Absorption and Scattering of Single Plasmonic Nanoparticles

by

Ji Won Ha

A THESIS SUBMITTED
IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE

Master of Arts

APPROVED, THESIS COMMITTEE:

Stephan Link, Chair
Assistant Professor of Chemistry and
Electrical and Computer Engineering

Seichi P. T. Matsuda
E. Dell Butcher Professor of Chemistry
and Professor of Biochemistry & Cell
Biology

Christy F. Landes
Assistant Professor of Chemistry

HOUSTON, TEXAS
May 2010
RICE UNIVERSITY

ABSORPTION AND SCATTERING OF SINGLE PLASMONIC NANOPARTICLES

by

JI WON HA

MASTER OF ARTS

HOUSTON, TEXAS
MAY 2010
ABSTRACT

Absorption and Scattering of Single Plasmonic Nanoparticles

by

Ji Won Ha

In this thesis, I present the absorption and scattering properties of single gold nanoparticles. I performed scanning electron microscopy (SEM) correlated dark-field scattering studies of gold nanorods (AuNRs). I found polarization-dependent scattering of a single AuNR. I studied the dependence of surface plasmon resonance (SPR) linewidth on both the refractive index of a surrounding medium and the adsorption of thiol groups onto the surface of AuNRs. I found that the SPR wavelength ($\lambda_{\text{max}}$) shifts to longer wavelength when increasing the refractive index of the surrounding medium, while the SPR linewidth remains almost constant when increasing the refractive index of the surrounding environment. I also found that SPR wavelength shifts to longer wavelength as thiol groups bind to the surface of a single AuNR and that the SPR linewidth is broadened.

I carried out photothermal imaging of gold nanospheres. The successful imaging of gold nanospheres down to 10 nm, which cannot be studied by conventional scattering-based methods, was achieved with a signal to noise (S/N) ratio of ~ 35. I studied the size dependence of the photothermal signal of gold nanospheres with diameters ranging from 30 to 250 nm. The experimental results showed a very good agreement with Mie calculations for absorption of nanospheres.
I further investigated polarization-dependent photothermal imaging of single AuNRs. It is observed that both gold nanowires and AuNRs show a polarization dependence in photothermal imaging. I utilized polarized photothermal imaging to determine the orientation of AuNRs. By selecting either the longitudinal or the transverse SPR mode, I precisely determined the orientation of individual AuNRs. Correlating SEM with photothermal images, the orientations of AuNRs were accurately measured. Most notably, I determined the orientation of an AuNR by exciting the transverse SPR mode which is not achievable by conventional scattering-based techniques.
ACKNOWLEDGEMENT

I would like to express my gratitude to my advisor, Dr. Stephan Link, for the opportunity to work in his group and for invaluable guidance and countless inspiration throughout the years. I would also like to thank Dr. Seiichi Matsuda and Dr. Christy Landes for being part of my thesis committee and for their support and helpful advice. I would also like to extend my thanks to my group members. They have been great help in many ways. In particular, I would like to thank Wei-Shun Chang whom I worked with in the photothermal imaging of gold nanoparticles. Liane Slaughter who helped me with the SEM work in my researches, and Alexei Tcherniak who helped me with the Mie and DDA calculations. I also want to thank Dr. Eugene Zubarev and Bishnu Khanal for providing me with a sample of gold nanowires. I am grateful to Minjung Cho for her love, support and understanding. Finally, I would like to thank my family (my father Dae Hong Ha, my mother Kyung Ja Yoon, my brother Gil Won Ha, my sister Mi Hyang Ha) for their endless love and unyielding support for many years.
TABLE OF CONTENTS

ABSTRACT ii
ACKNOWLEDGEMENT iv
LIST OF FIGURES vi
LIST OF TABLES xi

1. INTRODUCTION ............................................................................................................. 1

2. EXPERIMENTAL SECTION .......................................................................................... 6
   2-1. Materials and Chemicals ......................................................................................... 6
   2-2. Sample Preparation and Characterization ............................................................... 6
   2-3. SEM Correlated Dark-Field Single Particle Scattering Spectroscopy .................. 7
   2-4. Photothermal Imaging of Gold Nanoparticles ....................................................... 9

3. RESULTS AND DISCUSSION ...................................................................................... 12
   3-1. SEM Correlated Dark-Field Scattering Studies of Gold Nanorods ....................... 12
       3-1-1. Polarization Dependence of Scattering from Gold Nanorods .................... 14
       3-1-2. Surface Plasmon Resonance Linewidth of Gold Nanorods ....................... 16
   3-2. Photothermal Imaging of Gold Nanospheres ....................................................... 25
   3-3. Polarization-Dependent Photothermal Imaging of Gold Nanorods ................... 36
       3-3-1. Polarized Photothermal Imaging of Gold Nonowires .............................. 36
       3-3-2. Polarized Photothermal Imaging of Gold Nanorods .............................. 42

4. CONCLUSION .............................................................................................................. 60

5. REFERENCES ................................................................................................................. 62
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Schematic of the photothermal imaging setup</td>
<td>9</td>
</tr>
<tr>
<td>2. (A) SEM image of AuNRs, (B) Scattering image (20 × 20 μm²) overlaid with a SEM image (A). In the SEM image a magnified view of the NRs is shown in the colored boxes right next to the particles. A red square shows a dimer (side-by-side)</td>
<td>13</td>
</tr>
<tr>
<td>3. (A, B) Polarization-dependent scattering spectra for a single AuNR in the white circle in Figure 2A. Spectra were measured as a function of polarization angles. λ&lt;sub&gt;max&lt;/sub&gt; is 706.2 nm. (C) Intensity as a function of polarization angles. The red curve is a fit to Mcos(2θ - φ). (D) Scattering spectrum for a dimer in the red square in Figure 2A. λ&lt;sub&gt;max&lt;/sub&gt; is 658.8 nm</td>
<td>15</td>
</tr>
<tr>
<td>4. Single NR scattering spectra in air (blue), water (red) and oil (green) and Lorentzian fits (A). The SPR wavelength increases with increasing medium refractive index (B) while the linewidth remains constant (C). (D, E, F) Corresponding DDA calculations for a qualitative comparison. Note that the dimensions have not been optimized to exactly match the experimental results. However, the experimental trend is well reproduced</td>
<td>18</td>
</tr>
<tr>
<td>5. (A) Single NR scattering spectra in air (blue) and after adding 1-decanthiol (red). The scattering spectrum is red-shifted and strongly damped with an increased linewidth. The change in linewidth is better illustrated in (B) where the same spectra are normalized and plotted as a function of energy. The full width at half</td>
<td></td>
</tr>
</tbody>
</table>
maximum is 103 meV and 117 meV for the blue and red spectra, respectively

6. Photothermal image of 10 nm gold nanospheres. The scan area is 20 × 20 μm²

7. Photothermal images (20 × 20 μm²) of gold nanospheres with diameters ranging from 30 to 250. (A): 30 nm, (B): 50 nm, (C): 70 nm, (D): 90 nm, (E): 150 nm, (F): 250 nm

8. Intensity histograms for gold nanospheres with diameters ranging from 30 to 250 nm. (A): 30 nm, (B): 50 nm, (C): 70 nm, (D): 90 nm, (E): 150 nm, (F): 250 nm. Several hundred nanospheres were used to build the histograms for each sample. The red curves in panels E and F are fits to a sum of two Gaussian curves. The two distributions arise from the presence of single particles and small aggregates. The Gaussian curve corresponding to the smaller intensity values and hence the single particles were used to calculate the average absorption intensity.

9. Size dependence of gold nanospheres with diameters ranging from 30 to 250 nm. We measured several hundred nanospheres in air for each sample. A 532 nm laser was used as a heating beam. Red squares indicate the mean intensity for each size. The black line is a Mie calculation for absorption.

10. Photothermal images (20 × 20 μm²) of gold nanospheres ranging from 10 to 90 nm in diameter. Nanospheres were embedded in a PVA film. A 514 nm laser was used as a heating beam. (A): 10 nm, (B): 30 nm, (C): 50 nm, (D): 70 nm, (E): 90 nm
11. Intensity histograms for gold nanospheres inside a PVA film. The diameter of the gold nanospheres varied from 10 to 90 nm. Several hundred nanospheres were used to make the histograms for each sample. (A): 10 nm, (B): 30 nm, (C): 50 nm, (D): 70 nm, (E): 90 nm.

12. Size dependence of gold nanospheres with diameters ranging from 10 to 90 nm. We measured several hundred nanospheres in a PVA film for each sample. A 514 nm laser was used as a heating beam. Red squares indicate the mean intensity for each size. The black line is a Mie calculation for absorption.

13. Effect of thiol coating on the photothermal signal of 90 nm gold nanospheres. Photothermal images (20 x 20 \( \mu \text{m}^2 \)) of gold nanospheres without thiol coating (A) and with thiol coating (B) under the same condition. (C, D) Intensity histograms of bare gold nanospheres (C) and thiol coated gold nanospheres (D).

14. (A) TEM image of gold nanowires. The mean width and length of the nanowires are 25 nm and 2.5 \( \mu \text{m} \), respectively. Scale bar is 1 \( \mu \text{m} \). (B) Absorption spectrum of gold nanowires in aqueous solution. The transverse SPR peak appears around 509 nm.

