30 transmission electron microscope (TEM) operated at 200 kV in order to determine the average size and shape of the nanorods in the sample. Images were captured using a Gatan Orius SC 1000 CCD camera with 11 Megapixel (4008 X 2672) resolution and Gatan DigitalMicrograph.

**Results & Discussion**

The gold nanorods were initially characterized by UV-Visible spectrophotometry and TEM. The colloid displayed two distinct peaks, centered at 521 and 627 nm (Figure S2 in the SI). The strength of the signal at 521 nm suggests that the colloid contains a mixture of spheroids and nanorods. TEM analysis confirmed that the sample primarily consisted of nanorods with aspect ratios in the range of 2.0 – 2.2.

The expected position of the absorption maximum due to the dipolar, longitudinal LSPR wavelength is related to aspect ratio and the dielectric constant as follows:

\[
\lambda_l = (53.71R - 42.29)\varepsilon_m + 495.14
\]  

(4)
where $\lambda_l$ is the wavelength of the longitudinal LSPR, $R$ is the nanorod’s aspect ratio, and $\varepsilon_m$ is the dielectric constant of the surrounding medium. For nanorods with an aspect ratio between 2.0 and 2.2, $\lambda_l$ ranges from 610 to 630 nm for water ($\varepsilon_m = 1.77$) and from 645 to 670 nm for immersion oil ($\varepsilon_m = 2.30$). As explained below, the optical signal detected by a microscope is not merely a function of absorption. Instead, the signal varies amongst the techniques and polarizations that are employed.

The discussions throughout this technical note are based on spectra collected at multiple wavelengths and orientation angles. Data were collected by rotating the sample slide of fixed nanoparticles to different orientations and taking images with bandpass filters inserted in the light path. It should be noted that a spectrophotometer may be used to generate continuous wavelength spectra in dark field and simple polarization modes; however, there is no simple way to use a spectrophotometer with a DIC microscope due to DIC’s principle of interferometry. Therefore, all spectra were taken with bandpass filters to ensure consistency in the data.

**Linear and Crossed Polarization**

Images under linear and crossed polarizations were collected as a means of aiding in the interpretation of the DIC findings. A full discussion of these polarizations can be found in the SI. In brief, linear polarization produced a longitudinal LSPR at ~650 nm that repeated every 180° (Figure S3). At an angle of 90°, the nanorod came closest to being parallel to the polarizer. A weak, transverse LSPR peak (520 – 540 nm) appeared at angles near 0° and 170°. Under crossed polarization, the signal pattern
repeated itself every 90°. As shown in Figure S4, the longitudinal LSPR appeared at 660 nm at an angle ~130°, a shift of ~40 – 45° from the linear mode.

**Elliptical and Circular Polarization**

Using the DIC mode, the polarizer was initially set at an angle of +8° (± 0.5°) to the crossed polarizer position (CPP), to approximately match the angle used in Stender, et al. CPP settings are explained in Figure S1. Figure 1 compares a nanorod’s bright-side intensity in water versus oil. All values are >1, since the data are normalized to the average background. (The complementary dark-side intensities are <1 and provided in Figure S5.) The nanorod’s spectral behavior is complicated by the presence of additional bands of intensity at non-plasmonic wavelengths that are due to higher-order diffraction. In Figure 2A, the highest bright-side intensity occurs at 640 nm between angles of 120° and 135°. Because this feature has the strongest intensity and is ~45° shifted from the longitudinal LSPR under dark field microscopy, it is the longitudinal LSPR. This feature is also the zeroth-order diffraction, since the other features maintain symmetry relative to it. In Figure 2A, the band is ~60° wide at the FWHM, but it is closer to 90° wide in Figure S5A. It is difficult to point out the transverse LSPR from Figure 2A, but a broad feature appears in Figure S5A around 540 nm from 60° to 110°. Two symmetric bands arise at 620 and 660 nm to the left of the longitudinal LSPR peak (gray dotted lines in Figure 2A and S5A). The intensity is stronger at 620 nm, but the shapes are similar. Another band appears at 560 nm in a similar position. Two symmetric bands also appear at 580 and 700 nm to the right of the LSPR band (orange
dashed lines). It is not understood why the bands appear at these specific wavelengths, but as described below, the position of these bands can be altered by changing the medium or the polarizer setting.

After inspection in water, the medium was switched to immersion oil (Figure 2B and S5B). Oil’s higher dielectric constant caused a red-shift in the longitudinal LSPR, as described by equation (4). Signal intensity also increased. The strongest bright-side intensity appears again between 120° and 130°. The exact longitudinal LSPR wavelength is not as obvious, because the signal shape between 660 and 680 nm is different from the peak observed for water. Strong bands appear between 60° and 110° at 60 nm intervals (560, 620, 680, and 740 nm); another set of bands appear between 140° and 180° at 540, 580, 660, and 700 nm. All of these features are rotated by 90° in Figure S5B.

Switching the polarizer to a setting of +24° creates a new diffraction pattern with fewer distinct diffraction bands (See SI Figure S6). The maximum bright-side intensity appeared between 100° and 130° at 680 nm. A strong, secondary peak appears at 660 nm between 180° and 200°. Finally, a broad feature arises between 20° and 70° from 540 to 640 nm.

To further investigate the nanorod’s optical behavior, the nanorod was fixed at 130°, while the polarizer was adjusted to several equivalent settings on either side (±) of the CPP. These paired spectra are shown in Figure 2 A-C and SI Figure S7. In Figure 2A, the bright-side spectra at ±2° are shown to exhibit shape and magnitude symmetry centered around 670 nm. At +2°, the strongest signal appears at 680 nm, but it switches
to 660 nm at -2°. The dark-side intensities are basically identical. In the remaining figures, the paired spectra maintain the shape and magnitude symmetry centered around 660 nm. At positive polarizer settings, the intensity at 680 nm continues to dominate the bright-side spectra, but at negative polarizer settings, the dominant bright-side wavelength is variable.

With the polarizer at ±8° (Figure 2B), a secondary peak appears at 720 nm. At +8°, the peak is in the bright-side intensity, while at -8°, it appears in the dark-side signal. As the polarizer is rotated to higher positive settings, the secondary peak disappears, and the peak at 680 nm develops a gradual slope towards higher wavelengths. At negative polarizer settings, the dark-side peak at 720 nm shifts to 680 nm. In general, signal intensity decreases at higher polarizer settings. The dark-side signal gradually gains dominance over the bright portion of the signal, particularly at the longitudinal LSPR, due to the polarizer and analyzer being moved towards alignment.

In Figure 2 D-F, the signal intensities at 680 nm are shown for 200° rotations at the three positive polarizer settings displayed in Figure 2 A-C. At +2° (Figure 2D), the bright-side intensity is centered at ~130°, whilst the dark-side is centered at 40°. Because the background is close to maximum extinction, the bright-side signal is more intense than the maximum dark-side signal. At +8° (Figure 2E), the maximum bright and dark-side intensities are similar in amplitude to each other. The dark-side intensity becomes dominant by +24° (Figure 2F), and the centers of the two peaks have shifted ~10° – 15° to the left.
Spectral and rotational profiles were also collected with the polarizer at 45° (Figure S8). At this setting, the polarization is circular, but it continues to follow the trend observed under the various elliptical settings.

As stated above, de Sénarmont DIC microscopes utilize three birefringent components. It is possible that the prisms could introduce higher-order diffraction if they were offset, since this alignment would alter the polarization. Because both prisms remained fixed throughout the experiment, the QWRP was the component vital for inducing changes in polarization.

Based on the findings, it is clear that certain polarizer settings are preferred when the goal is to acquire quantitative data from anisotropic nanoparticles, such as nanorods. First, the polarizer should not be set >22.5° (halfway point to quarter-wavelength). At angles < 22.5°, the rotational profile of the longitudinal LSPR remained in good alignment with the dark field profile. Once the polarizer was rotated past 22.5°, DIC’s rotational profile drifted out of alignment. Image contrast also became poor, making it difficult to focus on the nanorod. Some researchers prefer to align the polarizer, such that the strongest white and black signals are equal in amplitude. To achieve this goal, researchers should position the polarizer between ~12° – 22°. Switching between the positive and negative sides of the CPP can cause confusion as to the actual LSPR wavelength. It seems advisable to stay at least 2° away from the CPP and to consistently work on one side of it.

Several additional recommendations are worth noting. When working with bandpass filters, a nanoparticle’s LSPR wavelength should lay within the range of the
filter’s FWHM. Thus, it is crucial to work with a highly uniform nanoparticle sample.

