GOLD NANOSPHERES AND GOLD NANORODS AS LOCALIZED SURFACE PLASMON RESONANCE SENSORS

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ABSTRACT OF THESIS

GOLD NANOSPHERES AND GOLD NANORODS AS LOCALIZED SURFACE PLASMON RESONANCE SENSORS

A novel localized surface plasmon resonance (LSPR) sensor that differentiates between background refractive index changes and surface-binding of a target analyte (e.g. a target molecule, protein, or bacterium) is presented. Standard, single channel LSPR sensors cannot differentiate these two effects as their design allows only one mode to be coupled. This novel technique uses two surface plasmon modes to simultaneously measure surface binding and solution refractive index changes. This increases the sensitivity of the sensor.

Different channels or modes can be created in sensors with the introduction of gold nanospheres or gold nanorods that act as receptor mechanisms. Once immobilization was achieved on gold nanospheres, the technique was optimized to achieve the same immobilization for gold nanorods to get the expected dual mode spectrum. Intricate fabrication methods are illustrated with using chemically terminated self assembled monolayers. Then the fabrication process advances from chemically silanized nanoparticles, on to specific and systematic patterns generated with the use of Electron Beam Lithography.

Comparisons are made within the different methods used, and guidelines are set to create possible room for improvement. Some methods implemented failed, but there was a lot to learn from these unsuccessful outcomes. Finally, the applications of the dual mode sensor are introduced, and current venues where the sensors can be used in chemical and biological settings are discussed.
KEYWORDS: Surface Plasmon Resonance Sensor (SPR), Dual Mode SPR Sensor, Acid Terminated Self Assembled Monolayer (SAM), Absorption vs. Wavelength, Patterned Gold Nanorods in Electron Beam Lithography (EBL).

Akil Mannan Matcheswala

Date: February 25th 2010

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GOLD NANOSPHERES AND GOLD NANORODS
AS LOCALIZED SURFACE PLASMON RESONANCE SENSORS

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2/25/2010
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GOLD NANOSPHERES AND GOLD NANORODS
AS LOCALIZED SURFACE PLASMON RESONANCE SENSORS

THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of
Master of Science in Electrical Engineering in the College of
Engineering at the University of Kentucky

By
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Lexington, Kentucky

Director: Dr. J. Todd Hastings, Associate Professor of Electrical Engineering
Lexington, Kentucky
2010

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DEDICATION

This thesis is dedicated to my family and everybody who stood steadfast beside me, and provided assistance I have received either directly or indirectly from them. If you put your mind and perseverance into it, the sky is the limit.
ACKNOWLEDGEMENTS

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1. CHAPTER 1 – INTRODUCTION

1.1. Basis of sensing capabilities

Chemical and biological sensors have been extensively studied due to their wide applications in contamination assessment, medical diagnosis and other areas. Surface plasmon resonance (SPR) methods have made an important contribution to the observation of bimolecular interactions [1]. For this type of sensor a relatively simple approach can be used by taking advantage of the nanoparticles of noble metals. For noble metal nanoparticles, the plasmon resonance band can be changed by changing certain physical characteristics such as the size, shape, composition, relative distance, and refractive index of the surrounding medium [2].

The collective oscillation of the conduction electrons within noble metal nanoparticles, referred to as a localized surface plasmon exhibits an absorption spectrum, which depends on the surrounding medium [1]. The ratio of the change in the absorption peak shift to the change in surrounding refractive index gives the sensitivity of the sensor, which is dependent on the surrounding refractive index itself and the binding methods used to attach the particles to a supporting substrate [3]. In our experiment, we immobilized the nanoparticles onto a substrate because the immobilized nanoparticles stabilized their position and orientation. The immobilization of the nanoparticles onto the substrate can be achieved by numerous chemical methods which will be described in this thesis. In particular, gold nanoparticles (AuNP) have been used in the form of gold nanospheres (AuNS) and gold nanorods (AuNR), and these immobilized particles could be used as refractive-index sensors.
1.2. SPR sensor in planar, array based form

This thesis presents an optical sensor that has several important advantages over a conventional SPR sensor. Some of these are easy fabrication, better nanoparticles pattern location, and simple implementation requiring only an UV-visible spectrometer. A SPR biosensor was developed using immobilized gold colloids and nanorods, on an optically transparent substrate (glass) for many reasons. Noble metal gold nanoparticles permit the absorption of light in the visible and near infrared regions of the electromagnetic spectrum [4]. An important property of colloidal and rod shaped SPR sensors is that they enable SPR to be performed in transmission mode or absorption mode, which can be detected by a UV-visible spectrometer [5]. This makes SPR sensing accessible to any researcher with an UV-visible spectrophotometer.