15. Photothermal images (3 x 3 \( \mu \text{m}^2 \)) of nanowires as a function of polarization angles. The arrows indicate the axis of polarization. (A - G) 488 nm excitation, (H - N) 514 nm excitation.
16. (A) Intensity as a function of polarization angles from 0 to 90° for both 488 nm and 514 nm excitation of the single nanowires in Figure 15. (B) The photothermal traces are normalized and fitted to $Mc\cos(\theta - \phi)$. 41

17. AuNRs ensemble extinction spectrum taken in aqueous solution (A) and single particle scattering spectra deposited on glass and surrounded by different media (B). The inst shows a TEM image of the AuNRs having an average length and width of 73 and 25 nm, respectively. 43

18. SEM (A) and photothermal (B) images of the same area showing two AuNRs and one NR dimer. The photothermal image was recorded with 675 nm excitation. 44

19. Polarization dependence of the photothermal intensity for the AuNRs shown in Figure 18. The insets show the photothermal signal of the individual particles as a function of excitation polarization angle $\theta$. 47

20. Histogram of modulation depths (A) and polarization anisotropy (B) for AuNRs excited at 675 nm. The NR orientations obtained from the modulation depth (C) and polarization anisotropy (D) analysis are in very good agreement with the angles measured from the correlated SEM images. The red lines are linear regressions. 51

21. Photothermal imaging of nanospheres and AuNRs with 514 nm excitation. (A) Comparison of modulation depth histograms for 30 nm nanospheres in PVA film (brown: $n = 1.5, <M_{514}> = 0.07\pm0.04$), 86 nm long AuNRs in water (yellow: $n = 1.3, <M_{514}> = 0.29\pm0.20$), 73 nm long AuNRs in water (green: $n = 1.3, <M_{514}> = 0.29\pm0.21$), and 73 nm long AuNRs in oil (blue: $n = 1.5, <M_{514}> = 0.42\pm0.22$).
Included is also the distribution measured for 73 nm long AuNRs in oil using a N.A. = 0.7 objective (red: n = 1.5, <M_{51,4}> = 0.52±0.25). (B) Photothermal image of 73 nm long AuNRs recorded with circularly polarized excitation at 514 nm. (C) Polarization dependence of the photothermal signal for the AuNRs highlighted by the colored squares in (B). The closed (open) circles correspond to excitation of the transverse (longitudinal) SPR absorption. (D) Simulated modulation depth as a function of the ratio k between transverse SPR and interband absorptions for objectives with N.A. = 1.3 and water as the surrounding medium (blue), N.A. = 1.3 and oil (green), and N.A. = 0.7 and oil (red). The open circles indicate the experimentally determined mean modulation depths.

22. Polarization dependence of the photothermal intensity for the same AuNR using different excitation wavelengths, numerical apertures, and media. (A): 675 nm, N.A. = 1.3, n = 1.3; (B): 514 nm, N.A. = 1.3, n = 1.3 (red) and 514 nm, N.A. = 0.7, n = 1.5 (blue).
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Comparison of the different damping contributions to the total SPR linewidth $\Gamma$</td>
<td>16</td>
</tr>
<tr>
<td>2.</td>
<td>Contribution of each decay process to the total SPR linewidth for an AuNR (AR=3). The calculated total linewidth as a function of the medium refractive index (air, water, oil) is demonstrated. The calculated values are compared to the experimental values for the AuNRs shown in Figure 4</td>
<td>23</td>
</tr>
</tbody>
</table>
1. INTRODUCTION

Determining the local orientation of synthetic and biological materials at the nanoscale using optical probes has attracted much attention.\textsuperscript{1-7} Single molecule spectroscopy has become an indispensable tool because it avoids ensemble averaging of the measurable observables in heterogeneous systems.\textsuperscript{4,6} With respect to problems in materials science, single molecule spectroscopy has, for example, been applied to measuring orientations and conformations of single polymer chains in isotropic environments\textsuperscript{4} and anisotropic liquid crystalline media.\textsuperscript{6} Single molecule imaging has also contributed tremendously to the detailed understanding of complex biological systems, such as RNA folding,\textsuperscript{8} the walking mechanisms of the molecular motors myosin and kinesin,\textsuperscript{9} and the production of proteins in cells.\textsuperscript{10} The orientation of the optical probe, as well as the local order of the environment, is obtained by measuring the fluorescence polarization anisotropy\textsuperscript{6} or polarization-dependent absorption\textsuperscript{4} of the probe. Fluorescent probes include organic dye molecules\textsuperscript{2,11} and conjugated polymers\textsuperscript{4,6} as well as inorganic semiconductor nanoparticles.\textsuperscript{12} A major disadvantage of these probes is the time-dependent fluctuations of the fluorescence signal (photoblinking)\textsuperscript{11,13} and a limited measurement time before irreversible photochemical changes occur (photobleaching).\textsuperscript{13,14} However, the most serious obstacle for many biological experiments, such as imaging in living cells, is the potential toxicity of both organic molecules and inorganic nanoparticles.\textsuperscript{15}

Metallic nanoparticles, and especially gold nanoparticles, have been used for labeling and visualization in biological systems due to their large scattering and absorption cross sections.\textsuperscript{16} The high photostability\textsuperscript{16}, lack of photoblinking\textsuperscript{17}, and
excellent biocompatibility of gold nanoparticles make them attractive probes for biological research. The high scattering and absorption cross sections of gold nanoparticles result from the SPR, which is the collective oscillation of the conduction band electrons. The SPR depends on the size and shape of the nanoparticles as well as the dielectric constant of the surrounding medium, which allows one to easily tune the frequency of the SPR. AuNRs have longitudinal and transverse SPR modes polarized parallel to the long and short axes of the AuNRs, respectively. The anisotropic shape of AuNRs together with the large extinction cross section and polarization anisotropy of the longitudinal SPR oscillation make them ideal probes for orientation sensitive single particle imaging. Several single particle techniques such as dark-field microscopy, confocal microscopy using higher order laser modes, spatial modulation extinction spectroscopy, and two-photon luminescence have been employed to measure the orientation of single AuNRs. Most widely used for single nanoparticle measurements is dark-field microscopy, a technique based on scattering. However, when the nanoparticle radius becomes less than 20 nm, the scattering cross section decreases below the detection limit for dark-field single particle imaging. Hence, only larger metallic nanoparticles can be used as probes even though a smaller probe with less impact on the system of interest is very much desirable.

Larger nanoparticles have been studied by scattering-based methods. Scattering-based methods allowed us to carry out SPR measurements on individual nanoparticles. Dark-field single particle scattering spectroscopy was used to investigate how the SPR linewidth of metallic nanoparticles depends on the particle size, shape, and environment. The first spectroscopic studies of single metallic
nanoparticles on the SPR linewidth were presented by the Schultz and Feldmann groups.\textsuperscript{27-29} They examined silver and gold nanospheres as well as AuNRs. In their measurements they showed a significant reduction in the SPR linewidth at a given resonance frequency for NRs as compared to nanospheres. This is due to the decrease in the radiation damping. Recently, Hu \textit{et al.} have employed scattering spectroscopy to investigate the linewidth of AuNRs.\textsuperscript{30} Their result shows that both electron-surface scattering and radiation damping can make significant contributions to the SPR linewidth. For the AuNRs, electron–surface scattering dominated for narrow NRs with a width less than 10 nm, and radiation damping dominated for thick NRs with a width greater than 20 nm. However, there have been no studies focused on how the refractive index of the environment affects the SPR linewidth of AuNRs. Therefore, we investigated the effect of the refractive index of the surrounding medium on the SPR linewidth of single AuNRs. For comparison with the experimental results, we performed calculations using the discrete dipole approximation (DDA) that yielded the optical properties of the NRs, including radiation damping.

Because the absorption cross section scales with \( R^3 \) instead of the \( R^6 \) scaling of the scattering cross section,\textsuperscript{36} absorption properties dominate for small nanoparticle sizes. This fact has been exploited by a few recent reports on single NR absorption and extinction measurements.\textsuperscript{17,24,25,36-39,40-42} Vallée and coworkers have developed a technique based on spatially modulated extinction spectroscopy and measured the extinction spectra of single AuNRs with lengths of 40-60 nm and widths of 15-20 nm.\textsuperscript{25} They also measured the polarization dependence of the extinction cross section for a single AuNR at the longitudinal and transverse SPRs. While the longitudinal SPR is
highly polarized, the transverse SPR shows only a very weak polarization dependence, which was attributed to the spectral overlap between the transverse SPR mode and interband transitions. Orrit, Lounis, and coworkers have developed a technique that is based purely on absorption, called photothermal imaging, to study the absorption properties of different nanostructures.\textsuperscript{17,40-42} Photothermal imaging detects the heat generated through nonradiative decay after photoexcitation of nanoparticles.

Strong absorbers, such as metallic nanoparticles with diameters as small as 2 nm, have been imaged,\textsuperscript{37} but semiconductor nanocrystals\textsuperscript{38} and single-wall carbon nanotubes\textsuperscript{39} can also be visualized by photothermal imaging. Photothermal imaging has proven to be a very versatile imaging tool with great promise for applications in biophysics using small nanoparticle probes. For example, single membrane proteins labeled with 5-10 nm gold nanoparticles have been tracked in cells\textsuperscript{40} and gold nanoparticle diffusion and binding in aqueous solution has been measured by photothermal absorption correlation spectroscopy.\textsuperscript{41} However, measuring the orientations of single AuNRs with polarization-sensitive photothermal imaging has not yet been studied in detail, although this combination of a non-photobleaching and non-photoblinking vector probe with a single particle absorption method promises to be highly sensitive for determining the orientation of macromolecules and the local order in biological systems.

In this thesis we present the first polarization-sensitive photothermal imaging of single AuNRs. We found that the longitudinal SPR absorption behaves as a pure single dipole which allowed us to accurately determine the orientations of single AuNRs. Agreement with the NR orientations as determined by SEM was excellent. If a low
numerical aperture (N.A.) objective was used, we obtained a large polarization
dependence of the transverse SPR absorption, from which the NR orientation could be
determined as well. Being able to establish the NR orientation from the transverse mode
is unique to the photothermal imaging experiments presented here and is furthermore
important because the transverse SPR is insensitive to aspect ratio (AR) of the NR,
allowing many different AuNR lengths to be probed with the same laser frequency.
2. EXPERIMENTAL SECTION

2-1. Materials and Chemicals

Gold nanospheres, 73 nm long (part #: 30-25-700) and 86 nm long (part #: 30-25-730) AuNRs were obtained from Nanopartz. 2.5 μm long gold nanowires were synthesized and obtained from Prof. Zubarev’s group at Rice University. Microscope cover glass (part#: 12-545-F) was purchased from Fisher Scientific. Indium tin oxide (ITO)-coated glass slides (25 × 25 x 1.1 mm) were purchased from Delta Technologies (Part#: X140, MN, USA). All chemicals such as acetone and methanol used for cleaning microscope cover glasses were purchased from Sigma (St. Louis, MO, USA).