Second, proper alignment of the optical components is critical for DIC microscopy. Even a minor misalignment in the polarizer, analyzer, or QWRP could cause a significant change in polarization and the observed behavior of a nanorod. These three components should be calibrated before starting data collection. Third, researchers should make it a common practice to document the polarizer or bias setting used for DIC experiments. Finally, further development of wavelength-dependent DIC imaging theory is obviously necessary for describing the diffraction behavior of anisotropic and plasmonic particles with sizes below the lateral shear. Previous DIC theory has focused on isotropic samples with diameters greater than the lateral shear.9,12

The presence of diffraction bands at non-plasmonic wavelengths is not necessarily a detriment. The diffraction bands can be manipulated by adjusting the polarization, but none of the settings used herein were able to completely remove the bands. It is important to not confuse a diffraction band with a LSPR band. For small nanorods, such as those employed here, only two bands are of significant interest: the transverse and longitudinal LSPR. These two bands run parallel to the optical axes of nanorods and are 90° out of phase with each other. By adjusting the polarizer, it becomes evident where the actual longitudinal LSPR band is centered, because the LSPR wavelength retains the highest signal intensity relative to other wavelengths. Moreover, the wavelength of the longitudinal LSPR does not shift, as with the diffraction bands. Finally, it seems reasonable that by setting the polarizer at a small angle to the CPP, the various diffraction bands could be utilized to obtain quantitative
orientation information about nanorods. However, the investigator would need to correlate the difference between the diffraction and zeroth-order bands to determine the nanorod’s true orientation.

**Conclusions**

In conclusion, polarization is a useful and powerful tool for determining the orientation of anisotropic nanoparticles. However, different microscopy techniques rely on distinctly different principles for gathering quantitative information about samples. Dark field microscopy remains a tried and true mode for studying nanorods in simple environments, but an increasing number of researchers are utilizing DIC microscopy when researching complex environments. At LSPR wavelengths, these two techniques provide similar information, but at non-plasmonic wavelengths, it becomes obvious that they are quite different in their approach to detection. By properly aligning the microscope (regardless of mode) and documenting the optical settings, confusion will be reduced when it comes to collecting quantitative information about anisotropic nanoparticles.

**References**


Figure 1. Bright-side intensity nanorod profile under DIC with QWRP at +8° (±0.5°). Spectral data collected at 7.5° increments from 0° – 195°, using wavelengths from 500 – 760 nm in 20 nm steps. Contours represent an increase in normalized intensity by steps of 0.05 AU. Data were normalized in relation to the average background, which has a value of 1 AU. Gray dotted and orange dashed lines represent higher-order diffraction bands. A) Nanorod in water. B) Nanorod in oil.
Figure 2. DIC spectra were collected at equivalent angles on either side of the CPP with the nanorod aligned to 130° and oil as the medium. Dashed lines represent spectra from the – (left) side of the CPP, and solid lines represent spectra from the + (right) side. Profile intensity >1 (<1) represents bright side (dark side) intensity of the nanorod. Polarizer set to: A) ± 2°, B) ± 8°, C) ± 24°. DIC rotational profiles were also collected at a wavelength of 680 nm, with the following polarizer settings: D) + 2°, E) + 8°, F) + 24°. Profile intensity >1 (<1) represents bright side (dark side) intensity.
Appendix of Supporting Information

Methods: Linear and Crossed Polarizations

Under dark field mode, the lenses consisted of a Nikon dark field condenser with a numerical aperture (NA) of 1.42 – 1.20 in oil and a Nikon Plan Fluor 100X 0.5 – 1/3 oil iris objective set to a NA of 0.7. A linear polarizer from Edmund Optics was fixed in place before the condenser.

To obtain the cross-polarized mode, the same objective, condenser, and analyzer were used as for the DIC mode, but the other DIC components were removed. The linear polarizer from Edmund Optics was used again, but no beam splitter was utilized, unlike traditional polarized light microscopy. The linear polarizer was set at an angle of 90° to the analyzer to achieve crossed polarization.

Results and Discussion: Linear Polarization.

Linear polarization is commonly used in conjunction with nanorod imaging under dark field microscopy, primarily to obtain information about nanorod geometry.1-5 Dark field microscopy reveals nanorod orientation by measuring the amount of scattering from an individual nanorod as functions of illumination wavelength and angle relative to the polarizer. In order to collect only scattered light, the condenser is designed with a light stop that prevents rays of light from passing directly through the center of the condenser. Sample detection is therefore accomplished via light rays that pass along the periphery of the condenser at oblique angles, interact with the sample, and reach the detector.
In this experiment, dark field images were captured in the visible range with immersion oil used as the medium. Normalized signal intensity is presented in Figure S3. A single, isolated nanorod of interest was found that displays a maximum signal at 640 nm. A rotational spectral profile was collected at 10° increments, and the angles were oriented afterwards, such that the greatest normalized intensity occurred at an angle of 90°. Thus, at 90°, the nanorod comes closest to being aligned parallel with the polarizer. Based on the shape of the intensity profiles (particularly Figure S3B), the actual longitudinal LSPR appears to lie at ~650 nm, since the intensities at 640 and 660 nm are roughly equivalent at all angles. A weak secondary peak (520 – 540 nm) that corresponds to the transverse LSPR appears at angles near 0° and 170°. This finding confirms that the long axis of the nanorod is well-aligned with the direction of the linear polarizer at 90°, and it is orthogonal to the polarizer at 0°. It should also be noted that the line width of the longitudinal LSPR peak remains relatively constant across all angles from 20 - 160°.

Finally, for a perfect dipole, no signal should be observed at the longitudinal LSPR under linearly polarized dark field conditions. However, it is possible for a particle to couple with the substrate, thereby producing a LSPR that is not purely dipolar. Such appears to be the case here, since some signal is present at the longitudinal LSPR at 0° and 180°. More importantly, the change in normalized signal intensity at the longitudinal LSPR (640 nm) under rotation of the nanorod fits the behavior expected of a single nanorod under linear polarization.
Crossed Polarization.

Before discussing elliptical and circular polarization, it is helpful to consider a simple crossed polarization scheme that does not utilize a beam splitter. The polarizer was set in the same orientation relative to the stage as with dark field, and the analyzer was set at a 90° angle to it. Thus, the nanorod was parallel to the polarizer at an angle of 90° and to the analyzer at 180°. Because the polarizer and analyzer are fixed in a crossed position, the background remains at maximum extinction at all times, and nanorods exhibit an intensity that varies with angle, similar to dark field. Since no light stop is employed in this mode, this technique detects both scattering and absorption.

As with dark field mode, the nanorod was observed only under immersion oil, and the signal intensity was normalized. The signal pattern repeats itself every 90° under this mode, thus the nanorod was only imaged from 70° – 180°. As shown in Figure S4, the longitudinal LSPR appears at 660 nm, and the FWHM is ~70° wide. The center of the maximum signal band is at ~130°, a shift of ~40 – 45° from the dark field mode. The longitudinal and transverse LSPR should produce their strongest signal at the same angle, but it is difficult to pinpoint the transverse LSPR under this mode.