The first half of this thesis explains a method that uses self-assembly of a monolayer of gold nanoparticles due to chemical binding, with the use of different chemicals. Such sensors can be easy to fabricate and the end result yields good sensitivity which can be easily reproduced. In the second half of this thesis, a more efficient, and definite method of generating an AuNR monolayer is discussed. The immobilized gold nanoparticles using both methods can be functionalized with a biological ligand and their interactions with receptor molecules can be observed. This interaction would produce a change in SPR behavior which can then be measured with an UV-visible spectrometer.
1.3. Noble metals as a primary choice for nanoparticles

Spherical silver and aluminum particles have more intense surface plasmon absorption peaks than gold or copper. However, absorption by silver and aluminum particles is much more dependent on shape than copper and gold particles [13]. This makes them very dependent on the exact spherical contour, size and shape. The absorption peaks are highest for silver, slightly more dampened for gold, and even more dampened for titanium. The best metals adapted for use in SPR sensors are gold or silver. Under the consideration of chemical stability, gold is preferred in the sensor structure over silver due to its low oxidation rate in addition to other properties discussed above.

1.4. Introduction to AuNP

Spherical gold nanoparticles display a single strong absorption band that is not present in the spectrum of a solid layer of deposited gold on substrate. In elliptical shaped or rod shaped gold particles, two separate absorption bands are observed due to transverse and longitudinal structures of the particle, as illustrated in Figure 1-1. The benefits of AuNR over AuNS are described in section 2.2. “These absorption bands occur when the incident photon frequency is resonant with the collective oscillation of the free electron cloud of the particle, and is known as localized surface plasmon resonance (LSPR)” [3]. As such, the absorbance and peak wavelength of the particle are directly proportional to change in this resonance frequency of the LSP. The sensitivity is dependent on the nanoparticle composition (type, size, shape), and refractive index of the surrounding solvent or medium.
Figure 1-1 Localized surface plasmon resonance phenomenon.
$E_T$ represents the electric field and electron motion along the transverse axis, and $E_L$ represents the electric field and electron motion along the longitudinal axis. [Dr. J. Todd Hastings, University of Kentucky]
2. CHAPTER 2 – DISCUSSIONS

2.1. Response of AuNP to refractive index of surrounding mediums

The strategy for this research was to examine the ability of the immobilized monolayer of gold nanoparticles, to transduce changes in the surrounding refractive index Figure 2.1. Both the wavelength shift and the change in absorbance curve can be used as stimulus for detecting change in refractive index [5].

Figure 2-1 Absorbance spectra of a monolayer of immobilized gold spheres on glass in two different mediums, methanol and ethanol. Change in refractive index of the solvents results in a change in absorption spectrum.
These results show that the absorbance spectrum of an immobilized monolayer of deposited gold is sensitive to the refractive index of the surrounding bulk medium. The nanorods lead to a dual mode, as opposed to a single resonance mode, expected from nanospheres. Details about what these modes mean and why dual mode is a better choice is explained in the next few paragraphs.

2.2. Benefits of AuNR and dual mode as opposed to AuNS and single mode

Molecules bind more strongly to AuNR than AuNS which aid in better sensing capability and usability variations. This improved binding occurs due to the presence of \{110\} facet in addition to the existing \{111\} and \{100\} facets which have a higher surface energy. This additional \{110\} facet makes the structural difference and is absent in gold nanospheres [12]. The limit-of-detection of the sensor is also improved because dual modes generated by AuNR can detect changes in two stimuli as opposed to one change detected in AuNP.