2-2. Sample Preparation and Characterization

The microscope cover glasses were cleaned by first sonicating in acetone for 15 min, and then in methanol for 15 min, followed by O₂ plasma cleaning for 1 min. After cleaning the slides, a pattern was created by evaporating a 5 nm Ti layer followed by a 20 nm Au layer through an indexed copper Transmission Electron Microscopy (TEM) grid (Ted Pella) placed on the cleaned glasses. This pattern aided in locating the same particles in SEM and optical microscopy. The solution containing nanoparticles was 10 x diluted and sonicated for 3 min to prevent the aggregation of the NPs. A drop of the diluted NP solution was then cast onto the cleaned and patterned glass slides and allowed to dry. Characterization was carried out using a FEI Quanta ESEM2 SEM operated under low vacuum in a water vapor atmosphere at 30 kV.
2-3. SEM Correlated Dark-Field Single Particle Scattering Spectroscopy

In this study, we correlated SEM images with optical images and spectra of single AuNRs. Dark-field scattering microscopy using a Zeiss microscope with a tungsten lamp as a light source was performed on a sample with a gold pattern that allowed us to locate the exact same AuNRs. The sample was placed in a sample holder that can be translated, and then mounted on a piezo-scanning stage. Images were then taken using an Epiplan objective (100x, N.A. = 0.75). Dark-field images were obtained by scanning the sample over a 50 micron pinhole in the image plane, which rejects excess excitation light and improves resolution. The scattered light passed through a polarizing beam splitter which split the orthogonal polarizations of light onto separate avalanche photodiode detectors (APDs, Perkin Elmer, SPCM-AQR).

The experiments were performed by first recording a scattering image of the sample. A motorized flipper mirror was placed in the detection path so that light could be redirected to the spectrometer for taking spectra once a suitable particle in the image had been selected. Scattering spectra were acquired with a spectrometer (SP2150i, Princeton Instruments) and a CCD camera (PIXIS 400BR, Princeton Instruments). When taking a spectrum, the 3-D piezoscanner moved the sample to the desired location so that only the scattered light from the selected single particle could pass through the pin hole. The scattered light was refocused at the entrance of the spectrometer by a lens, dispersed by a grating inside the spectrometer, and detected by the CCD camera. For polarized scattering measurements, the spectra for single AuNRs were obtained as a function of polarization direction. The spectra were averaged over six frames, each having an exposure time of 30 s for the single AuNRs. The background was measured at a region
without any particles; dark counts were measured with the lamp off. Data analysis was performed with specially designed Matlab programs. The spectra were fitted to a Lorentzian function \( I(\omega) = C_0 / [(\omega - \omega_0)^2 + \Gamma^2 / 4] \), to determine the linewidth \( \Gamma \) and the resonance frequency \( \omega_0 \).
2-4. Photothermal Imaging of Gold Nanoparticles

The photothermal imaging method requires a combination of two laser beams: A time-modulated heating beam (514 nm) and a non resonant probe beam (632 nm) as illustrated in Figure 1.

Figure 1. Schematic of the photothermal imaging setup.
The heating beam is overlaid with the probe beam and focused on the sample by means of a microscope objective. The probe beam produces a frequency shifted scattered field as it interacts with the time-modulated variations of the refractive index around the absorbing nanoparticle. The scattered field is then detected via its beatnote with the probe field which plays the role of a local oscillator. The signal is easily extracted by a lock-in amplifier and is directly proportional to the absorption cross section of the nanoparticle.

Photothermal imaging of gold nanospheres were performed as follows. A 514 nm Ar$^+$ laser and a 532 nm laser were used as a heating beam for exciting the SPR mode of nanospheres in this study. As a probe beam, a 633 nm He-Ne laser (JSD Uniphase) was employed. The intensity modulation of the beam (328 kHz) was carried out by an acousto-optic modulator (IntraAction) and a function generator. Both an Epiplan objective (air spaced, 100×, N.A. = 0.75) and a Fluar objective (oil immersion, 100×, N.A. = 1.3) were used for focusing the beams into the sample. In the measurement of 10 nm gold nanospheres, we measured nanospheres inside a 0.5 % (wt) polyvinyl alcohol (PVA) matrix and placed glycerol on top of the sample. The power of the heating beam varied from 100 $\mu$W to 1 mW. The power of the probe beam was 10 mW. The integration time was 20 ms.

Polarization-dependent photothermal imaging of AuNRs was carried out as follows. We employed two heating beams, close to resonance, for exciting two different SPR peaks of the AuNRs. A 514 nm Ar$^+$ laser was used as a heating beam for exciting the transverse SPR mode of AuNRs. A 675 nm diode laser was used as a heating beam for the longitudinal SPR mode of the NRs. The polarization of the heating beams was
controlled using half and quarter waveplates. A 633 nm He-Ne laser was employed as a probe beam. The heating and probe beams were carefully overlaid each time after changing the polarization of the heating beam. Intensity modulation of the heating beam at 400 kHz was carried out with an acousto-optic modulator. The laser beams were directed into an inverted microscope (Zeiss) and focused on the sample with a Fluar or a Plan-Apochromat (oil immersion, 63×, N.A. = 0.7) objective. The photothermal signal was detected by a 125 MHz photoreceiver (New Focus) and fed into a lock-in amplifier (Princeton Applied Research, Oak Ridge, TN, USA), which was connected to a surface probe microscope controller (RHK Technology). Photothermal images were acquired using a three-dimensional piezo scanning stage (Physik Instrumente). Photothermal polarization traces of multiple AuNRs were constructed from a series of 20 × 20 μm² images taken with different excitation polarizations using an automated particle finding routine developed in Matlab.
3. RESULTS AND DISCUSSION

3-1. SEM Correlated Dark-Field Scattering Studies of Gold Nanorods

We performed SEM correlated scattering measurements of AuNRs. Because optical properties of gold nanoparticles are strongly dependent on both size and shape, it is important to correlate the optical image and spectra with structural information. Therefore, we used SEM for structural characterization, and it is correlated with optical images and spectra of single gold nanoparticles. Through correlated structural and optical imaging we further ensured that we measured single particles or dimers within our diffraction-limited optical detection area. In our study we used a gold pattern to find the exact same nanoparticles.

First, we obtained a sample where single NRs were well distributed in a 20 × 20 μm² scan area. Figure 2A shows a SEM image of AuNRs (25 × 73 nm). As seen in Figure 2A, single AuNRs were well separated. A magnified view of the single AuNRs is shown in the colored boxes right next to the particles in the SEM image. We then took a scattering image for the same AuNRs. Figure 2B shows a scattering image that is overlaid with a SEM image. As shown in Figure 2B, a SEM image was successfully correlated with an optical image and this allowed us to study specific AuNRs we are interested in. As we can see in Figure 2A, there is one dimer (side-by-side).
Figure 2. (A) SEM image of AuNRs, (B) Scattering image ($20 \times 20 \, \mu m^2$) overlaid with a SEM image (A). In the SEM image a magnified view of the NRs is shown in the colored boxes right next to the particles. A red square shows a dimer (side-by-side).
3-1-1. Polarization Dependence of Scattering from Gold Nanorods

We studied the polarization dependence of the scattering from an AuNR which is circled in Figure 2. As shown in Figures 3A and 3B, we obtained scattering spectra for a single AuNR as a function of polarization angles. Only the longitudinal SPR mode was observed in the scattering spectrum of a single AuNR. By measuring the polarized light scattering intensity from an AuNR, we were able to determine its orientation. To do this we first plotted the scattering intensity as a function of polarization angles as shown in Figure 3C. Then we fitted this dependence to \( M \cos(2\theta - \phi) \). By fitting we obtained the modulation depth \( M \) and 2-D orientation \( \phi \) of a NR on a glass slide. The fitted \( \phi \) was 161 degrees, which shows a very good agreement with 158 degrees measured from the SEM image in Figure 2A.

It is very interesting to measure a dimer (side-by-side) in a SEM image and to compare it to a single NR. This is because we can investigate near field interaction of SPRs of two NRs in a dimer. Therefore, we measured a scattering spectrum for a dimer in the red square in Figure 2. Interestingly we observed that a SPR peak (\( \lambda_{\text{max}} \): 658.8 nm) for a dimer aligned in a side-by-side configuration is blue-shifted when comparing with a SPR peak (\( \lambda_{\text{max}} \): 706.2 nm) for a single AuNR in Figures 3A and 3B. This result is consistent with a previous study by Funston and co-workers.\(^43\) The blue-shift occurs as a result of the SPR coupling in the dimer and can be explained by plasmon hybridization. In this configuration, the longitudinal SPRs of the two NRs interact repulsively, which leads to an overall blue-shift in the spectrum. In addition, it is worth noting that the SPR linewidth for a dimer is broader than that of a single AuNR. This is mainly due to the increase in radiation damping because of a larger volume of the dimer compared to the
individual AuNRs.