References


Supplemental Figures

**Figure S1.** The Quarter Wavelength Retardation Plate (QWRP) used in this study. The QWRP is mounted in a semi-permanent position at the top of the housing. The polarizer is directly underneath the QWRP and can be adjusted to the right or to the left with the aid of the thumb screw. The thumb screw can also be utilized to temporarily fix the polarizer in place. The position of the polarizer can be identified by a vertical pipe with a dot atop it. Vertical pipes are spaced at 5° intervals on the polarizer holder. The Crossed Polarizer Position (CPP) can be identified on the holder by a black, inverted V. In the current experiment, a shift of the polarizer away from the CPP along the right side is considered a positive (+) adjustment, while a similar shift along the left side is deemed a negative (-) shift. Accuracy of the polarizer reading is within ±0.5°. In the displayed image, the polarizer is set at an angle of +13° in relation to the CPP.
**Figure S2.** UV-Vis spectrum for the colloidal gold nanorods used in this study. The two maxima are located at 5121 and 627 nm. Inset: TEM image of gold nanorods from the original colloid.
**Figure S3.** Linear-polarization, dark field nanorod profile. A) Spectral data collected at 10° increments from 0° – 200°, using wavelengths from 500 – 760 nm in 20 nm steps. Contours represent an increase in normalized intensity by steps of 0.1 AU. Data were normalized in relation to the highest observed intensity. The nanorod is closest to being aligned parallel to the polarizer at 90°. B) Spectral cross-sections at 0°, 30°, 60°, and 90°.
**Figure S4.** Crossed-polarization nanorod profile. A) Spectral data collected at 10° increments from 70° – 180°, using wavelengths from 520 – 740 nm in 20 nm steps. Contours represent an increase in normalized intensity by steps of 0.1 AU. Data were normalized in relation to the highest observed intensity. The nanorod is closest to being aligned parallel to the polarizer at 90° and to the analyzer at 180°. B) Spectral cross-sections at 0°, 30°, 60°, and 90°.
Figure S5. Dark-side intensity nanorod profile under DIC with QWRP at +8° (±0.5°). Spectral data collected at 7.5° increments from 0° – 195°, using wavelengths from 500 – 760 nm in 20 nm steps. Contours represent an increase in normalized intensity by steps of 0.05 AU. Data were normalized in relation to the average background, which has a value of 1 AU. Gray dotted and orange dashed lines represent higher-order diffraction bands. Peaks are shifted by 90° relative to the peaks displayed in Figure 1. A) Nanorod in water. B) Nanorod in oil.
Figure S6. Elliptical polarization, DIC nanorod profile in immersion oil with QWRP at +24° (±0.5°). Spectral data collected at 10° increments from 0° – 200°, using wavelengths from 500 – 760 nm in 20 nm steps. Remaining details are identical to those in Figure 1. A) Bright-side intensity. B) Dark-side intensity. It is worth noting that the secondary maximum that appears at 540 nm in Figure S6B coincides well with the expected position of the nanorod’s transverse LSPR.
Figure S7. Details similar to Figure 2. Spectral profiles under DIC mode with the polarizer set to A) ± 12°, B) ± 18°, and C) ± 36°.
Figure S8. Details similar to Figure 2, with the polarizer set at ± 45° (circularly polarized conditions). A) Spectral profile. B) Rotational profile. It is worth noting that the bright-side intensity is quite weak at this setting, and the rotational peaks are centered at 100° – 110°. Overall, the findings here follow the trend observed at the previous polarizer settings.
CHAPTER 5. THE PLASMONIC BEHAVIOR OF SINGLE GOLD DUMBBELLS AND SIMPLE DUMBBELL GEOMETRIES

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Abstract

Dumbbell-shaped nanoparticles are similar in size to their nanorod counterparts, but their optical properties have not been studied as extensively as the nanorod. In this paper, the spectra of a single dumbbell, several dumbbell dimers, and a pentamer were collected experimentally and compared with simulated spectra. Surface charge density plots were also obtained in order to elucidate the nature of the plasmonic modes. The dumbbell is shown to be a particle that acts as a transition from the nanorod to the nanosphere. Because the dumbbell shape allows adjacent particles to interlock like
puzzle pieces, dumbbells can be thought of as optical building blocks that can combine into designs that are capable of supporting localized hot spots, Fano resonance, and tunable plasmon peaks.

**Introduction**

Gold nanorods are a remarkable and versatile platform for experimentation on the nanoscale. Some of the more common applications for nanorods include: bottom-up self-assembly,\(^1\)\(^-\)\(^6\) orientation-based sensing,\(^7\)\(^-\)\(^{12}\) redox reactions,\(^13\)\(^-\)\(^{15}\) and tracking within biological environments\(^16\)\(^-\)\(^{21}\). In comparison, dumbbell-shaped nanoparticles, which look like two spheres connected to the ends of a cylinder, have not proven to be a widely-utilized nanoparticle to date. However, the Liz-Marzán group has recently investigated the optical effects of gold dumbbells coated with silver, and they have also shown that gold dumbbells can assemble into interesting structures due to steric hindrance effects.\(^22\)\(^-\)\(^{24}\) Similarly, we have observed in the course of this work that dumbbells tend to interlock with each other as opposed to lining up side-to-side or tip-to-tip as nanorods do. Such configurations of dumbbells should present interesting optical behavior, such as mode-mixing (and hence Fano resonance), hot spots, and highly mobile plasmon peaks, all of which are influenced by particle geometry and polarization angle. Thus, the optical behavior of single gold dumbbells and simple dumbbell aggregates deserves a closer investigation at this time.

Gold dumbbells can be formed through one of several synthetic routes.\(^25\)\(^-\)\(^{28}\) Some of these pathways are nearly identical to that of nanorod synthesis, and as a result,
the final size and shape of the particles is dependent upon proper timing during key steps in the synthesis.\textsuperscript{27,28} Moreover, the crystallography of nanorods was carefully determined in a pair of recent studies.\textsuperscript{29,30} Interestingly, the facets along the sides differ from the facets near the nanorod tips. Facets near the tips have a higher index (i.e. have higher energy and form a more open structure) than the facets along the sides.\textsuperscript{27-30} Higher index regions are able to grow at a faster rate than the sides of a nanorod, and under the proper synthetic conditions, dumbbell formation is highly favored. Previous work has also shown that the general appearance of the spectral profiles of colloidal dumbbells is quite similar to that of colloidal nanorods; however, the formation of lobes does cause a red-shift in the transverse and longitudinal localized surface plasmon resonance (LSPR) wavelengths (i.e. shift to lower energy).\textsuperscript{27}

Furthermore, we have observed that cetyltrimethylammonium bromide (CTAB) coated dumbbells often form small aggregates when prepared on substrates for transmission electron microscopy (TEM). Because of the shape of dumbbells, they don’t simply line up side-to-side as do nanorods. Instead, they tend to interlock like puzzle pieces, with the rounded lobes of one dumbbell snapping into the notched section of its neighbor.

The optical behavior of single nanorods and of simple nanorod geometries with respect to polarization angle has been well explored,\textsuperscript{6-12} but such an in-depth study of dumbbells has not been previously reported. For the purposes of this paper, we investigated the optical characteristics of single dumbbells and simple dumbbell aggregates, in order to compare the optical properties of nanorods, spheres, and
dumbbells. We utilized both experiments and simulations for this study. To provide a complete story regarding the optical behavior of the aggregates, it was necessary to extend the wavelength range of the simulations deeper into the IR than could be achieved with the optical microscope.

**Results & Discussion**

Optical images were collected with a Nikon Eclipse 80i microscope using the differential interference contrast (DIC) mode. The principles of DIC microscopy have been explained in previous papers. In brief, DIC microscopy relies on a pair of crossed polarizers, a pair of Nomarski prisms, and a quarter-wavelength retardation plate to generate an image. Because light from the source is split into two orthogonal polarization beams before passing through the sample, it is possible to determine the orientation of anisotropic particles. Individual particles appear as black and white shadow-cast objects on a gray background. For an anisotropic particle, the intensity of the black and white portions of the signal are anti-correlated and varies as the particle is rotated in and out of alignment with the two polarization beams. Thus, when collecting spectra for an anisotropic particle, it is preferable to align the particle with one of the two polarization beams, because the longitudinal and transverse LSPRs will be aligned with opposing beams and appear as opposite colors (white vs. black). These beams will be referred to as the white or black polarization beam herein. In the case of idealized spheres, the black and white intensities remain constant during the particle’s rotation.
DIC microscopy was utilized instead of dark field microscopy for three important reasons. In a similar experiment involving nanorods on TEM substrates, it was shown that the dark field signal was negatively influenced by depolarization effects between the particle and the substrate, whereas DIC microscopy has no such problem.11 Furthermore, DIC microscopy has a shallower depth of field and a higher lateral resolution than dark field microscopy, thus the DIC signal is less affected by out-of-focus signal than dark field.31 Finally, dark field collects its signal from particle scattering, but for small nanoparticles with a LSPR in the wavelength range of 500 – 900 nm, the scattering signal from individual nanoparticles can be quite weak compared to the absorption signal.32,33 DIC also has its drawbacks. For example, it is common to incorporate a grating, a spectrometer, or a laser light source into a dark field microscope.34 However, it is not a simple task to incorporate any of these into a DIC microscope.21 Thus, it is difficult to improve the signal or decrease the background noise in a standard DIC microscope, such as the one used here.

Simulations were carried out using the Finite Element Method (COMSOL) in order to elucidate greater details about the optical behavior of dumbbell geometries. For the experiments, bandpass filters with a full width at half maximum (FWHM) of 10 nm were used to collect data. These filters covered 500 – 900 nm and were separated by 20 nm intervals (e.g. 500, 520, 540, etc.). For the rotational experiments, data were collected at either 5° or 7.5° angles, as reported below. To simplify the simulations, simulated dumbbells were designed to be uniform in size and shape even though the TEM images suggested minor differences between particles. As discussed below, this
assumption introduces slight differences in peak positions between model and simulation. However, the primary purpose of the model was to qualitatively describe the modes that were observed with the microscope.