2.3. Transverse and Longitudinal structure of AuNR

The optical absorption spectrum of gold nanoparticles is dependent on size, surrounding medium, and mainly the shape of the nanoparticle. The aspect ratio is the value of the long axis (length) divided by the short axis (width) of a cylindrical or rod-shaped particle. Increase in aspect ratio increases the energy separation between the resonance frequencies of the long and short bands [11]. For our experiments, we were aiming for an aspect ratio of ‘3’. This could be achieved by a nanorod feature in the range of 30 nm length, and 10 nm width, giving us an aspect ratio of ‘3’.
The absorption band around 520 nm corresponds to the oscillation of the electrons perpendicular (\(\perp\)) to the major (long) rod axis, and is referred to as the transverse plasmon absorption (\(\text{SP}_{\text{Trans}}\), at a shorter wavelength) \textbf{Figure 2-2}. This absorption band is relatively insensitive to the nanorod aspect ratio, but is sensitive to the surrounding medium and refractive index change in that medium. The other absorption band occurs at longer wavelengths around 760 nm - 830 nm. This is caused by the oscillation of the electrons in parallel (\(\parallel\)) to the major (long) rod axis, and is known as the longitudinal surface plasmon absorption (\(\text{SP}_{\text{Long}}\), at a longer wavelength) \textbf{Figure 2-2}. This absorption band is dependent on size of the particle and very sensitive to aspect ratio which leads to a shift towards the infrared spectrum. This will be explained more in theoretical detail in \textbf{2.4.2}. It can be observed from \textbf{Figure 2-2}, that the \(\text{SP}_{\text{Trans}}\) does not change a lot due to increase in size, but \(\text{SP}_{\text{Long}}\) change is more pronounced due to increase in major axis length of the nanorod. \textbf{Figure 2-3} shows a similar arrangement where the \(\text{SP}_{\text{Trans}}\) change is less prominent due to increase in aspect ratio ‘R’. A more pronounced change is observed in \(\text{SP}_{\text{Long}}\) due to increase in major axis length of the nanorod along with the aspect ratio change.
Figure 2-2 Size-dependence for surface plasmon absorption of gold nanorods. Optical absorption spectra of AuNR with constant mean aspect ratio at ‘3’ and size dimensions at 60 nm x 20 nm (—) and 120 nm x 40 nm (---) are shown. The transverse surface plasmon band is rather insensitive towards the nanorod size increase, in contrast with the longitudinal surface plasmon band, there is a more pronounced shift towards the infrared with increasing major axis size doubled from 60 nm to 120 nm.
Calculated extinction cross-sections for Au NS in water with aspect ratios ‘R’ ranging from 2, 3, and 4. As aspect ratio increases the $\text{SP}_{\text{Long}}$ resonance shifts towards the red spectrum and the $\text{SP}_{\text{Trans}}$ resonance slightly shifts to the blue spectrum. [Electrostatic approximation with optical properties of gold taken from Johnson and Christy. Plots courtesy: Dr. J. Todd Hastings, University of Kentucky].

2.4. Theoretical understanding of single and dual modes

To further understand theoretically how we get the single and dual modes, we first need to understand the concept of extinction. “Extinction is defined simply as the sum of the Absorption and Scattering of light” [13].

$$C_{\text{ext}} = C_{\text{abs}} + C_{\text{sca}}$$

Equation 1 Cross section for Extinction
The breakup of light into absorption and scattering will be explained, and how the resonant electron frequencies translate into a strong single or dual absorption bands for different AuNP such as AuNS or AuNR.

2.4.1. Theory for single mode due to AuNS [13]

The cross sections for absorption and scattering of light for a single mode LSPR sensor due to light introduced on AuNS are defined in Equation 2 and Equation 3.

\[
C_{abs} = k_0 4\pi a^3 \left( \frac{\varepsilon_m - \varepsilon_d}{\varepsilon_m + 2\varepsilon_d} \right)
\]

Equation 2 Cross section for Absorption due to AuNS

\[
C_{sca} = k_0 4 \frac{8\pi}{3} a^6 \left( \frac{\varepsilon_m - \varepsilon_d}{\varepsilon_m + 2\varepsilon_d} \right)^2
\]

Equation 3 Cross section for Scattering due to AuNS

\(\varepsilon_m\) - Permittivity of surrounding medium.

\(\varepsilon_d\) - Permittivity of sphere or elliptical rod.

\(a\) - Size parameter

\(k_0\) Defined as \(k_0 = \frac{2\pi N}{\lambda}\) where \(N\) - refractive index of medium

Extinction is dominated by absorption for small particles because \(C_{abs}\) is proportional to ‘\(a^3\)’, and scattering for large particles because \(C_{sca}\) is proportional to ‘\(a^6\)’. Both absorption and scattering are strongest when \(\varepsilon_m \approx -2\varepsilon_d\) in Equation 2 and Equation 3. However, since \(\varepsilon_m\) is a complex number, \(\varepsilon_m \neq -2\varepsilon_d\) for any practical use, but absorption
still peaks when $\varepsilon_m$ is very close to $-2\varepsilon_d$. The strongest absorption point is achieved when the oscillation frequency of electrons are in resonance with the frequency of the incident photon. This is why we get one strong absorption peak when using AuNS.