Figure 3. (A, B) Polarization-dependent scattering spectra for a single AuNR in the white circle in Figure 2A. Spectra were measured as a function of polarization angles. \( \lambda_{\text{max}} \) is 706.2 nm. (C) Intensity as a function of polarization angles. The red curve is a fit to \( M \cos(2\theta - \phi) \). (D) Scattering spectrum for a dimer in the red square in Figure 2A. \( \lambda_{\text{max}} \) is 658.8 nm.
3-1-2. Surface Plasmon Resonance Linewidth of Gold Nanorods

The SPR broadening is a very interesting phenomenon in single particle spectroscopy and it is important to figure it out because the SPR linewidth is a direct measure of the plasmon dephasing, i.e. loss of coherence. Dark-field spectroscopy has been used to evaluate the SPR linewidth of single metallic nanoparticles with different shapes by several groups.\textsuperscript{30-35} However, in order to assign the measured plasmon resonance maxima and linewidths to specific particle shapes and dimensions, it is necessary to correlate the optical spectra with structural information.\textsuperscript{44,47}

Here we carried out SEM correlated dark-field single particle scattering studies of the SPR linewidth for AuNRs. The SPR linewidth $\Gamma$ is controlled by lifetime broadening due to various decay processes.\textsuperscript{48} As summarized in Table 1, the different decay processes include bulk metal damping, electron-surface scattering, radiation damping, and chemical damping due to adsorption of molecules (chemical effect).\textsuperscript{30,31,49}

| Plasmon Broadening Mechanism | 1) Intrinsic bulk metal damping\textsuperscript{30}:  
\[ \Gamma_{\text{in}} = \frac{2\varepsilon}{|\partial\varepsilon/\partial\omega|} \]  
frequency dependent  
dielectric function  
2) Electron-surface scattering\textsuperscript{30}:  
\[ \Gamma_{\text{es}} = \frac{A\nu}{L_\text{ef}} \]  
\( \nu \) = Fermi velocity  
\( A \) = scattering parameter  
\( 0 \leq A \leq 1 \)  
3) Radiation damping\textsuperscript{30}:  
\[ \Gamma_{\text{rad}} = \frac{h\kappa V}{2} \]  
\( V \) = volume  
\( \kappa \) = proportionality constant  
4) Chemical effects |

Table 1. Comparison of the different damping contributions to the total SPR linewidth $\Gamma$.  
16
It is well known that for small nanoparticles with diameters below \( \sim 10 \text{ nm} \) electron-surface scattering causes a broadening of the SPR.\(^{30}\) Similarly, for large nanoparticles with diameters larger than \( \sim 20 \text{ nm} \) the SPR linewidth increases due to radiation damping. But there have been no studies focused on how the surrounding environment affects the SPR linewidth from radiation damping in single AuNRs. Therefore, we performed dark-field single particle scattering studies to elucidate the effect of the refractive index of the surrounding media on the SPR linewidth in single AuNRs. Through this study we will have more understanding about the effect of the refractive index of the environment on the broadening of the SPR linewidth.

In our study we took scattering spectra of a single AuNR (25 x 73 nm) in three different environments (air, water, oil). Figure 4 summarizes the results and shows the single particle scattering spectra of an AuNR deposited on a glass coverslip and surrounded by air, water, and oil. As shown in Figure 4A, the spectra were well fitted with a Lorentizian function. As seen in Figure 4B, the SPR wavelength increased as the refractive index increased from air to oil. This trend for the SPR wavelength as a function of refractive index is in agreement with previous reports.\(^{20}\) Furthermore, we also found that the SPR linewidth remains almost constant as the refractive index increases for a single AuNR as shown in Figure 4C. We performed DDA\(^{45,50,51}\) calculations that account for retardation effects such as radiation damping. Figures 4D, 4E and 4F show the corresponding DDA calculations for a qualitative comparison with experimental results. DDA calculations fully reproduced the experimental trend in Figure 4. Therefore, it is observed that the SPR wavelength increases with increasing
medium refractive index and that the SPR linewidth remains almost constant with increasing medium refractive index.

Figure 4. Single NR scattering spectra in air (blue), water (red) and oil (green) and Lorentzian fits (A). The SPR wavelength increases with increasing medium refractive index (B) while the linewidth remains constant (C). (D, E, F) Corresponding DDA calculations for a qualitative comparison. Note that the dimensions have not been optimized to exactly match the experimental results. However, the experimental trend is well reproduced.
As summarized in Table 1, the total linewidth is mainly due to bulk dephasing, electron-surface scattering, and radiation damping. The bulk term is approximately equal to 73 meV for gold in the 1-2 eV spectral region.\textsuperscript{31,52} Electron-surface scattering dominates for smaller particles (width < 10 nm). However, as the size of the particles increases, the coupling of the SPR oscillation to the radiation field can become an important energy loss mechanism. This effect is known as radiation damping, which is roughly proportional to the volume $V$, according to $\Gamma_{\text{rad}} = \frac{h k V}{2}$. Here $k$ is a constant that describes the magnitude of radiation damping. $\hbar$ is the Dirac constant given by $\frac{\hbar}{2\pi}$.

There has been no equation to show the relationship between $k$ and the refractive index $n$. Therefore, we derived an equation that shows the relationship between the linewidth from radiation damping and the refractive index of the surrounding medium. We started from equation (1) below for the radiation damping time. Equation (1) was taken from the literature.\textsuperscript{53}

\begin{equation}
\frac{1}{\tau_{\text{rad}}} (s^{-1}) = \frac{2\omega_p^4}{9c^3} \left( \frac{\sqrt{\varepsilon_i}}{1 + 2\varepsilon_i} \right) R_o^3 \tag{1}
\end{equation}

where $\tau_{\text{rad}}$ is the radiation damping time of the SPR oscillations, $\omega_p$ is the frequency of the SPR oscillation in bulk, $c$ is the speed of light, $\varepsilon_i$ is the dielectric constant of the medium, $R_o$ is the radius of a spherical particle. Equation (1) can be written in a form that demonstrates the volume dependence of the damping rate for a particle as shown in equation (2).
\[
\frac{1}{\tau_{\text{rad}}} \text{ (s}^{-1}) = \frac{\omega_p^4}{6\pi^3} \left( \frac{\sqrt{\varepsilon_1}}{1 + 2\varepsilon_1} \right) V \quad (2)
\]

\(V\) is the volume of a particle \(\left(\frac{4}{3}\pi R_p^3\right)\). \(\omega_p\) is related to the SPR frequency \(\omega_{sp}\) by the expression 
\[\omega_{sp} = \frac{\omega_p}{\sqrt{1 + 2\varepsilon_1}}.\] Therefore, we obtain the following equation (3) including \(\omega_{sp}\).

\[
\frac{1}{\tau_{\text{rad}}} \text{ (s}^{-1}) = \frac{\omega_p^2}{6\pi^3} \omega_{sp}^2 nV \quad (3)
\]

\(n \ (n = \sqrt{\varepsilon_1})\) is the refractive index of environment. Spectroscopically, the lifetime is characterized by the homogeneous linewidth \(\Gamma = \frac{2h}{\tau}\), which is connected to the dephasing time \(\tau\).\(^{48,54}\) Therefore, equation (3) can be expressed in terms of \(\Gamma_{\text{rad}}\).

\[
\Gamma_{\text{rad}} (eV) = \frac{\hbar \omega_p^2}{3\pi^3} \omega_{sp}^2 nV \quad (4)
\]

\(\omega_p h\) is about equal to 8.9 eV for gold.\(^{55}\) Therefore, \(\omega_p\) can be expressed as \(\frac{8.9}{h}\). By plugging \(\omega_p\) into equation (4), we get the following equation (5).

20
\[ \Gamma_{rad}(eV) = \frac{8.9^2}{3\pi^3 \hbar} \omega_p^2 n V \]  

(5)

\( \omega_p \): Bulk plasmon frequency for gold \( (s^{-1}) \), \( \omega_p \hbar = 8.9 \text{ eV} \)
\( \omega_{sp} \): SPR frequency \( (s^{-1}) \), \( \omega_{sp} = c/\lambda_{sp} \)
\( c \): Speed of light, \( 3 \times 10^8 \text{ m/s}^1 \)
\( \lambda_{sp} \): SPR wavelength \( (10^{-9} \text{ m}) \)
\( V \): Volume of a particle \( (\text{m}^3) \)
\( n \): Refractive index of the medium, \( n = \sqrt{\varepsilon_1} \)
\( \varepsilon_1 \): Dielectric constant of the medium
\( \hbar \): Dirac constant, equal to \( \frac{\hbar}{2\pi} \), \( 6.582 \times 10^{-16} \text{ eV s} \)
\( \h \): Planck constant

Therefore, we obtained an equation that relates the linewidth from radiation damping to the refractive index of the medium \( n \). According to equation (5), radiation damping is proportional to both the refractive index \( n \) and the volume of a particle \( V \). If we equate equation (5) with \( \frac{\hbar k V'}{2} \), \(^{30}\) we can obtain the following equation with respect to \( k \).

\[ k = \frac{2 \times 8.9^2}{3\pi^3 \hbar^2} \omega_{sp}^2 n \]  

(6)

We took scattering spectra of three AuNRs with AR of 3 in air medium \( (n = 1.25, \because \text{the NR sits on a glass slide } (n = 1.5)) \). In our calculation, we assumed that an
AuNR is 25 nm wide and 75 nm long (AR=3). The effective path length ($L_{eff}$) for the electrons was calculated by modeling a NR as spherically capped cylinders; specifically, $L_{eff} = D(1 - D/3L)$, where $D$ is the width and $L$ is the total length. Therefore, $1/L_{eff}$ has large values for thin NRs and small values for thick NRs. The total linewidth and SPR wavelength were determined by fitting the spectra to a Lorenzian function. We determined experimentally the average total SPR linewidth of 108.4 meV for three AuNRs in air. Then we used equation (5) to calculate the different contributions to the total linewidth. The calculated linewidth from radiation damping was 4.5 meV. The calculated $k$ was $3.69 \times 10^{-7}$ fs$^{-1}$nm$^{-3}$ and it shows a good agreement with the literature value $k$ of $4 \times 10^{-7}$ fs$^{-1}$nm$^{-3}$ for an AuNR with AR of 3. The linewidth from bulk damping was fixed at 73 meV. The linewidth from electron-surface scattering was determined to be 30.9 meV (108.4 - 73 - 4.5 = 30.9). In addition to $k$, we also determined the scattering parameter $A$ of 0.37 which shows a good agreement with the literature value $A$ of 0.30±0.03 for AuNRs with ARs between 2 and 3. Therefore, in this study, we were able to assign each contribution of the total linewidth by using equation (5).