**Single Dumbbell**

For the experiments, gold dumbbell particles were acquired from Nanopartz, Inc. Characterization with TEM revealed that the amount of tip overgrowth (i.e. lobe diameter) was variable between individual particles. A single isolated dumbbell was selected with significant lobe overgrowth and a longitudinal LSPR wavelength below 850 nm. This dumbbell was characterized as 91 nm in length and 30 nm wide at the midpoint with 40 nm wide angular-shaped lobes. The TEM image of this particle is shown as an inset in Figure 1.

Experimental data for the single dumbbell is given in Figure 1. The data are normalized to the average background value, which is assigned a value of zero. As a result, the black component’s intensity is assigned a negative value, and spikes in intensity along this polarization beam appear as dips in the intensity profile. The white side intensity appears as a positive value. The normalization process is described fully in the Methods section. In Figure 1, the longitudinal (transverse) axis was aligned with the white (black) polarization beam. At that angle, the dumbbell exhibited two peaks: at 840 nm (longitudinal LSPR) and between 540 – 560 nm (transverse LSPR). No other significant peaks are apparent in the profile.
In the first set of simulations (Figure 2), the model was used to compare the absorption spectra of seven different nanoparticles: 2 nanorods with different aspect ratios, 3 dumbbells with different lobe sizes and shapes, a pair of overlapping spheres, and a sphere. All particles were 91 nm in length. Exact dimensions of each particle can be found in the Supporting Information as Figure S1. The second dumbbell has angular lobes (red solid line in figure) and matches the experimental dumbbell from Figure 1. To resemble the experimental data, the signal intensity along the longitudinal axis is represented with a positive value (i.e. the white polarization beam in the DIC data in Figure 1), and the transverse signal is represented as a negative value (i.e. DIC’s black polarization beam). The first nanorod has a larger aspect ratio (3.0 vs. 2.3) and exhibits an 87 nm red-shifted longitudinal LSPR and a 6 nm blue-shifted transverse LSPR compared to the second nanorod. The first two dumbbells have longitudinal and transverse LSPR that are red-shifted in comparison to both nanorods, even though the dumbbells have an average aspect ratio that lies in-between the aspect ratios of the two nanorods. The dumbbell with rounded lobes undergoes a greater red-shift than the dumbbell with angular lobes. All three dumbbells display a multipolar mode along the longitudinal axis, which has previously been reported in overlapping spheres. As the rounded lobes grow in size (dumbbell #3 and the pair of overlapping spheres), both LSPR shift towards the position of the single LSPR for the sphere. As a result, dumbbells can be thought of as a transitional shape between the nanorod and the nanospheres.
Surface charge density (SCD) plots were also generated by the model to further explain the behavior of the single dumbbell with angular lobes (Figure 2 C-E). In the plots, red and blue represent equal and opposite charge (i.e. +ve and –ve). For polarization along the longitudinal axis, the dumbbell exhibits a dipolar mode at 835 nm and an octopolar mode at 545 nm. Figure 1E reveals a more complicated situation for transverse polarization at 540 nm. This mode is highly dipolar along the transverse axis, but weak spots or wrinkles appear in the surface charge wherever the lobes come into contact with the cylindrical section of the particle.

In addition, the dumbbell in Figure 1 was rotated with respect to the polarization field at the transverse LSPR wavelength in order to determine the optical properties of a dumbbell with respect to the polarization field. Images were collected at 5° intervals as the dumbbell was rotated a full 180°. Figure 3A reveals that the rotational behavior of the dumbbell is quite similar to the sinusoidal behavior observed previously with nanorods. As a reference, at 45° and 135°, the particles are aligned with either of the DIC’s polarization beams. Figure 3B shows the simulated data at 10° intervals for the dumbbell and for the first nanorod from Figure 1. To best match the experimental data, the simulated data were sinuized against the maximum data point within its own data set. (Only the white-side intensities are shown, since the black side intensities are identical.) Both profiles are sinusoidal, and in fact, both profiles are quite similar in shape and amplitude. These findings reveal that single dumbbells and single nanorods exhibit identical rotational behavior and could be used interchangeably for experiments that rely on observing a particle’s rotational behavior in real time. The dumbbell was modeled
and imaged at the longitudinal LSPR as well, but those data are presented in the Supporting Information as Figure S2, since the amplitudes of the dumbbell and nanorod are nearly identical.

To further study the optical distinctiveness between a single dumbbell and nanorod, a series of dumbbells with spherical lobes were simulated. These spectra and SCD plots are presented in Figure S3 of the Supporting Information. These data show that plasmonic behavior is greatly influenced by lobe size.

**Dimers**

Four distinctive dumbbell pairings were investigated in a fashion similar to the single dumbbell. These pairings are shown in Figures 4 and 6, and they include dumbbells that were aligned in end-to-end, L-shape, and side-by-side configurations. In each case, TEM imaging showed that the dumbbell pairs were separated by a distance of 1 – 2 nm, which is reasonable for CTAB-coated particles. Thus, classical mechanics applies to these cases. Dimer spectra collected at two orthogonal polarization angles: parallel (red line) and perpendicular (blue line) to the interparticle axis.

The first two dimer cases are presented in Figures 4 and 5. Figure 4A and 4B exhibit good agreement between the experimental and simulated spectra of tip-tip dumbbells tilted at an angle of 159°. Peaks of interest are labeled with Roman numerals, and their corresponding SCD plots are found in Figure 5A. For polarization along the interparticle axis (red arrow in TEM image), coupling between dumbbells is attractive in nature. The minor peak at 542 nm (indicated as (i)) is classified as a coupled higher-
order mode. Peak (ii) at 1005 nm is the bonding dipole mode and exhibits an obvious red-shift (~ 170 nm) compared to the longitudinal mode of the single dumbbell, due to attractive coupling across the dimer’s gap region. In contrast, when the polarization runs orthogonal to the interparticle axis (indicated by blue arrow), two peaks appear the spectra. Peak (iii) results from the coupling of transverse dipole modes, whilst peak (iv) results from coupling of the individual longitudinal dipole modes. Both of these peaks oscillate in-phase and interact repulsively, thus they are anti-bonding modes, and both peaks exhibit a blue-shift compared to the longitudinal and transverse modes of a single dumbbell.

The experimental and modeling results for an L-shaped dimer forming an angle of 102° are shown in Figure 4C and 4D. Similar to the first case, the dumbbells exhibit a bonding mode for polarization along the interparticle axis and an anti-bonding mode for the orthogonal polarization (Figure 5B). The resonance wavelengths of the bonding and anti-bonding modes exhibit little variation between these two dimers, meaning the modes are weakly dependent on the angles. However, signal intensities vary greatly with the angle change, signifying that intensity is closely tied to configuration angle. This behavior is quite similar for nanorod dimers. 39

The next two dimers are side-to-side configurations, but the pairings form different angles. Figures 6A and 6B display the experimental and simulated spectra for the parallel dumbbells presented in the TEM image. When the polarization is parallel to the interparticle axis, peaks appear near 600 nm and 700 nm. At the other polarization angle, a minor peak appears at 680 nm, whilst a large peak appears beyond 800 nm. The
simulation (Figure 6B) reveals that the main peak is centered at 870 nm. The minor peaks along both polarization beams match well between the simulation and experiment.

Unlike nanorod dimers, it is difficult to obtain symmetrically aligned dumbbells experimentally. Although a perfectly symmetric side-by-side dumbbell dimer was not located for the experiment, it is helpful to consider such an ideal dimer to explain the side-by-side dimer above. This ideal case is presented as Figure 7, and it exhibits a pair of peaks along each polarization angle. Like the other dimers, the two particles make contact at two exact locations, but here, the contact occurs at the widest points on the lobes.

Figure 8A shows the SCDs plots for our side-to-side dimer. Peaks (i) and (ii) are bonding modes, consisting of coupled quadrupolar and dipolar modes, respectively. These two modes are quite similar to the idealized dimer in Figure 7, but the symmetry breaking here results in a minor blue-shift (~ 10nm) in these two peaks. When the polarization runs orthogonal to the interparticle axis, the optical behavior varies significantly between the ideal and the symmetry-breaking dimers. For the ideal dimer, the resonant wavelengths of peaks (iii) and (iv) are anti-bonding modes, which result from the repulsive interactions between longitudinal octopolar and dipolar modes of the two dumbbells. In the case of our dimer, which exhibits broken symmetry, attractive coupling occurs between the individual longitudinal modes. The SCD plots for these peaks exhibit resonance wavelengths of 670 nm and 870 nm, respectively (Figure 8A).