2.4.2. Theory for dual modes due to AuNR [13]

The cross sections for absorption and scattering of light for a dual mode LSPR sensor due to light introduced on AuNR are defined in Equation 4 and Equation 5. These equations represent approximate solutions in the limit that the particle is much smaller than the wavelength of light. This assumption might be questionable for some of the large particles considered here (~150 nm along their longest dimension); however, these analytic solutions still provide insight into their behavior.

\[
C_{abs} = k_0 \nu \left[ \frac{\varepsilon_m - \varepsilon_d}{L_i (\varepsilon_m + 2\varepsilon_d)} \right]
\]

Equation 4 Cross section for absorption due to AuNR

\[
C_{sca} = \frac{k_0^4 \nu^4}{6\pi} \left[ \frac{\varepsilon_m - \varepsilon_d}{L_i (\varepsilon_m + 2\varepsilon_d)} \right]^2
\]

Equation 5 Cross section for scattering due to AuNR

\[
L_1 = L_L = \frac{1-e^2}{e^2} \left(-1 + \frac{1}{2e} \ln \left( \frac{1+e}{1-e} \right) \right), \quad L_2 = L_3 = L_T = 1 - 2L_L, \quad e = 1 - \frac{b^2}{a^2}; [e\text{-ellipticity}]
\]

$\nu$ - Particle volume

$L_i$ is the geometrical factor of the $i^{th}$ resonant.

$\varepsilon_m = -\varepsilon_d (1 + \frac{1}{L})...L \rightarrow [0,..,1]$
The geometrical factor $L$ is dependent on the aspect ratio of the rod. The value is between 0 and 1, but not exactly 0 or exactly 1. As the AuNR becomes more elliptical, the value for $L$ increases causing the absorbance band shift towards the infrared. This is why the $SP_{\text{Long}}$ absorption band is very sensitive to size along major axis, and aspect ratio which was introduced earlier in 2.3 and Figure 2-2.

The sensor theory leads us to the conclusion that gold nanorods support two surface plasmon resonances. These two resonances can form the basis for a sensor that distinguishes between background refractive index changes and surface-binding of an analyte. By measuring the change in resonance wavelength and peak extinction for both resonances of a nanorod, one can separately quantify changes in solution index and surface concentration of the analyte. This novel sensor configuration is presented in 

CHAPTER 3 – EXPERIMENTAL SETUP.
3. CHAPTER 3 – EXPERIMENTAL SETUP

**Figure 3-1** shows a substrate with immobilized nanoparticles and a bound analyte. Light passes through the structure and the binding of the analyte changes the absorbance. “LSPR wavelengths are a function of AuNR length, width, thickness, interim spacing and surrounding refractive index” [2]. A change in any of these parameters provides a visible variance in the absorbance change and is the basis of our sensing ability.

Figure 3-1 Physical principal underlying the sensor and its working.

Two primary experimental setups are described here that enabled us to detect these changes in the refractive index of surrounding medium. The basic concept of gold deposition on glass substrate remained the same for both experimental setups. Citrate stabilized colloidal gold has high affinity for thiol and amine groups. In our experiments, a glass substrate was functionalized by the formation of amine or thiol terminated Self Assembled Monolayer (SAM) on the glass surface. Next, the surface was immersed in a solution of colloidal gold. Such a chemical attachment of gold nanoparticles to the treated glass surface is strong enough to prevent their detachment from the surface. The self assembled gold nanoparticles on glass were finally characterized by a Scanning Electron Microscope (SEM) to check the quality of the monolayer. The formation of multilayers of such gold nanoparticles on glass would be reflected in the UV-visible spectrum as an increased absorbance at the appropriate wavelengths.
3.1. Basic experimental setup

3.1.1. Structure of the basic experimental setup

The basic structure was a simple tabletop setup that consisted of a transparent vial containing the amine terminated glass substrate with the gold nanospheres attached to it. Light from a halogen lamp (Model DH-2000, Ocean Optics, Inc.) was introduced in from one end ensuring that light passes through the densely populated gold deposition. The other end of the light beam was routed to a spectrometer (Ocean Optics model HR-4000) that would determine the presence of the gold deposition layer with an increased absorbance at the appropriate wavelength. Spectrum analysis was performed by using OOIBase32 Version 2.0.6.

Figure 3-2 Basic experimental setup

3.1.2. Measurement technique

Initially the path of incidence was determined and the sample in the vial was correctly positioned. The spectrum analysis was performed by first turning off the input light and recording a dark spectrum to account for background light and detector noise. Then the
light source was turned on and a plain glass sample spectrum was taken as the reference. The dark spectrum was subtracted from all future measurements. Figure 3-3 and Figure 3-4 are examples of the waveforms gathered from a basic setup for nanospheres and nanorods respectively.