It is also interesting that we can predict the dependence of the SPR linewidth on the refractive index of the surrounding medium (air, water, and oil) using equation (5). Table 2 summarizes the contributions of each decay process to the total SPR linewidth for an AuNR (AR=3). The calculated values are also compared to the experimental values for the AuNRs used in Figure 4.
The calculated linewidth of 4.5 meV from radiation damping in air is influenced by changing the refractive index of the medium to water and oil. We used equation (5) to predict the change in the linewidth from radiation damping in different media (water, oil). As shown in Table 2, the SPR linewidth remained almost constant with increasing refractive index for an AuNR with AR=3. The trend for the linewidth as a function of medium dielectric constant is in agreement with both the experimental data and DDA calculations as shown in Figure 4.

The broadening of the linewidth can also be due to chemical interactions. This is called chemical interface damping. This effect on the broadening is not well studied and understood. We therefore studied the chemical effect on the broadening of the SPR linewidth. In our study, we measured the single AuNR scattering spectra after adsorption

<table>
<thead>
<tr>
<th>Process</th>
<th>Air (n = 1.25)</th>
<th>Water (n = 1.41)</th>
<th>Oil (n = 1.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Gamma_{\text{bulk}}$</td>
<td>73 meV</td>
<td>73 meV</td>
<td>73 meV</td>
</tr>
<tr>
<td>$\Gamma_{\text{surf}}$</td>
<td>30.9 meV</td>
<td>30.9 meV</td>
<td>30.9 meV</td>
</tr>
<tr>
<td>$\Gamma_{\text{rad}}$</td>
<td>4.5 meV</td>
<td>4.1 meV</td>
<td>4.0 meV</td>
</tr>
<tr>
<td>$\Gamma_{\text{tot}}$</td>
<td>108.4 meV</td>
<td>108.1 meV</td>
<td>107.9 meV</td>
</tr>
<tr>
<td>$\Gamma_{\text{exp}}$</td>
<td>108.4 (±5.9) meV</td>
<td>112.9 (±9.8) meV</td>
<td>104.3 (±5.0) meV</td>
</tr>
</tbody>
</table>

Table 2. Contribution of each decay process to the total SPR linewidth for an AuNR (AR=3). The calculated total linewidth as a function of the medium refractive index (air, water, oil) is demonstrated. The calculated values are compared to the experimental values for the AuNRs shown in Figure 4.
of 1-decanthiol to the surface of an AuNR. As demonstrated in Figure 5, we observed that the thiol causes both a red-shift and a broadening of the SPR. While a change in the refractive index can explain the red-shift, the damping is inconsistent with a dielectric effect, which a comparison to the experiments and DDA calculations in Figure 4 shows. We therefore conclude that the increase in the SPR linewidth is due to specific interactions of the adsorbed thiol molecules with the SPR oscillation.

![Figure 5](image)

**Figure 5.** (A) Single NR scattering spectra in air (blue) and after adding 1-decanthiol (red). The scattering spectrum is red-shifted and strongly damped with an increased linewidth. The change in linewidth is better illustrated in (B) where the same spectra are normalized and plotted as a function of energy. The full width at half maximum is 103 meV and 117 meV for the blue and red spectra, respectively.
3-2. Photothermal Imaging of Gold Nanospheres

The most commonly used methods for studying metallic nanoparticles are scattering-based methods. But, the scattering methods are limited to the study of large nanoparticles above 30 nm in diameter. Recently, an absorption-based method called photothermal imaging was developed for measuring particles less than 10 nm in diameter.\textsuperscript{37,56}

In our laboratory, we successfully implemented the highly sensitive photothermal imaging technique. We measured 10 nm gold nanospheres to check if our setup is optimized and sensitive enough to perform polarization measurements with AuNRs (25 × 73 nm). As shown in Figure 6, we successfully measured 10 nm gold nanospheres by our photothermal imaging setup with a S/N ratio of about 35. In this measurement gold nanospheres were embedded in a PVA film and we added glycerol on top of the sample. Addition of glycerol on the sample insures homogeneity of the heat diffusion. The refractive index of glycerol and its thermal conductivity are close to those of common glasses. Therefore, there is no sharp discontinuity neither for the thermal parameters nor for the refractive indices at the glass–oil interface, and we can consider that the NPs are embedded in a homogeneous medium. In this measurement the sample was obtained by spin casting of a PVA solution containing 10 nm nanospheres and a Fluar objective (100×, N.A. = 1.3) was used to focus laser beams. The power of the heating beam (514 nm) and the probe beam (633 nm) was 1.18 mW and 10.1 mW, respectively.
Figure 6. Photothermal image of 10 nm gold nanospheres. The scan area is $20 \times 20 \, \mu m^2$. 
Furthermore, we studied the size dependence of the absorption cross section of gold nanospheres with diameters ranging from 30 to 250 nm at 532 nm, close to the transverse SPR. Figure 7 shows photothermal images (20 × 20 μm²) of gold nanospheres with diameters of 30 nm (A), 50 nm (B), 70 nm (C), 90 nm (D), 150 nm (E), and 250 nm (F). We measured several hundred nanospheres for each sample under the same conditions. For each sample, a histogram of the signal amplitudes was then generated as shown in Figure 8. Finally, we compared the mean intensities with Mie theory for absorption of spherical nanoparticles in air. As shown in Figure 9, we found a good agreement of our experimental data with Mie calculations for nanospheres ranging from 30 to 250 nm in diameter.

We also measured several hundred nanospheres with diameters ranging from 10 to 90 nm at 514 nm. In this measurement nanospheres were embedded in a PVA film and glycerol was added on top of the samples. An oil immersion objective was used to focus the laser beams onto the samples. Figure 10 shows photothermal images (20 × 20 μm²) of gold nanospheres with diameters of 10 nm (A), 30 nm (B), 50 nm (C), 70 nm (D), and 90 nm (E). For each sample, a histogram of the signal amplitudes was then made as shown in Figure 11. We found a narrower width of the intensity distribution for nanospheres inside a PVA film compared to nanospheres in air. This results from the difference in homogeneity of the heat diffusion. Finally, we also compared the mean intensities to Mie theory for absorption of nanospheres inside a PVA film (n = 1.5). As shown in Figure 12, we found a good agreement.
Figure 7. Photothermal images (20 × 20 μm²) of gold nanospheres with diameters ranging from 30 to 250 nm. (A): 30 nm, (B): 50 nm, (C): 70 nm, (D): 90 nm, (E): 150 nm, (F): 250 nm.
Figure 8. Intensity histograms for gold nanospheres with diameters ranging from 30 to 250 nm. (A): 30 nm, (B): 50 nm, (C): 70 nm, (D): 90 nm, (E): 150 nm, (F): 250 nm. Several hundred nanospheres were used to build the histograms for each sample. The red curves in panels E and F are fits to a sum of two Gaussian curves. The two distributions arise from the presence of single particles and small aggregates. The Gaussian curve corresponding to the smaller intensity values and hence the single particles were used to calculate the average absorption intensity.
Figure 9. Size dependence of gold nanospheres with diameters ranging from 30 to 250 nm. We measured several hundred nanospheres in air for each sample. A 532 nm laser was used as a heating beam. Red squares indicate the mean intensity for each size. The black line is a Mie calculation for absorption.
Figure 10. Photothermal images (20 × 20 μm²) of gold nanospheres ranging from 10 to 90 nm in diameter. Nanospheres were embedded in a PVA film. A 514 nm laser was used as a heating beam. (A): 10 nm, (B): 30 nm, (C): 50 nm, (D): 70 nm, (E): 90 nm.
Figure 11. Intensity histograms for gold nanospheres inside a PVA film. The diameter of the gold nanospheres varied from 10 to 90 nm. Several hundred nanospheres were used to make the histograms for each sample. (A): 10 nm, (B): 30 nm, (C): 50 nm, (D): 70 nm, (E): 90 nm.
Figure 12. Size dependence of gold nanospheres with diameters ranging from 10 to 90 nm. We measured several hundred nanospheres in a PVA film for each sample. A 514 nm laser was used as a heating beam. Red squares indicate the mean intensity for each size. The black line is a Mie calculation for absorption.
We also studied the enhancement of the photothermal signal by coating the particle surface with thiols. To do this, we first made two samples with 90 nm gold nanospheres. In this experiment we used 1-decanethiol. To introduce the thiol, we added two drops of 10 μM thiol solution onto the top of one of the two samples and allowed enough time for drying. Then we measured two samples under the same experimental conditions and compared the photothermal signal. In this experiment a 532 nm laser was used as a heating beam and a low excitation power of 145 μW was employed. In addition, we used an Epiplan objective (100x, air spaced) and the nanospheres were measured in air.

Figure 13 shows photothermal images of 90 nm gold nanospheres without thiol coating (A) and with thiol coating (B). As shown in the photothermal images, the photothermal signal was enhanced for thiol-coated 90 nm gold nanospheres. To make this clearer, we constructed intensity histograms from many particles for each sample. Figures 13C and 13D show intensity histograms for bare gold nanospheres and thiol-coated gold nanospheres, respectively. The photothermal signal was roughly 7 times improved by coating the particle surface with thiols.

There are some possible explanations for the signal enhancement. The first possible explanation is that it might be due to an optical effect. If the particle surface is capped with thiols, the refractive index of the surrounding environment will be changed and the SPR peak will shift. This can affect the energy absorbed by the nanospheres. The second possible explanation is that the enhancement is due to a chemical effect from the thiol capping. The gold nanosphere surface is capped by decanethiols, which have a relatively long chain. The generated heat by laser irradiation could be kept inside the
layer and released into the glass substrate where the gold nanosphere is touching. The thiol layer would therefore help to prevent heat loss to the air. Thus, almost all the converted heat would be released to the glass substrate. This would cause an increase of the local temperature at the glass surface and an enhanced photothermal signal.