In the second side-by-side case, the dumbbells form an angle of 23° (Figure 6C and 6D). For this case, we aligned the polarization parallel and perpendicular to the
longitudinal axis of the dumbbell on the left, which serves as a “plug-in location” for the second dumbbell. Figure 8B reveals that the mode splitting of this dimer is more complicated than the previous side-by-side case. When the polarization angle is parallel to the left dumbbell’s transverse axis, a transverse mode is activated. The right dumbbell can support either the transverse and longitudinal mode at this polarization. Peak (v) is the coupling of two transverse quadrupolar modes, whilst peak (vi) is an interaction of the transverse dipole mode of the left dumbbell with the longitudinal dipole mode of the right dumbbell. It is reasonable that this hybridized mode red-shifts compared with a single dumbbells’ longitudinal mode. When the polarization is rotated 90°, the longitudinal mode can be easily excited in both dumbbells. Peak (vii) results from coupling between a quadrupole mode (left dumbbell) and a dipole mode (right dumbbell). Because of strong near-field coupling, the quadrupole can couple back to and lead to the deviation in the right dumbbell, resulting in the observed mode mixing. Similar deviations effects have also been found in dolmen structures and have been attributed to Fano resonance. Mode mixing can also be found for peak (viii). This mode should consist wholly of the individual longitudinal modes, but instead, due to strong near-field coupling, a small deviation arises on the corner of the right dumbbell.

From a geometric standpoint, the two side-by-side geometries appear to be more stable than the L or tip-to-tip cases. From an optical point of view, the behaviors of all four dimers are indeed important to consider. Focusing on the main four peaks in each profile, the red-shifted peak along the interparticle axis displays the greatest range in position (>300 nm) and size. The two peaks along the other polarization angle also shift
considerably. The largest signal for side-by-side dimers is generated when the polarization is orthogonal to the interparticle axis, but rotating the two dumbbells into an L or a tip-to-tip configuration causes the strongest signal to be generated along the interparticle axis and at near-IR wavelengths. By accounting for the sizes and positions of these three important peaks, it should be possible to identify dimer geometries.

**Pentamer**

The final structure we investigated was a pentamer of dumbbells: five dumbbells aligned side-by-side (Figure 9). Such a structure is interesting to consider, because the multiple interactions between particles allow for more degrees of freedom in which plasmon resonances can occur. The TEM image in the inset of Figure 8 shows that each pairing of dumbbells has 2 junction points, and in each case, the dumbbells are separated by 1 – 2 nm. For simplicity, it was assumed that all dumbbells are equal in size and separation (1 nm). Data were collected both parallel and perpendicular to the interparticle axis. Experimental and modeled spectra are shown in Figures 9A and 9B, respectively.

The pentamer spectra are more complex than the spectra observed from the side-by-side dimers. When observed under the microscope, the signal is strong across the entire spectrum and at both angles (Figure 9A). With the dimers, when strong signals were observed, they were limited to a narrow band of wavelengths and to one orientation of the dimer. For polarization along the pentamer’s interparticle axis, the main signal
spikes are centered at 700 and 840 nm. Orthogonal to the interparticle axis, major peaks appear at 680 and 780 nm.

Modeling along both angles produced more peaks than observed with the dimers, and the maximum intensities were similar in scale to the dimers’ (Figure 9B). As observed in the experimental data, the intensity between peaks is stronger than in the dimer or single dumbbell cases. The locations of peaks vary between model and experiment, likely due to the simplifying assumption that all dumbbell junctions were 1 nm.

Focusing on the pentamer’s SCD plots, the broad peak at 850 nm (peak i) was associated with a highly dipolar mode of the five longitudinal axes, as shown in Figure 9C. Similar to the parallel dumbbell case above, deviations in the surface charges appear at the interior of the plot. Only the dumbbell tips that lie entirely outside of the structure avoid showing deviations along the interior of the SCD profile. Along the interparticle axis, the largest peak appeared at 830 nm (Figure 9C ii). This was not due solely to an attraction between transverse dipolar modes, but it was a bonding mode. A highly dipolar attraction of the individual transverse modes is associated with the small peak at 930 nm (Figure 9C iii).

We also utilized the simulation to investigate the local electric field enhancement (so-called “hot spot”) in the gap regions of the pentamer. Because these “hot spots” can exhibit larger field enhancement than isolated nanoparticles, they have been widely investigated in other plasmon-coupled nanoparticle dimers, such as discs$^{35}$ and nanorods$^{41}$. The “hot spot” is highly dependent on the geometry and separation distance
of the nanoparticles as well as the polarization direction. For example, when light is polarized along the interparticle axis of a dimer of nanospheres, a “hot spot” is created due to the bonding mode that is excited (accompanied by strong attraction) between the two particles. A dimer of nanospheres is only capable of producing a single “hot spot” due to the ultra-small gap volume. Side-by-side nanorod dimers have been previously shown to produce multiple “hot spots” within their gap region. However, the exact number and location of these “hot spots” are difficult to determine.

Figure 9D displays the electric field enhancement distribution (|E/E₀|) for peaks (ii) and (iii). The “hot spots” are marked with red circles. The maximum field enhancement (|E/E₀|) observed is a value of 398. One advantage of the dumbbell pentamer geometry compared with a nanorod cluster is that the dumbbell structure’s “hot spots” are distinct and easy to locate as with a grouping of nanospheres. Interestingly, two gaps in peak (ii) have been identified as “cold spots”, because of the drastic reduction in the electric field there, (|E/E₀|=7.8). At peak (iii), the former “cold spots” become “hot spots”. Thus, the existence and location of hot/cold spots is directly tied to the coupled plasmon modes. Such selectivity is extremely promising for biosensing, plasmon-enhanced fluorescence, Fano-resonance research, and other applications.

In this paper, we have systematically studied the optical properties of single dumbbells and dumbbell clusters. TEM imaging revealed that the dumbbells within clusters were not in direct contact but were separated by gaps > 1 nm, which was due to their CTAB coating. Thus, electron tunneling was not a concern, and we were able to
rely on classical electromagnetic calculations to model the optical properties. Due to the proximity between adjacent particles in the clusters, excitation and coupling of higher order modes is commonplace and influential on the optical properties. Some variations arise between the simulated and experimental data, and these are most likely due to variations in size, shape, and separation distance of the actual dumbbells compared to the singular “standard” dumbbell size and distance used in all of the simulations.

Due to the puzzle-piece geometry of dumbbell nanoparticles, they can interlock with one another and other particles when forming aggregated structures. The dumbbell’s shape also makes it easier to pinpoint “hot spots” within aggregates, since “hot spots” are co-located with the narrow junction points between individual dumbbells. Furthermore, in some configurations, mode mixing between dipolar and higher modes is enabled. In essence, individual dumbbell particles can be thought of as optical building blocks that can be integrated with other particles to form assemblies of optical interest. Furthermore, since the dumbbell can be viewed as a transitional shape between the nanorod and the sphere, the dumbbell could prove to be a powerful tool for endeavors in which neither spheres nor nanorods are effective.