Figure 3-3 Absorbance vs. Wavelength plot in basic setup for AuNS
Good example of an absorbance vs. wavelength plot for 20nm gold nanospheres with APTES bonding and integration time set to 10ms. The sample is placed in the vial and the spectrometer characterizes the absorbance plot

Figure 3-4 Absorbance vs. Wavelength plot in basic setup for AuNR
Example of an ideal spectrum analysis of a concentrated nanorod solution tested directly in the vial and the subsequent measured distribution [Spectrum provided by Ted Pella for 60 nm x 20 nm purchased AuNR].
3.1.3. Disadvantages of the basic experimental setup

The basic setup was a wonderful tool to quickly determine the absorbance/transmission vs. wavelength of particles in a vial. However it would be advantageous to have a setup where the contents of the vial could quickly and easily be populated by different kinds of liquids with different refractive indices, to determine change in the absorption wavelength. In the basic setup, once a sample has been tested in some liquid, the contents would need to be emptied and both the sample and vial would need to be cleaned and dried before reuse. This was not a feasible step for our experiment, because there was no way to guarantee that the same spot of the sample would be illuminated with the light source. In turn we would not get a one-on-one comparison between liquids. For this reason, we began to use the flow cell structure for future experimental work. Another disadvantage was that the light was unpolarized and there were no optics available to polarize the light in this configuration.

3.2. Flow cell structure

The structure of the flow cell was conducive to testing the AuNP coated glass substrate for refractive index changes through many different liquids without much setup disturbance or restructuring. Another benefit in using the flow cell was that it could be used in conjunction with a polarizer (ThorLabs, Inc.) to create either a transverse or longitudinally polarized wave on the sensor.
3.2.1. Structure of the flow cell setup

The gold particles deposited on the glass substrate here are referred to as a sensor. The sensor was clamped in a custom made acrylic flow cell sealed with silicone o-rings. The flow cell was custom made to have three independent channels on the sensor. Different solutions could be introduced into each of the independent channels Figure 3-7. Liquids were introduced to the sensor through Teflon tubing using a pump (Ismatec, Inc). A selection valve was used to avoid air bubbles during the switching of one solution to another. This selection setup helped in easily changing between different solutions. Elimination of pockets of air bubbles in the liquid was also important, since it would be detected as a big variation in refractive index. This air bubble elimination step took a lot of practice and repeated trials to perfect. Figure 3-5 and Figure 3-6 show examples of plots where the error due to air bubbles occur.

Figure 3-5 Existence of air bubbles in the methanol solution yields a big variation in refractive index as opposed to the reference AuNS in air and methanol without air bubbles.
Figure 3-6 AuNS sample with methanol and ethanol in liquid for instances taken with and without bubbles.

Figure 3-7 Flow cell setup.
Shows three channels for a gold nanoparticles deposition on gold layered AET (Aminoethanethiol Hydrochloride). [Actual setup had polarizer and input light at exactly horizontally straight angles at 180°, and sensor rotated 90° for light to pass through it. Current setup shown just for better camera viewing angle]
The sensor assembly was mounted on a custom designed optical transmission measurement system where the angle could be varied. Light from a halogen lamp was introduced into the measurement setup using a multi-mode optical fiber. A collimating lens directed the light from the fiber through a polarizer onto the setup. The polarizer was mounted in such a way that it could be adjusted to create either a transverse or longitudinally polarized wave incident on the sensor. The transmitted light was collected by another lens and coupled to a fiber which transmitted the spectrum to a spectrometer [29].

3.2.2. Measurement technique

The flow cell was placed in the optical assembly for performing the sensing operation. The assembly could be adjusted to make the light incident on any one of the three independent channels. When the intensity of the light obtained was at its maximum, the flow cell and the optical assembly was clamped at that position to prevent any movement during the experiment. In this way, different liquids could be introduced into separate channels without changing the setup.
After the flow cell was positioned, the input light was introduced onto the intended region, and using the polarizer, transverse or longitudinally waves were spectrum analyzed using the spectrometer.
4. CHAPTER 4 – Sensor fabrication with chemically treated acid terminated self assembled monolayer

The surface of the glass substrate is covered using a self assembly process by immersion in a liquid which contains silanes that attach to the glass layer. This is possible because glass have properties that displace the aloxy group on the silane, forming a strong covalent adhesion. There are numerous ways to get AuNP to stick to silanized glass substrate. Transparency is a key requirement for allowing light to pass through the glass substrate to get a clear reading from the spectrometer. The next few sections will discuss in detail the chemical methods used to allow bonding of AuNP onto glass substrate. Initially a lot of time and resources were spent on immobilizing AuNS onto the glass substrate and then once that was achieved, we attempted to immobilize AuNR on the glass substrate using the same procedure. This plan was implemented because AuNS were cheaper and easier to obtain than AuNR.