Figure 13. Effect of thiol coating on the photothermal signal of 90 nm gold nanospheres. Photothermal images (20 × 20 μm²) of gold nanospheres without thiol coating (A) and with thiol coating (B) under the same condition. (C, D) Intensity histograms of bare gold nanospheres (C) and thiol coated gold nanospheres (D).
3-3. Polarization-Dependent Photothermal Imaging of Gold Nanorods

3-3-1. Polarized Photothermal Imaging of Gold Nanowires

AuNRs have unique optical properties. Their dipole plasmon wavelength can be tuned from the visible to the infrared by changing the AR of the NR as well as the refractive index of the environment\(^{3,57,58}\). Because of the size and shape-controlled scattering and absorption properties, the AuNRs have promising applications in cancer diagnosis\(^{59}\) and in photothermal destruction of cancer cells.\(^{60}\) The dipole plasmon resonance of metallic NRs has been intensively studied, both experimentally and theoretically. However, there have been fewer studies on the multipolar SPR mode of nanowires.

Multipolar SPR modes have been investigated on nanowires in aqueous solution by Mirkin and co-workers.\(^{61}\) They found that the multipolar mode can be excited for the both even and odd order modes due to the random orientation of the NRs in the aqueous solution. They also simulated the spectra of multipolar SPR modes by the DDA method. The simulated results agreed well with experimental results. Khlebtsov and co-workers have studied multipolar SPR modes theoretically by the T-matrix-DDA method.\(^{62}\) They have studied the absorption, scattering and extinction properties of AuNRs with different ARs. They found that the scattering intensities of the multipolar longitudinal modes are stronger than the absorption of the transverse mode for NRs with a diameter of 80 nm. On the other hand, the absorption of the transverse mode is dominant for a NR with a diameter of 20 nm. They also simulated the wavelength shift of the multipolar modes as a function of the refractive index of the environment. The higher order modes are less sensitive to the refractive index of the surrounding medium. The scaling of the peak
wavelength of multipolar modes with size (length, width) is also discussed in their work. It is crucial to investigate the multipolar longitudinal modes to understand the fundamental properties of the SPR in nanowires. However, most studies so far were done by exploring the SPR extinction which is the combination of scattering and absorption.\textsuperscript{61,63-66}

We carried out polarization-dependent photothermal imaging of nanowires exciting the multipolar modes. Figure 14 shows a TEM image of nanowires and the absorption spectrum in aqueous solution. The mean width and length of the nanowires are 25 nm and 2.5 \( \mu \text{m} \), respectively. The photothermal signal presents the absorption property of the nanowire. In order to probe the transverse and multipolar SPR modes separately, a linearly polarized heating beam was employed and the polarization dependence with respect to the long axis of a nanowire was performed. In our experiment we used excitation wavelengths of 488 and 514 nm. According to the absorption spectrum of the nanowires as shown in Figure 14B, the transverse SPR mode is peaked at 509 nm. However, the wavelength of the transverse SPR mode is blue-shifted as the refractive index of the surrounding environment decreases. The excitations using 514 and 488 nm will be close to the wavelength of the transverse SPR. In addition to the transverse SPR mode, we also expect to excite multipolar SPR modes along the long axis of the nanowire with the two excitation wavelengths. It is well known that the dipole plasmon shifts to the red with increasing the length \( L \) of a NR at a fixed diameter \( D \) while a new SPR peak appears at a shorter wavelength, corresponding to the 3\textsuperscript{rd} order multipolar SPR mode \( (l=3) \).\textsuperscript{61,62} If \( L \) further increases, more multipolar SPR modes
From a calculation performed by Encina et al., we expect that with our excitation beams we would excite the 13th order multipolar SPR mode ($l=13$).

Figure 14. (A) TEM image of gold nanowires. The mean width and length of the nanowires are 25 nm and 2.5 μm, respectively. Scale bar is 1 μm. (B) Absorption spectrum of gold nanowires in aqueous solution. The transverse SPR peak appears around 509 nm.
Figure 15 shows the polarization-dependent photothermal images of single nanowires measured with 488 nm and 514 nm laser beams. Photothermal images (3 x 3 μm²) with different polarization angles θ are shown in Figure 15. Multipolar SPR modes and the transverse SPR mode of the nanowire are excited with θ = 0° and 90°, respectively.

Figure 16A shows the intensity as a function of polarization angle from 0 to 90° for 488 nm and 514 nm excitations on the single nanowires shown in Figure 15. The photothermal traces were normalized and fitted to \( \cos^2(\theta - \phi) \) as shown in Figure 16B. The experimental condition was the same for both excitations. It was observed that the photothermal signal is maximum at \( \theta = 0° \) and minimum at \( \theta = 90° \) for both excitation wavelengths. This result suggests that the absorption of the multipolar SPR mode is more intense than that of the transverse SPR mode for both excitation wavelengths. As shown in Figure 16A the absorption intensity of the multipolar SPR mode with 488 nm excitation is higher than that with 514 nm excitation. In addition we observe that the absorption intensity of the transverse SPR mode with 514 nm is greater than that with 488 nm. This suggests that 514 nm is closer to the transverse SPR wavelength.

We can perform further photothermal experiments with more excitation wavelengths to gain deeper insight into the absorption spectrum of longitudinal and transverse modes of nanowires. Therefore, in this work, we obtained polarization-sensitive photothermal images of nanowires and studied absorption properties of their multipolar SPR modes.
Figure 15. Photothermal images (3 × 3 μm$^2$) of nanowires as a function of polarization angles. The arrows indicate the axis of polarization. (A - G) 488 nm excitation, (H - N) 514 nm excitation.
Figure 16. (A) Intensity as a function of polarization angles from 0 to 90° for both 488 nm and 514 nm excitation of the single nanowires in Figure 15. (B) The photothermal traces are normalized and fitted to $M\cos^2(\theta - \phi)$. 
3-3-2. Polarized Photothermal Imaging of Gold Nanorods

Polarization-sensitive photothermal imaging was performed on AuNRs with a mean width of 25 nm and a mean length of 73 nm. As shown in Figure 17A, the ensemble extinction spectrum of these AuNRs in aqueous solution had broad longitudinal and transverse SPR bands, peaking at 700 nm and 514 nm, respectively. For this NR size, absorption dominates over scattering in the extinction spectrum, especially for the transverse SPR. Therefore, a technique based on absorption is expected to be better suited in probing both longitudinal and transverse SPR modes compared to dark-field scattering spectroscopy.

For photothermal imaging of single AuNRs the sample was deposited on a glass coverslip at low concentration to achieve a coverage of less than one NR per \( \mu \text{m}^2 \). To excite the longitudinal and transverse SPR absorptions, we used lasers with wavelengths of 675 nm and 514 nm as heating beams. Because of the strong dependence of the longitudinal SPR on the medium refractive index \( n \), we first had to ensure that the 675 nm laser was able to efficiently excite the longitudinal SPR. To address this issue, we measured the scattering spectra of the same single AuNR on a glass coverslip surrounded by air \((n = 1)\), water \((n = 1.3)\), and index-matched oil \((n = 1.5)\), which is shown in Figure 17B. The scattering spectra illustrate how the maximum of longitudinal SPR red-shifts with increasing medium refractive index. Although the relative cross sections differ for SPR absorption and scattering, the SPR absorption maxima for these AuNRs coincide with the scattering maxima within a few nanometers. According to the scattering spectra, the longitudinal SPR was most efficiently excited with 675 nm when the AuNRs were covered with water. The water also allowed for a better thermal conductivity.
between the AuNRs and the surrounding medium leading to an enhanced photothermal signal. Efficient excitation and heat transfer were important in order to avoid partial photothermal reshaping of the AuNRs. We independently confirmed the absence of any NR shape changes by measuring both scattering spectra and SEM images before and after the photothermal absorption experiments. Unless specifically stated otherwise, all photothermal measurements were carried out with AuNRs deposited on glass and surrounded by water.

![Figure 17. AuNRs ensemble extinction spectrum taken in aqueous solution (A) and single particle scattering spectra deposited on glass and surrounded by different media (B). The inset shows a TEM image of the AuNRs having an average length and width of 73 and 25 nm, respectively.](image)
To correlate the orientations of individual AuNRs measured by SEM with those determined from polarization-sensitive photothermal imaging and to ensure that we were probing isolated AuNRs, we fabricated an identification pattern on the glass substrates using indexed TEM grids as masks and evaporating thin metal layers of 5 nm Ti followed by 20 nm Au. Figure 18 shows SEM (A) and photothermal (B) images of the same two AuNRs and one NR dimer. This result demonstrates our ability to locate the same AuNRs with SEM and photothermal imaging. In Figure 18B the NR longitudinal SPR was excited by a circularly polarized 675 nm heating beam, which resulted in comparable photothermal intensities of all AuNRs regardless of the NR orientation. The vertical axis of the SEM image was selected as the reference for determining the orientation of AuNRs in the SEM images. We defined the orientation angle \( \phi \) as the angle between the long axis of a NR and the reference axis. The orientation angle increased in the counter clockwise direction as shown in Figure 18A. For the two AuNRs highlighted by the green and red squares in Figure 18A, we obtained values of \( \phi = 11^\circ \) and \( 105^\circ \) following this procedure.

Figure 18. SEM (A) and photothermal (B) images of the same area showing two AuNRs and one NR dimer. The photothermal image was recorded with 675 nm excitation.
Polarization-sensitive photothermal imaging was carried out by linearly polarizing the 675 nm excitation beam and changing the polarization from 0° to 180° with respect to the reference axis in 30° increments. The photothermal intensities are plotted as a function of polarization angle in Figure 19A and 19B for the same two AuNRs marked by the green and red squares in Figure 18B. The insets show magnified photothermal images of the single AuNRs for the different polarization angles θ. It can clearly be seen from these photothermal polarization traces that the photothermal response of single AuNRs is highly polarized with a large contrast when excited at the longitudinal SPR. The observed polarization dependence of the photothermal intensity furthermore confirms that the longitudinal SPR behaves as single dipole absorber and indicates that polarization-sensitive photothermal imaging can be used to determine the orientation of single AuNRs. For the two AuNRs in Figure 18A the photothermal polarization traces are 90° out of phase in agreement with their orthogonal orientation (φ = 11° and 105°).