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Figure 1. TEM inset is the single dumbbell from which spectral data were collected. Scale bar = 50 nm. Data are normalized to the average background value. Red (blue) line represents signal intensity along longitudinal (transverse) axis. Red (blue) line is referred to as the white (black) DIC polarization beam discussed in the main text.
**Figure 2.** Seven nanoparticles were simulated with COMSOL, each with a length of 91 nm. Fuller details are given in Supporting Information. Inset: TEM image of dumbbell described in Figure 1 and represented here by solid red lines in spectra. Scale bar = 50 nm. Nanorods are represented as dashed lines, dumbbells by solid lines, spherical particles as dot-dashes. The first nanorod has the same aspect ratio as the dumbbells. Radiation is polarized along the A) longitudinal and B) transverse axis of each particle. To match the normalized DIC data in Figure 1, the longitudinal data are given a positive value, while the transverse data are plotted as a negative value. SCD plots of imaged dumbbell (red line in A,B) at C) dipolar longitudinal mode (840 nm), D) octopolar longitudinal mode (540 nm), and E) dipolar transverse mode (540 nm). Colorbar represents charge scale in SCD plots.
Figure 3. Rotational profiles. A) Data collected for the single dumbbell under rotation with respect to the polarization beams at 550 nm using the Nikon polarizers at 5° increments. B) Simulation results. Comparison of first nanorod in legend of Figure 2 (black, dashed) and the dumbbell imaged with TEM (red, solid) at 10° increments at the transverse LSPR along the white polarization beam. Each data point is normalized to the maximum raw data point of its respective data set (value = 1 at maximum).
**Figure 4.** Spectra of the end-to-end and L-shaped dimers, accompanied by their TEM images. Scale bars = 100 nm. A) Experimental and B) modeling results of the dimer for 159° configuration. C) Experimental and D) modeling results of the 102° dimer. Red (blue) arrows represent direction of polarization associated with data along red (blue) lines in experimental (A,C) and simulated (B,D) data. Roman numerals are used to identify peaks discussed in Figure 5.
Figure 5. SCD plots for the four major peaks affiliated with each dimer in Figure 4. Red (blue) arrows represent polarization direction and correspond to the line colors in Figure 4. Roman numerals identify specific peaks in the simulated data of Figure 4. Colorbar represents charge scale.
Figure 6. Spectra of the side-to-side dimers, accompanied by their TEM images. Scale bars = 100 nm. Red (blue) arrows represent angle of polarization associated with data along red (blue) lines in experimental (A,C) and simulated (B,D) data. Because these two dimers were imaged early on with visible range polarizers (and not the Glan polarizers), spectral data is not available past 780 nm for the experiment. Roman numerals identify peaks discussed in Figure 7.
Figure 7. Modeled spectra of a symmetrically aligned side-by-side dumbbell dimer. The red and blue curve represents the spectra when the polarization is parallel and perpendicular to the interparticle axis, respectively. Roman numerals indicate the peaks of interest, and their SCDs plots have been shown below. Peaks (i) and (ii) are classified as bonding modes, peak (i) is the coupling effect between two quadrupolar modes, and peak (ii) results from the coupling between two dipolar modes. Peaks (iii) and (iv) are both classified as anti-bonding modes. Peak (iii) is the coupling between two octopolar modes, and peak (iv) is the coupling of two dipolar modes.
Figure 8. SCD plots for the four major peaks affiliated with dimers in Figure 6. Red (blue) arrows represent polarization angle and correspond to the line colors in Figure 6. Roman numerals identify specific peaks in the simulated data of Figure 6.
Figure 9. Pentamer case. TEM scale bar = 200 nm. The colored arrows with TEM image represent direction of polarization and correspond to colored curves in panels A and B. Roman lower case numerals in panel B correspond to the peaks displayed in panels C and D. A) Experimental spectra. B) Simulated spectra. C) SCD plots for peaks at (i) 850, (ii) 830, and (iii) 930 nm. D) Electric field enhancement images for peaks (ii) and (iii).
Appendix

Figure S1. Nanoparticles used in Modeling Experiments

The following images depict the single nanoparticles that were used for the COMSOL modeling in Figure 2. In each case, the east-west (longitudinal) axis is 91 nm to match the dumbbell imaged with TEM. Dumbbell #2 is the best match to the dumbbell imaged with TEM. The north-south (transverse) axis was variable in length and is labeled in each image.
Figure S2. Rotation of Single Dumbbell at Longitudinal LSPR

This is a companion figure for Figure 3, but it concerns the optical behavior at the longitudinal LSPR during $180^\circ$ rotation. Because the transverse and longitudinal axes are orthogonal to each other, the peak for this curve is $90^\circ$ out of phase with the peak in Figure 3. A) For the experimental dumbbell data, the IR polarizers were utilized, and images were collected at 7.5° intervals. The experimentally determined rotational profile matches well with the simulated case, shown in B. B) In this case, the simulated absorbance values for the nanorod (black, dashed) and dumbbell (red, solid) are practically identical in shape and amplitude. Because of the strong correlation between the two curves, the nanorod curve was offset by a value of 0.05 in order to display both curves on one graph. Only the white side intensity is displayed.
Figure S3. Additional Dumbbell Modeling with Varying Lobe Diameter

For this set of simulations (Figure S3A), we started with a 30 nm x 91 nm nanorod (identical to nanorod #1 in other simulations) and gradually increased the diameter of the spherical lobes while retaining the 91 nm length. Lobe diameters are stated in the figure. The longitudinal LSPR shifts to the red in very small increments as the lobe diameters increase to 40 nm. Once the lobes grow beyond 40 nm in diameter, the longitudinal LSPR undergoes a blue-shift. Because the lobes are connected by the cylindrical section, this is not identical to the case of two spheres in close proximity.
undergoing a plasmonic interaction.\textsuperscript{1} For cases with small and relatively distant lobes, the plasmonic behavior mirrors that of separated spheres. However, that behavior eventually breaks down as the lobes grow in size. It should also be noted that once the lobes come into contact and begin to overlap, the longitudinal LSPR undergoes a more dramatic blue-shift.

SCD plots are provided for three of the modeled particles under polarized illumination along the longitudinal axis. For the nanorod and first dumbbell, the particle tips carry the strongest charges, and a broad region of low or neutral charge appears at the center of each particle. For the dumbbell with 50 nm wide lobes, the charges within the lobes are weaker and spread across a larger region than for the previous two particles. Furthermore, the region of neutral charge is restricted to the 1 nm wide cylinder section in between the two lobes.

**Materials & Methods.**

**Materials and Sample Preparation.**

The methods here closely follow those previously reported by our group.\textsuperscript{2} The gold nanoparticles used for these experiments were obtained in colloidal form from Nanopartz, Inc. (Loveland, CO). Prior to imaging, an aliquot of nanoparticles was centrifuged for 10 minutes at 6000 rpm in order to remove any excess CTAB. Afterwards, the supernatant was removed and replaced with 18.2 MΩMilli-Q water. Shortly prior to imaging, 5 µL of nanoparticle solution were cast onto a holey-carbon TEM substrate. After a couple of minutes, the substrate was dabbed against a piece of
filter paper to remove the solution, but many particles and aggregates were left behind on the TEM substrate.

Immediately following the TEM analysis, the substrate was placed atop a pre-cleaned glass microscope slide. One droplet of Carl Zeiss Immersol 518F immersion oil (refractive index = 1.518 and permittivity = 2.30) was dropped onto the substrate. The substrate was then covered with a coverslip, and nail polish was used along the edges of the cover slip to prevent the oil from leaking out.

**Imaging.**

For TEM imaging, a Philips CM30 was operated at a voltage of 200 kV. An 11 megapixel (4008 x 2672 pixel imaging array) GatanOrius SC 1000 CCD camera was used to collect the TEM images with the aid of GatanDigitalMicrograph. A series of high to low magnification images were taken of each particle or grouping in order to characterize the sample and to identify key landmarks that could be used to find the same area under the optical microscope.

Optical microscopy was accomplished with an upright Nikon Eclipse 80i microscope using de Sézarmont type differential interference contrast (DIC) mode with the polarizer set at ~7° to the right of the cross-polarization point. In the DIC mode, the microscope relies on a Nikon 100X 1.40 NA Plan Apo VC oil immersion objective and a 1.40 NA oil immersion condenser. Bandpass filters from Thorlabs, Inc. (Newton, NJ) were used for obtaining spectroscopic information. The filters covered the optical range of 500 – 900 nm in 20 nm steps (e.g. 500, 520, 540, etc.), and each filter had a full width
at half-maximum of 10 nm. In order to obtain quality images with accurate detail beyond 780 nm, the Nikon’s visible-range polarizers had to be replaced with two Glan-type polarizers. Except as noted for two of the dimer cases in the main text, the Glan-type polarizers were used for collecting data at all wavelengths and orientations. Two cameras were used for collecting the optical images. In early experiments (i.e. the dimers that are parallel and offset by 23°), a PhotometricsCoolsnap ES CCD camera (1392 x 1040 imaging array with 6.45 x 6.45 µm pixels) was used. For later (and the majority of) experiments, a Hamamatsu C11440-10C, Orca-Flash 2.8 CMOS camera (1920 x 1440 imaging array with 3.63 x 3.63 µm pixels) was utilized instead. The two cameras were found to yield similar particle spectra over the range of wavelengths employed.

For imaging the single dumbbell, the DIC’s white polarization beam was aligned with the longitudinal axis, thus the black polarization beam was simultaneously aligned with the transverse axis. For the pentamer and dimers, the white beam was aligned with the interparticle axis, and the black beam was at the orthogonal angle, except as noted in the text. For this paper, the interparticle axis is defined as the line that connects the center point of two nanoparticles.

Data Normalization.

Optical data were analyzed with ImageJ. The data were normalized by dividing the white (or black) signal intensities against the average background value. Since the white (black) signal is higher (lower) in value than the background intensity, the
normalized white (black) data consisted of data points greater than (less than) a value of 1. These normalized values were subtracted by 1, in order to assign the average background a value of zero. This process does not alter the size of the experimentally determined peaks. However, peaks on the black side of the signal appear as dips in the spectra while peaks on the white side still do appear as peaks. In order to directly compare experimental data with the simulated data, the data affiliated with the black polarization direction were assigned a negative value.