4.1. Aminoethanethiol Hydrochloride (AET)

The method presented here is for depositing a thin layer of gold onto a glass substrate (almost transparent) and then using an agent that could chemically modify the surface. 2-Aminoethanethiol Hydrochloride was used as the modifying agent. In this section the chemistry of the attachment technique is explained and the procedure of depositing the AET onto the gold surface is discussed.
4.1.1. Discussion of how AET attachment works for AuNP

Once gold is deposited onto the glass substrate, it is functionalized with AET. “… AET forms a peptide bond with carboxylic acid groups on [the] surface, resulting in a thiol-terminated surface” [14]. “… The lengths [of the molecular chains are] determined by the number of methylene groups in the hydrocarbon chains” [7]. The molecules adhere onto the surface with its S-H head on the Au substrate, and the free amine end ready to immobilize the gold nanoparticles.

![Figure 4-1 Front and end chain of AET.](image1)

This was the expected outcome because of the unique tendency of sulfur to have a strong binding with gold. In this way, a SAM layer is created on the glass surface. The free amine ends can then bond to the free gold nanospheres and in this way we can assemble the spheres onto the thin gold substrate as illustrated below. The reflection of the gold spheres onto the gold substrate simulates the presence of gold nanorods and we hoped to produce dual modes by simply using AuNS for sensing capabilities.

![Figure 4-2 Schematic illustration of an AET functionalized nanostructured multilayer system for gold on gold deposition.](image2)
4.1.2. Reagents and materials

Glass slides (dimensions – 25 mm x 25 mm x 0.17-0.25 mm) were purchased from Fisher Scientific - Fisherbrand. Deionized (DI) water was also used in addition to other chemicals such as 2-aminoethanethiol hydrochloride 98% (Acros Organics), and ethanol (200 proof). The colloidal gold nanoparticles of 20-nm diameter were used (Ted Pella, Inc.)

4.1.3. Procedure for depositing AET

We adopted the procedure of Okamoto and Yamaguchi. First the gold substrates were rinsed thoroughly with DI water and then sonicated three times with ethanol. In this way the substrate was free of any oil or impurities on the surface and was ready to be functionalized with the AET layer. “… [After drying, the cleaned gold coated substrate was] immersed in a 10 mM (10 millimolar) ethanol solution of AET for 1 h” [6].

AET was in powdered form, so to determine how much AET would be needed, the following calculations were performed:

\[
\text{Molecular Weight of AET in use} = 113.61 \text{ g mol}^{-1}
\]

\[
\text{Molarity} = \frac{\text{# of moles of solute}}{1 \text{ liter of solution}}
\]

\[
113.61 \text{ g mol}^{-1} \times 10 \text{ mM} = 0.11361 \text{ g mol}^{-1} \times 10 = 1.1361 \text{ mg ml}^{-1}
\]

Based on the above calculations, 1.136 mg of AET was taken for every 1ml of ethanol and the gold coated substrates were immersed for 1 hour. In this process, the thiol end of AET attaches to the gold surface and forms its monolayer on the gold surfaces. After the
AET immersion, the substrates were rinsed with distilled water and dried with a nitrogen gun. The substrates coated by AET were then immersed in a colloidal gold suspension for the free amine ends to bind to the nanoparticles. They were immersed for 15 hours based on previous work that recommended 8-20 hours with frequent agitation [6]. After immersion, the samples were rinsed with distilled water again and dried with a nitrogen gun.

4.1.4. Results for AET use

This process should have strongly immobilized colloidal gold particles onto the gold substrate, because of the affinity of the amino group to the gold. However the process did not work ideally for our purposes and we did not get the expected dual mode. Instead, the output we got was consistent with the gold film alone and not with gold nanospheres deposited on the substrate. AET as a SAM layer might be possible in some instances, but did not work well for our purposes. After a few more unsuccessful trials, decision was made to immobilize gold nanoparticles with a different type of surface modifier.