For a quantitative analysis of the NR orientations, we fitted the photothermal polarization traces to \( I(\theta) = N(1 + M \cos 2(\theta - \phi)) \). This equation has been successfully applied before to extract the orientation and conformation of single conjugated polymer chains, which due to a limited exciton delocalization are composed of multiple single dipole chromophores. The modulation depth \( M \) represents the average anisotropy of the absorption dipoles projected onto the sample plane. \( M = 1 \) if only a single dipole is present or all chromophores are aligned in a linear chain. On the other hand, \( M = 0 \) corresponds to a conformation in which all dipole projections are evenly distributed in the sample plane. \( \phi \) represents the angle of the longest projected dipole axis with respect to a reference frame, thereby giving the in plane orientation of the major absorption
dipole moment. We applied this formulism to the SPR absorption of AuNRs because the transverse mode can be regarded as two orthogonally polarized oscillations, which furthermore overlap with unpolarized interband transitions. Before we elaborate more on the transverse SPR mode, we first present a quantitative analysis of the longitudinal SPR absorption.

Our analysis confirms that for single AuNRs deposited on a glass substrate the longitudinal SPR mode behaves like a single dipole absorber and that the direction of the SPR oscillation is orientated parallel to the long NR axis. This result is in agreement with previous single NR scattering and extinction measurements. The solid lines in Figure 19 are fits of the measured polarization traces giving modulation depths of $M_{675} = 1.05$ and $0.95$ for the AuNRs shown in Figure 19A and 19B, respectively. Interestingly, the modulation depth of a NR dimer is much smaller than 1 and the fit in Figure 18C gives $M_{675} = 0.33$. This is consistent with a hybridization of the longitudinal and transverse SPR modes in a side-by-side NR dimer; which reduces the modulation depth.
Figure 19. Polarization dependence of the photothermal intensity for the AuNRs shown in Figure 18. The insets show the photothermal signal of the individual particles as a function of excitation polarization angle $\theta$. 

$M_{675} = 0.33$  
$\phi_{675} = 36^\circ$

$M_{675} = 0.95$  
$\phi_{675} = 85^\circ$
In order to back up our conclusions with a statistically meaningful data set, we measured the modulation depths for ~200 single AuNRs with 675 nm excitation. Figure 20A displays the histogram of measured modulation depths for the longitudinal SPR absorption. The distribution has a maximum for $M = 1$ further confirming the single dipole behavior of the longitudinal SPR absorption. The width of the distribution is attributed to mainly errors in the measurement rather than an intrinsic variation in the modulation depth. Changes in the excitation polarization in between scanned photothermal images caused small deviations in the overlap between heating and probe beams, which in turn led to variations in the signal intensities giving a less perfect fit. An automated beam stirring setup could in principal correct for these fluctuations in future experiments. However, despite a finite width in the distribution of modulation depths, our results demonstrate that polarization-sensitive photothermal imaging can accurately determine the dipole characteristics of the longitudinal SPR absorption for single AuNRs even when using a high N.A. objective.

The orientation angles obtained from the photothermal imaging agree well with those measured by SEM. The fits in Figure 19A and 19B yield orientation angles of $\phi = 5^\circ$ and $85^\circ$ compared to the SEM values of $11^\circ$ and $105^\circ$. The systematic deviation between these two techniques can be explained by the error introduced when overlaying corresponding photothermal and SEM images to determine the reference axis. Minor depolarization of the excitation light after passing through the high N.A. objective could have also contributed. Figure 20C shows the correlation of orientation angles for 50 AuNRs determined independently by photothermal imaging and SEM. The solid line is a linear regression yielding $\phi_{\text{SEM}} = 0.95 \times \phi_{\text{photothermal}} + 10.5^\circ$ with $r^2 = 0.97$. The excellent
correlation between the orientation angles obtained by the two techniques validates that
the orientation of single AuNRs can be accurately determined by polarization-sensitive
photothermal imaging. Note that the intercept of the linear fit is $10.5^\circ$ which is consistent
with the systematic deviation discussed already. Determining the orientation of single
AuNRs by optical means is very important for live cell imaging when electron
microscopy cannot be used.

The polarization-sensitive photothermal imaging results have verified that the NR
longitudinal SPR absorption can be treated as a single dipole oscillation and that we are
able to measure a modulation depth of unity. It is thus possible to determine the absolute
value of the orientation angle from the measurement of only two orthogonally polarized
intensities $I_x$ an $I_y$ by defining the polarization anisotropy $x y, P = \frac{I_x - I_y}{I_x + I_y}$, as is
conventionally done for the determination of the dipole orientation of a single dye
molecule. This approach is significantly easier and faster because only two instead of
seven images have to be measured. To further test this conclusion, we calculated the
photothermal polarization anisotropy (Figure 20B). The polarization anisotropies for the
randomly orientated AuNRs are distributed evenly between the minimum and maximum
values of -1 and +1 in agreement with the polarization anisotropy distribution of single
dye molecules in an isotropic environment. Similar to the correlation analysis shown
in Figure 20C, Figure 20D is a plot of orientation angles of the same 50 AuNRs
determined from the polarization anisotropy compared to the corresponding angles
obtained from the SEM images. Because only two polarized intensities are used for this
analysis, we cannot distinguish between $+\phi$ and $-\phi$ with respect to the reference axis and
therefore the absolute values of the orientation angles are shown in Figure 20D. A linear
regression of the correlation plot gives $|\phi|_{\text{SEM}} = 0.87 \times |\phi|_{\text{photothermal}} + 8.2^\circ$ and $r^2$ of 0.78.

This result demonstrates good agreement for orientation angles obtained from the photothermal polarization anisotropy and SEM, although the analysis is not as accurate as fitting the modulation depth to 7 polarization angles.

With $M = 1$ we measured one of the two extreme values of the modulation depth by polarization-sensitive photothermal imaging. Next, we also tested if the other extreme value, i.e. $M = 0$, can be detected for a single nanoparticle with randomly orientated dipole absorptions. We therefore acquired polarized photothermal images for 30 nm gold nanospheres embedded in a PVA film with 514 nm excitation. The almost isotropic shape of an Au nanosphere results in a polarization independent SPR excitation. The photothermal traces were nearly independent of the polarization angle leading to a very small modulation depth close to zero. The distribution of modulation depths shown in Figure 21A peaks at ~0 with a mean value of $<M_{514}> = 0.07\pm0.04$ for thirty 30 nm gold nanospheres, confirming that polarization-sensitive photothermal imaging is capable of measuring modulation depths over the entire range from 0 to 1. Small deviations from $M = 0$ are due to both an error in the fit as well as a slight anisotropy in the nanoparticle shape.
Figure 20. Histogram of modulation depths (A) and polarization anisotropy (B) for AuNRs excited at 675 nm. The NR orientations obtained from the modulation depth (C) and polarization anisotropy (D) analysis are in very good agreement with the angles measured from the correlated SEM images. The red lines are linear regressions.
The modulation depth of the transverse SPR absorption is expected to be nonzero but also smaller than 1 for AuNRs because of the polarization independent interband transitions that overlap energetically with the transverse mode. Using spatial modulation spectroscopy, Muskens et al. have measured a polarization contrast ratio of 1.1 for the transverse SPR from the extinction spectrum of single AuNR with a diameter of ~20 nm. As expected from ensemble measurements, they also found that the maximum of transverse SPR extinction occurs at a polarization angle that is perpendicular to the longitudinal resonance. However, such a small polarization dependence suggests that it is difficult to extract the NR orientations from the transverse SPR. For NR diameters of ~20 nm or smaller, dark-field spectroscopy is not sensitive enough to record the scattering of the transverse SPR (Figure 17B). The high sensitivity of photothermal imaging to visualize single metal nanoparticles smaller than 10 nm in diameter and the ability to determine the entire range of modulation depths therefore makes this technique ideally suited to test the polarization dependence of the transverse SPR absorption. Determining the orientation of AuNRs also from transverse mode is significant because, unlike the longitudinal SPR, the transverse SPR can be excited with the same laser frequency independent to AR of the NR and refractive index of the surrounding environment.

Figure 21B shows a photothermal image of AuNRs on glass and covered with water excited by 514 nm, which coincides with the peak of the transverse SPR mode (Figure 17A). The high signal to noise ratio exceeding 500 confirms that photothermal imaging can probe the transverse SPR absorption of these AuNRs with a high sensitivity. The photothermal polarization traces for the two AuNRs marked by the colored squares
in Figure 21B are shown in Figure 21C for both 514 nm (closed symbols) and 675 nm (open symbols) excitations. The traces are 90° out of phase in agreement with orthogonal polarizations of the two SPR modes. Similar to the results by Muskens et al., the modulation depth for the transverse SPR absorption is much smaller with $M_{514} = 0.24$ and $0.32$ compared to $M_{675} = 0.92$ and 1.06 for the AuNRs highlighted by the orange and magenta squares, respectively. In addition, it is important to note that for these conditions we had problems fitting some photothermal polarization traces accurately to obtain $M$ and especially $\phi$ because of the small polarization dependence of the transverse SPR. This makes it difficult to determine the NR orientations with 514 nm excitation.