The maximum and minimum background intensities were similarly normalized and is reported here as the average error in the background intensity. The average normalized background intensity is $\pm 0.08$ with a standard deviation of 0.01. This is higher than previously reported by our group,\textsuperscript{4} but in that experiment, the particles were placed directly atop a glass slide; no TEM grid was present. A glass slide would be expected to provide a flatter imaging surface than a holey carbon TEM grid, and it has been established that a non-flat surface will cause an increase in the background noise.\textsuperscript{5} Thus, higher background noise would be expected here.

**Modeling.**

Theoretical profiles of single particles and aggregated structures were calculated with COMSOL Multiphysics as a comparison to the observed experimental data. The calculations were based on optical absorbance and relied on the permittivity values from Johnson and Christy.\textsuperscript{6} A permittivity value of 2.30 was used for the homogeneous surrounding medium, which matches that of the immersion oil used. For rotational
profiles, data were calculated at 10° intervals. Furthermore, it was necessary to normalize the raw rotational data in a manner that was consistent with how the experimental data is normalized. As such, the simulated data was normalized by dividing each data point by the maximum value along its curve. Thus, the maximum normalized value has a value of 1. Because of the significant overlap along the longitudinal rotational profile, the nanorod curve was offset by a value of 0.05 to show both curves on one plot.

References


CHAPTER 6. DYNAMIC BEHAVIOR OF NOBLE METAL NANOPARTICLE ASSEMBLIES IN SOLUTION

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Abstract

Nanoparticle functionalization and assembly is undergoing a period of challenging yet exciting development. Much of this research effort has been focused on the development of new functionalities that might enable strategically-directed assembly of nanoparticles into structures that can be utilized in other important applications.

In order to determine the success of such experiments, researchers often prepare a dried sample and study the assembly patterns with electron microscopy. However, these imaging techniques can be expensive and do not provide a complete illustration of what the three dimensional nanoparticle assemblies truly look like in solution. Moreover, sample preparation presents its own challenges. Most notably, sample preparation may cause alteration to the individual assemblies or unwanted aggregation of the assemblies upon removal of the solution.

To address these concerns, assemblies of anisotropic nanoparticles were tracked free-floating in solution with differential interference contrast (DIC) microscopy. DIC microscopy is an optical technique based on interferometry with high lateral resolution
and shallow depth of field. After functionalizing gold and silver nanoparticles for self-
assembly, aliquots of the nanoparticle solutions were examined in real-time.
Nanoparticle assemblies were observed undergoing rotations, internal vibrations,
structural modifications, and interactions with other assemblies. Observations of the
dynamic behaviors of nanoparticle assemblies serve as a complement to imaging with
electron microscopy and provide new insights into the actual assembly process.

Introduction

Directed assembly of anisotropic nanoparticles has garnered much research
attention in recent years. Many researchers view directed assembly as a means for
building structures or designing sensors that are not made possible through
manufacturing techniques. Although some kinetic studies have been completed on
nanoparticles during the assembly process, real time imaging of the assembly process
and of free-floating aggregates is lacking from the literature.

Recently it was shown that differential interference contrast (DIC) microscopy is
well-suited for tracking individual nanoparticles within complex environments. \(^1\) DIC
microscopy also provides a shallow depth of field and has a high lateral resolution. Due
to the presence of two orthogonal polarizers and two Nomarski prisms along the light
path, nanoparticles have a black and white “shadow-cast” appearance when viewed at
their plasmonic wavelengths. An anisotropic nanoparticle has varying degrees of white
and black signal as the particle is rotated within the x, y plane. This variance in the
signal follows a sinusoidal relationship and can be used to determine the orientation of
the particle. It has also been shown recently that aggregates can display an anisotropy that resembles that of a single particle.\(^2\)

In the series of experiments described here, DIC microscopy was utilized to observe the assembly process of anisotropic nanoparticles. Separate solutions of nanoparticles were functionalized with biotin or streptavidin prior to mixing the two groups of particles and viewing their interactions under the microscope. In addition to observing capture events, large dendritic structures displayed rigidity in solution, but individual sections of these structures were able to rotate freely. From these experiments, it can be stated that DIC microscopy is well-suited for real time imaging of nanoparticle assemblies in solution.

**Experimental Methods**

**Materials**

Gold nanorods (25 x 60 nm) and gold dumbbells (30 x 85 nm, ~20% wider at lobes) used in this study were purchased from Nanopartz, Inc. (Salt Lake City, UT). The silver-coated gold nanorods (~15 x 50 nm) were prepared by and acquired from Dr. Yasuro Niidome.\(^3\) Small aliquots (50 – 100 µL) of nanoparticles were functionalized for each experiment with Streptavidin (Life Technologies), Neutravidin (Thermo Scientific), or EZ-Link HPDP-Biotin (Thermo Scientific) in following with the methods published by the C.J. Murphy group.\(^4,5\) Biotinylated lipid solutions (Avanti Polar Lipids, Inc.) were prepared several days in advance of the lipid-based experiments.
For lipid experiments, channels were built atop glass microscope slides using double-sided tape and cover slips. Biotinylated lipid solutions were introduced to the chamber via syringe and excess solution was rinsed out after 20 minutes. Neutravidin-functionalized gold nanorods were then injected into the chamber and viewed under optical microscopy as the particles attached to the lipid surface.

For the aggregate imaging experiments, gold dumbbells and silver-coated gold nanorods were utilized, because biotinylated gold nanorods tended to flocculate too readily. Aliquots of biotinylated nanoparticles were mixed with an aliquot of streptavidinized particles. After several minutes to several hours, an aliquot of the mixed solution was placed on a glass microscope slide and imaged under an optical microscope while the aggregates were mostly still floating in the solution.

**Instrumentation**

Optical imaging was performed on a Nikon Eclipse 80i upright microscope in DIC mode using a magnification of 100X. This microscope relies on a 12 V – 100 W halogen lamp, a Nikon 100X 1.40 NA Plan Apo VC oil immersion objective, and a Nikon 1.40 NA oil immersion condenser. Images were captured with a Hamamatsu C11440-10C Orca-Flash 2.8 CMOS camera, which has a 1920 x 1440 pixel imaging array (3.63 μm pixel size). Because DIC microscopy is diffraction limited, single nanoparticles have an apparent diameter of ~600 nm.² Movies were collected at 20 or 25 frames per second while using a bandpass filter corresponding to one of the localized surface plasmon resonance wavelengths for the nanorods (540 or 650 nm). For further
A series of experiments were performed in order to gain an understanding of the actual real time assembly behavior of individual nanoparticles. In the first experiment, a glass microscope slide chamber was coated with a layer of biotinylated lipids. Gold nanorods functionalized with neutravidin were then introduced to the chamber. The microscope was focused on the lipid layer, while individual gold nanorods were captured by the biotin in the lipid layer. Capture events happen almost instantaneously. Nanorods could be observed floating in solution away from the lipid layer, if the microscope was focused on the solution instead of the lipid layer. Because the microscope was focused on the lipid layers during this experiment, the nanorods did not come into view until the binding process had begun. In other words, nanorods did not float along the surface and in the focal plane, looking for a place to bind. Instead, as soon as the nanorods came close to the lipid layer, they quickly found a binding site. As shown in Figure 1, two types of capture events were witnessed in this experiment. In the first event, the nanorod was captured by the lipid layer and continued to rotate indefinitely. This is apparent by the strong fluctuations in the intensity of both the white and black component to the signal. Such behavior suggests that the nanorod was captured in only one location along its surface. In the second event, the nanorod was
captured and almost immediately locked into place. Both the black and the white side intensities become stable upon capture. In this case, multiple linkages between the lipid layer and the nanorod occurred simultaneously, thus locking the nanorod into place.

In the second experiment, biotinylated dumbbells and streptavidinized dumbbells were mixed in solution and aggregate growth was observed in real time. Figure 2 shows a V-shaped aggregate that became attached to the glass slide. Over the course of several minutes, the aggregate continued to grow in size as further nanoparticles or small aggregates floated by and came into contact with the main structure. With the exception of the last attachment (frames 4 and 5), these capture events also happened suddenly and without previous observation of the incoming segment. For the last capture event, the incoming segment slowly tumbled towards the main aggregate for 1.5 seconds before capture occurred. It is also noteworthy that upon capture, the individual segments mostly retain their independent plasmonic identity.

During the course of the experiments discussed in the previous paragraph, an interesting phenomenon was observed that is recounted in Figures 3 and 4. Large dendritic structures commonly form. However, the behavior of these structures in solution is not apparent without real time imaging techniques. When individual nanoparticles and aggregates come together to form a larger structure, rigidity is introduced. However, not all of the individual segments are frozen into place. Instead, many of the individual segments are able to rotate freely. This is demonstrated in Figure 3. Three consecutive images reveal that most segments within this particular branched structure are frozen along the structure’s backbone. However, two segments are allowed
to rotate, which is made apparent by each segment’s change in color from frame to frame. These observations suggest that some segments are attached to the main structure at a single location while others are attached at multiple locations, just as with the single gold nanorods in Experiment #1.