4.2. (3-Mercaptopropyl) Trimethoxysilane (MPTMS)

The method presented here is for using a surface modification agent of (3-Mercaptopropyl) Trimethoxysilane. The chemistry and procedure of the attachment technique is discussed. This section is concluded with results as to why MPTMS does not work as well as other modifying agents.
4.2.1. Discussion of how MPTMS attachment works for AuNP

Molecules of 3-mercaptopropyltrimethoxysilane react with gold nanoparticles to form a gold monolayer on a glass substrate. MPTMS is a compound with mercapto and silane terminated ends which reacts with the hydroxyl groups. This reaction is important because it causes Si-O covalent bonding which provides the adhesion necessary to bind the MPTMS to glass. It is resultant from the pre-cleansing treatment of glass slides with \( \text{H}_2\text{O}_2 \) and \( \text{H}_2\text{SO}_4 \), see Figure 4-3 with the surface of glass substrate [8].

![Figure 4-3 Front and end chain of MPTMS.](image)

“The gold nanoparticles are then attracted to the … sulfhydryl groups by electrostatic interactions” [8].

![Figure 4-4 Molecular structure of MPTMS compound.](image)

This way we can deposit a layer of MPTMS onto a cleaned glass substrate. If colloidal gold nanoparticles are used, spheres will be attached to the linker molecules providing a single absorbance band.
4.2.2. Reagents and materials

Glass slides (dimensions – 25 mm x 25 mm x 0.17-0.25 mm) were purchased from Fisher Scientific - Fisherbrand. The following chemicals (3-mercaptopropyl) trimethoxysilane (Sigma-Aldrich), ethanol (200 proof), sulfuric acid (H₂SO₄) conc., and hydrogen peroxide (H₂O₂) at 30% aqueous grade were used. DI water was used from the filtration system and the colloidal gold nanoparticles were approximately 20 nm diameter (Ted Pella, Inc.).

4.2.3. Procedure for depositing MPTMS

For this process, we adopted the procedure of Chen et al. Glass slides were cleaned with acetone, ethanol and DI water, and air dried with a nitrogen gun. These would remove any organic impurities. “… [Then they] were cleaned by immersion in a piranha solution consisting of [30% H₂O₂ to 70% H₂SO₄] at 80°C for 1 h” [3]. After cooling to room temperature, they were moved out, rinsed with DI water, and then sonicated three times for 3-5 minutes each by replacing contents with fresh ethanol. “… Slides were placed in an oven at 100°C for 1 hour and were then immersed in a MPTMS ethanol solution [at ratio] (V_{MPTMS} : V_{EtOH} = 1 : 20)” [3]. The MPTMS ethanol solution was frequently agitated for the 30 minutes recommended time period.

Then, the slides were rinsed again with DI water and ethanol, and air-dried at room temperature. The MPTMS-functionalized glass slide was placed in a small clean beaker and completely immersed in the colloidal gold nanoparticles. The time duration allowed for immobilization of gold nanoparticles via gold–thiol bond is debatable and is...
explained in detail in section 4.2.5.1. The treated and coated glass slide was rinsed with DI water. A graphical representation of the process is shown in Figure 4-5 and Figure 4-6.

![Diagram](image)

Figure 4-5 Schematic illustration of a MPTMS functionalized nanostructured multilayer system for gold on glass deposition.

![Diagram](image)

Figure 4-6 Another schematic illustration of a MPTMS functionalized nanostructured multilayer system for gold on glass deposition.

4.2.4. Results for MPTMS

The results for the absorption spectra were not very encouraging. An analysis of the absorbance vs. wavelength plot revealed no definite reason for the distribution of the peak. A possible explanation for the discrepancy might be due to agglomeration of colloidal particles into clusters of particles. However the results attained did not seem consistent with this either. Reasons why expected deposition of AuNP on MPTMS layer might have failed are discussed in the next section.
4.2.5. Discussion for possible failures due to MPTMS use

4.2.5.1. MPTMS undergoes self-polymerization

Although the gold-thiol bonds in MPTMS are very strong bonds and cannot be easily broken, research proves (Kwarnpong Muanpho, Piyasan Praserthdam, and Varong Pavarajarn [8]) from experimental observations that AuNS were hardly detected in the MPTMS modified surface. “Sulfur detection on the surface was low which indicated that crucial sulphydryl group in MPTMS was eliminated during the surface modifying process. This occurs due to the tendency of MPTMS to undergo self-polymerization” [8]. When MPTMS undergoes this process, it forms small sized particles which reduce the number of sulphydryl groups in it. This self-polymerization process could explain the reason why large agglomerated particles can be observed on and around the surface of the glass container containing concentrated amounts of the used MPTMS solution illustrated in Figure 4-7.