In order to exclude that a spectral overlap from the tail of the much more intense and orthogonally polarized longitudinal SPR absorption did not lead to a depolarization and hence smaller measured modulation depth for the transverse SPR mode, we also measured polarization-dependent photothermal images of longer AuNR ($25 \times 86$ nm) with 514 nm excitation. According to the ensemble extinction spectra in aqueous solution, the transverse SPR maxima are at 514 nm for both $25 \times 73$ nm and $25 \times 86$ nm AuNRs, while the longitudinal SPR maximum was red-shifted from 700 nm to 730 nm for the longer AuNRs. However, we found that the modulation depths of both NR samples for 514 nm excitation and with water as the partially surrounding medium were nearly identical. The mean modulation depth for the $25 \times 86$ nm AuNRs was $<M_{514}> = 0.29 \pm 0.20$ and the distribution is also given in Figure 21A. It can be concluded from these results that a contribution from the tail of the longitudinal SPR can be ignored for 514 nm excitation.
Figure 21. Photothermal imaging of nanospheres and AuNRs with 514 nm excitation. (A) Comparison of modulation depth histograms for 30 nm nanospheres in PVA film (brown: \( n = 1.5, \langle M_{514} \rangle = 0.07\pm0.04 \)), 86 nm long AuNRs in water (yellow: \( n = 1.3, \langle M_{514} \rangle = 0.29\pm0.20 \)), 73 nm long AuNRs in water (green: \( n = 1.3, \langle M_{514} \rangle = 0.29\pm0.21 \)), and 73 nm long AuNRs in oil (blue: \( n = 1.5, \langle M_{514} \rangle = 0.42\pm0.22 \)). Included is also the distribution measured for 73 nm long AuNRs in oil using a N.A. = 0.7 objective (red: \( n = 1.5, \langle M_{514} \rangle = 0.52\pm0.25 \)). (B) Photothermal image of 73 nm long AuNRs recorded with circularly polarized excitation at 514 nm. (C) Polarization dependence of the photothermal signal for the AuNRs highlighted by the colored squares in (B). The closed (open) circles correspond to excitation of the transverse (longitudinal) SPR absorption. (D) Simulated modulation depth as a function of the ratio \( k \) between transverse SPR and interband absorptions for objectives with N.A. = 1.3 and water as the surrounding medium (blue), N.A. = 1.3 and oil (green), and N.A. = 0.7 and oil (red). The open circles indicate the experimentally determined mean modulation depths.
Finally, we also examined the effect of a high N.A. objective. It is well known that the polarization at the focus of a high N.A. objective has changed from that of the incident beam by acquiring polarization components that are perpendicular to the image plane and the original polarization direction.\textsuperscript{71} In fact, this depolarization can be used to determine the 3-D orientation of single chromophores because transition dipoles aligned perpendicular to the sample surface can also be excited. The transverse SPR of the cylindrically shaped AuNRs can be approximated as two dipole oscillation polarized perpendicular to each other, i.e. SPR oscillations parallel and perpendicular to the sample plane. Using a 514 nm heating beam and a high N.A. objective, both these oscillations were excited in our experiments. However, the dipole absorption perpendicular to the sample plane surface is independent of the excitation polarization resulting in a smaller modulation depth. In addition to the N.A., the cone of excitation light and hence the out-of-plane polarization components are also determined by the refractive of the laser light at the interface between the glass substrate \((n = 1.5)\) and the medium covering the AuNRs. For example, the smaller refractive index of water \((n = 1.3)\) compared to index-matched oil \((n = 1.5)\) causes a larger illumination cone. We indeed found that the out-of-plane polarization component was responsible for the low modulation depth of the transverse SPR absorption and that it was possible to increase the modulation depth by changing to a low N.A. objective and a higher refractive index of the medium covering the AuNRs. As shown in Figure 21A, the modulation depths of the same AuNRs increased from \(<M_{514}> = 0.29\pm0.21\) to \(<M_{514}> = 0.42\pm0.22\) when water was replaced with and oil. An additional increase to \(<M_{514}> = 0.52\pm0.25\) was achieved by changing to an objective with N.A. = 0.7. It is worth noting that a red-shift of the transverse SPR absorption away from
the interband transitions when changing from water to oil is expected to lead to a smaller modulation depth as the transverse SPR is peaked at 514 nm in water. This trend is opposite to what we observed and hence only plays a minor role if any.

Because the depolarization caused by the optical elements can be calculated from the N.A. of the objective and the medium refractive index, we quantitatively modeled the modulation depths of the transverse SPR absorption (Figure 21D). Assuming that only unpolarized interband transitions and two perpendicular dipole oscillations of the transverse SPR contribute to the NR absorption at 514 nm, we obtained the intrinsic degree of polarization of AuNRs at the excitation wavelength. Defining the ratio of transverse SPR and interband absorptions as $k$, we can write the modulation depth as

$$M = \frac{(C_x - C_y)k}{(C_x + C_y + 2C_z)k + 2}$$

where $C_x$, $C_y$, $C_z$ are the microscope correction factors in an $x,y,z$ lab frame in which the $z$-axis is perpendicular to the image plane. Figure 21D shows the simulated modulation depth as a function of $k$ for the three different experimental conditions of objectives and surrounding media, i.e. N.A. = 1.3 and $n$ =1.3 (blue), N.A. = 1.3 and $n$ =1.5 in oil (green), N.A. = 0.7 and $n$ =1.5 in oil (red). Changing from oil to water as the surrounding medium for the low N.A. objective causes only a very minor decrease in the modulation depth which is important for imaging biological samples. The open circles indicate the experimentally determined mean modulation depths from the histograms. The simulations then yield the corresponding $k$ values of 3.1, 3.1, and 2.7, which are in excellent agreement for the different experimentally conditions. We can therefore conclude that the transverse SPR absorption is on average 3 times stronger than the interband absorption at 514 nm for this AuNR sample. It should be noted that average modulation depths have been assumed for these calculations, but that
the distribution for 514 nm excitation is actually non-Gaussian unlike the modulation depth distribution for 675 nm excitation. The shape of the latter is consistent with the experimental errors discussed above. However, the reason for the asymmetric modulation depth distribution for 514 nm excitation is not clear at the moment and more detailed modeling of the transverse plasmon absorption taking into account variations in the shape of the NR ends are necessary.

The effects of a high N.A. objective and the refractive index mismatch with the surrounding medium on the modulation depth can be even better illustrated by comparing the photothermal polarization traces for the same NR using different excitation conditions. Figure 22A shows the polarization trace of an AuNR for 675 nm excitation covered with water using a N.A. = 1.3 objective. Despite the large N.A., any excitation light that becomes polarized perpendicular to the long NR axis (parallel and perpendicular to the sample plane) will not be absorbed by the NR as the transverse SPR mode absorbs at shorter wavelength than 675 nm. We therefore obtained a modulation of unity ($M_{675} = 1.03$) and the NR orientation of $\phi = 155^\circ$ agrees well with the angle measured by SEM ($\phi = 160^\circ$). However, when the transverse SPR of the same AuNR covered with water is excited at 514 nm using the same high N.A. objective, out of plane polarized light is absorbed by the transverse mode independent of the polarization angle yielding a small modulation depth of $M_{514} = 0.04$ (Figure 22B, red symbols). A smaller N.A. and a refractive index matched medium cause a smaller illumination cone and reduce the component of excitation light with a polarization vector perpendicular to the image plane. Therefore, measuring the photothermal polarization trace for the same NR covered with oil and using a N.A. = 0.7 objective, yields a much a higher modulation depth of $M_{514} =$
0.65 and better fit with an orientation angle of $\phi = 58^\circ$ in very good agreement with the one measured using 675 nm excitation, considering the $90^\circ$ phase shift for the two SPRs. As discussed above, determining the NR orientation by exploiting the polarization dependence of the transverse SPR is important because the transverse mode is mostly insensitive to changes in the environment and AR of the NR compared to the longitudinal mode. Thus, only a single excitation wavelength is necessary to probe the orientations of different AuNRs regardless of the NR size and medium refractive index.
Figure 22. Polarization dependence of the photothermal intensity for the same AuNR using different excitation wavelengths, numerical apertures, and media. (A): 675 nm, N.A. = 1.3, n = 1.3; (B): 514 nm, N.A. = 1.3, n = 1.3 (red) and 514 nm, N.A. = 0.7, n = 1.5 (blue).
4. CONCLUSION

In this thesis we report on SEM correlated dark-field scattering studies on single AuNRs. We successfully correlated SEM images to optical images and spectra. We clearly observed polarization-dependent scattering of a single AuNR, and its orientation was precisely determined by fitting the polarization dependence to \( M \cos(\theta - \phi) \). We further studied the dependence of the SPR linewidth on both the refractive index of the surrounding medium and the adsorption of thiol groups onto the surface of AuNRs. We found that the SPR wavelength shifted to longer wavelengths when increasing the refractive index of the surrounding medium as expected. It is more interesting to note that the SPR linewidth remained almost constant while increasing the refractive index of the surrounding environments from air to oil. The experimental results showed a good agreement with DDA calculations. We also found that thiol groups cause both a red-shift and a strong damping with an increased SPR linewidth. The increase in SPR linewidth is thought to be due to specific interactions of the adsorbed thiol molecules with the SPR oscillation.

We carried out photothermal imaging of gold nanospheres down to 10 nm with high sensitivity. Furthermore, we studied the size dependence of the SPR absorption of gold nanospheres with diameters ranging from 30 to 250 nm at 532 nm, close to the transverse SPR. We measured several hundred nanospheres for each sample under the same condition. For each sample, a histogram of the signal amplitudes was then generated. Finally, we compared with Mie calculations and found a good quantitative agreement of our experimental data.
We have, for the first time, demonstrated the polarization-dependent photothermal imaging of AuNRs. It is confirmed that both nanowires and AuNRs show a polarization dependence in photothermal imaging. The polarization dependence of the AuNRs has been exploited to develop a new absorption-based single particle method that allows us to accurately determine the orientation of individual AuNRs by selectively exciting either of two SPRs. In particular, our results show that by exciting the transverse SPR when using a low N.A. objective, the NR orientation can be accurately probed, which is impossible with conventional dark-field scattering spectroscopy and significant because of the invariance of the transverse SPR absorption with respect to AR of the NR. Correlated SEM imaging furthermore independently verified the AuNR orientations obtained from the polarization-dependent photothermal images.
5. REFERENCES


18. Murphy, C. J.; Gole, A. M.; Stone, J. W.; Siscot, P. N.; Alkilany, A. M.;


43. Funston, A. M.; Novo, C.; Davis, T. J.; Mulvaney, P. Plasmon Coupling of Gold Nanorods at Short Distances and in Different Geometries *Nano Lett.* 2009, 9, 1651-1658.