Another example of aggregate rigidity and flexibility is demonstrated in the series of images displayed in Figure 4. A T-shaped aggregate was observed over the course of 25 seconds. Initially, the two arms of the structure pointed away from each other, but the arm pointing towards the east suddenly swung upwards until it hooked the arm pointing towards the west (frame 2). As soon as the two arms became hooked together, they slowly rotated clockwise (frame 3) until the east arm unhooked itself (frame 4). At that time, the east arm continued to rotate clockwise until it was pointing towards the west. The other arm is on the opposite side of the structure in frame 5 and out of focus. As pointed out previously, these movements are indicative of a narrow point within the structure where each of the two segments was connected by only a single linkage.

In a final example, a V-shaped aggregate was attached to the glass microscope slide at the apex of the V. The images in Figure 5 display another form of structural flexibility. The structure was able to rotate quite freely around the attachment point, and as the structure rotated, the central segment changed color, as expected with an anisotropic feature under a DIC microscope. Similar behavior was noticed in several other aggregates as well. Of greater interest here is the behavior that is exhibited between frames 2 and 5. In frame 2, the arms on this structure were pointing towards the
west. During the next 0.20 seconds, the central segment remained relatively steady while the arms reversed their positions such that they were now pointing towards the east. After another 0.40 seconds, the arms flipped back, and the entire structure started to rotate, as evidenced by a change in color of the central segment. These images suggest that the arms consist of one long rigid structure that cannot rotate independently of the center point, but they can flip back and forth without affecting the orientation or signal of the center point.

Conclusions

We have demonstrated herein that DIC microscopy can be utilized to observe the real time behavior of growing plasmonic aggregates in solution. During these experiments, several key types of behavior were noticed. Because biotin and streptavidin form a strong and essentially irreversible bond, aggregated structures can undergo rotational stress without breaking apart. In several examples presented, structures were shown to have very rigid backbones. However, sections of these structures were allowed to rotate or swing freely in solution without breaking free of the larger structure. These sections maintained their freedom of movement over time. This finding suggests that the biotin and/or streptavidin coatings are indeed strong but not dense or uniform, which agrees with earlier reports.\textsuperscript{4,5} Such freedom of movement within aggregated structures could potentially affect the ability of other researchers to use these linkers to form large directed-assembly structures. Conversely, it could allow
researchers to design robotic structures that can perform simple tasks upon proper stimulation.

An additional important observation is that when capture events occur, the individual segments retain their plasmonic identities instead of morphing into a new segment with a very different plasmonic signal. As such, these individual segments act like optical building blocks that work together to form a large structure while maintaining their original optical independence. This behavior makes it possible to simultaneously observe the real time behavior of small segments and the overall structure with DIC microscopy.

References

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Figure 1. Time series of two capture events of neutravidin functionalized gold nanorods by a biotinylated lipid layer. Black (top) line represents intensity of the white side of the nanorod, and the blue (bottom) line represents the black side intensity. Time series starts prior to each capture event. Data are normalized to the background value, which is set to 1.
Figure 2. Images of aggregate growth. Gold dumbbells were utilized. The main aggregate forms a V-shape in image 1. The separate black particle to the southeast is not part of the aggregate. Frames 2, 3, and 5 show changes in the aggregate’s shape due to capture events. Frame 4 is taken 0.85 seconds prior to the final capture event and reveals the presence of the incoming segment that is captured by frame 5. Scale bar = 1800 nm.
Figure 3. Images taken 0.05 seconds apart of an aggregated structure. Most of the individual segments are frozen in place. The white (black) arrow shows a segment that appears white (black) in frame 1 but rotates to a different orientation in frames 2 and 3. Scale bar = 1000 nm.
**Figure 4.** Select images of the movements of a T-shaped aggregate during a 25-second span of time. The two arms that point to the right and left are able to swing freely. Scale bar = 1300 nm.
Figure 5. Series of images of a V-shaped aggregate attached to the glass slide as it rotates and flips around its center point (indicated by white arrow in frame 1). The time elapsed between each image is 0.20 seconds, except the last two frames where the time lapsed is 0.10 seconds. Scale bar = 1000 nm.
CHAPTER 7. CONCLUDING REMARKS

As demonstrated in the previous pages, gold nanorods and gold dumbbells can be treated as simple optical building blocks. Once the behavior of a single particle is well-understood, then it becomes easier to distinguish a single particle from the simplest of nanoparticle structures. It also becomes easier to begin the process of putting nanoparticles together to form grander pre-planned structures. However, it is also necessary to understand the instrumentation with which you are conducting your experiments in order to avoid foolish mistakes along the way that could be damaging to the science.

For the moment, gold nanoparticles are preferred for many imaging experiments, particularly for those that require long imaging times. Furthermore, gold is relatively non-toxic, and it can easily be used within biological systems without upsetting normal intracellular activity. Perhaps gold’s biggest drawback is its cost. In order for gold nanoparticles to find widespread usage in the medical field, large quantities would have to be synthesized or recovered from patients. Because of these sorts of concerns, as well as for other reasons, some researchers are focusing their efforts on discovering a better plasmonic material that could replace the noble metals under many circumstances.

Going forward, research into gold nanoparticles needs to continue, and this particular research project could stem into one of several directions at this point:
1) Silver-gold hybrid nanoparticles

Silver has been shown to have a different LSPR wavelength than gold, and in many instances, silver has also been shown to have a stronger signal. As a result, for non-biological research, silver certainly would have its benefits in imaging. While silver nanospheres or cubes can be easily synthesized, the synthesis of stable rod-shaped silver particles remains somewhat elusive at this time. Alternatively, some researchers have synthesized gold nanorods or gold dumbbells, and then fully coated the particles with layers of silver. In such instances, the optical behavior of the particle is dependent on the thickness of the silver overcoat. It would be interesting to synthesize a gold nanorod and then attach silver to the tips only, in order to form a gold dumbbell particle with silver lobes. Such a particle should exhibit the LSPR behavior at the wavelengths for both gold and silver, and because of the anisotropic shape, the particle should be useful as an orientation sensing probe.

2) Assemblies of Multiple Types of Nanoparticles

As demonstrated in several sections of this dissertation, nanoparticles like to form aggregates. In many cases, this behavior is highly problematic and undesirable. However, such assembly of particles often leads to new and interesting optical behavior, some of which could be utilized in optical circuitry.

In the appendix of Chapter 5, a brief discussion was included on the interesting aggregation behavior that occurs between nanospheres and dumbbells. Only two sizes of spheres were used in that study, and no simulations or surface charge density plots
were prepared to add to that specific discussion. However, it would be of great interest in the field of plasmonic circuitry to understand the optical behavior that is taking place, because it could be helpful in designing junctions and advanced waveguides. Furthermore, it would be interesting to study the optical behavior of the dimers that form between dumbbells and nanorods. The options for mixing particle sizes and shapes seems endless and monotonous, and as such, the focus should be on utilizing shapes and sizes that would be interesting to applied research efforts.

3) New Materials with Plasmonic Metals at the Core

On the application side, many physicists and engineers view plasmonic materials as a medium for transmitting electronic signals. Unfortunately, metals are viewed as being a ‘lossy’ material, because the movements and collisions of electrons lead to a rapid dissipation of energy. This seems to be a greater concern to people working with films than with nanoparticles, but as a result of these concerns, some researchers are focusing on the construction of better plasmonic materials.

The design of new plasmonic materials is seemingly headed along several lines of attack at the moment, many of which fall into the category of materials known as optical metamaterials. Metamaterials are broadly defined as artificially manufactured materials that display properties not found in nature. Depending on how the material is designed, they can exhibit any number of optical characteristics, such as invisibility, optical magnetism, or a negative refractive index. Quite often, metamaterials rely on plasmonic materials as part of their design.
Of particular interest is the design of nanoparticles with metamaterial characteristics. The Halas group at Rice designed nanoshells and nanocups, two types of metamaterial nanoparticles which incorporate a silica core surrounded by a thin gold layer.\textsuperscript{4,6,7} Going forward, it is important to fully investigate the optical properties of metamaterial nanoparticles as they become available. One advantage of working with plasmonic nanoparticles is that they are highly visible over a tunable range of wavelengths, but metamaterials could prove to be even more highly tunable or more sensitive than simple plasmonic materials. If that proves to be the case, metamaterial nanoparticles could easily displace simple plasmonic ones in many applications.

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