Figure 4-7 Self-polymerization process of MPTMS.
In two stored containers (a) Extreme case with \( V_{\text{MPTMS}} : V_{\text{EtOH}} = 1 : 2 \), and (b) less polymerized case \( V_{\text{MPTMS}} : V_{\text{EtOH}} = 1 : 10 \)
It can be observed that in Figure 4-7 (b) that even though the ratio was five times higher than that in Figure 4-7 (a), there were still distinct features of self-polymerization at the bottom of the container. The ideal ratio used was 1:20 ($V_{\text{MPTMS}} : V_{\text{EtOH}} = 1 : 20$). MPTMS in standalone state is a transparent liquid. This self-polymerization process would also be the reason why gold does not stick to glass well, and the ones that do stick are very scanty. Comparison experiments were performed by Kwarnpong Muanpho, Piyasan Praserthdam, and Varong Pavarajarn, to determine the deposition of gold nanoparticles with MPTMS and APTMS. They observed that for the same 12 hour deposition time, type, and deposition procedure for the AuNP, deposition density was very different and much denser for APTMS. Also the AuNP deposition uniformity was very scarcely distributed on MPTMS-modified substrate, while the monolayer deposition was more uniformly coated on APTMS-modified surface [8].

4.2.5.2. Dependence of time criterion for MPTMS and gold nanoparticles

The time to set the cleaned glass sample into the MPTMS solution is debatable. This is one of the key steps for getting a good deposition of AuNP onto the glass substrate. Many different papers recommend different time limits for MPTMS surface modification and later for AuNP deposition. One paper recommended putting the glass in a MPTMS solution for 4 hours and having the functionalized glass immersed in the gold nanoparticles solution for another 4 hours [8]. Another paper recommended leaving the glass in MPTMS for 45 minutes and exposing the functionalized glass to the particles for 3 minutes [3]. The sample was agitated by sandwiching two glass samples together. These differences in time indicate that more research is needed to determine the optimum
conditions for AuNP deposition using MPTMS. In our work, MPTMS was eliminated as a possible modifying agent mainly due to the polymerization characteristic reducing the affectivity of adhesion as described earlier.

4.3. (λ-Aminopropyl) Triethoxysilane (APTES)

After the use of AET and MPTMS, the use of APTES as a SAM finally led to success in deposition of AuNP on glass. The use of (λ-Aminopropyl) Triethoxysilane as a surface modifying agent is described here. “The glass slide is silanized with APTES to present terminal amine groups at the surface, which has a high affinity to gold. The self-assembly of these AuNP is driven by electrostatic attraction between positively charged amine group on the surface and negatively charged citrate ions around nanoparticles, as well as by electrostatic repulsion between negatively charged nanoparticles” [2]. The formation of a high-quality silane monolayer is critical to prevent aggregation of gold nanoparticles on the APTES-functionalized glass, which would otherwise contaminate the readings.

4.3.1. Discussion of how APTES attachment works for AuNP

Molecules of APTES react with gold nanoparticles to form a gold monolayer on a glass substrate. APTES is a compound with amine and silane terminated ends which reacts with the hydroxyl groups on the glass surface. This reaction is important because it causes Si-O covalent bonding which provides the adhesion necessary to bind the MPTMS to glass. It is resultant from the pre-cleansing treatment of glass slides with H₂O₂ and H₂SO₄, see Figure 4-8 with the surface of glass substrate [8].
The gold nanoparticles are then attracted to the amine groups by electrostatic interactions.

As noted earlier, colloidal gold nanospheres were used mainly since they are easily obtained and almost four times less expensive than nanorods with similar specifications. Once success was achieved with nanospheres, we planned to switch to nanorods.

4.3.2. Reagents and materials

Glass slides (dimensions – 25 mm x 25 mm x 0.17-0.25 mm) were purchased from Fisher Scientific - Fisher brand. In chemicals, (λ-aminopropyl) triethoxysilane (Sigma-Aldrich), ethanol (200 proof), sulfuric acid (H₂SO₄) conc., and hydrogen peroxide (H₂O₂) at 30% aqueous grade were used. DI water was used from the filtration system and colloidal gold nanoparticles of 20-nm diameter were used (Ted Pella, Inc.). For a few experiments and
trials, we also used hydrochloric acid (HCl) conc. with a (1 : 1) ratio of methanol for 30 min and immersed overnight for 60 °C.

4.3.3. Procedure for depositing APTES

For this process, we adopted the procedure of Chen et al. Glass slides were cleaned with acetone, ethanol and DI water, and air dried with a nitrogen gun. These would remove any organic impurities. “… [Then they] were cleaned by immersion in a piranha solution consisting of [30% H₂O₂ to 70% H₂SO₄] at 80°C for 1 h” [3]. After cooling to room temperature, they were moved out, rinsed with DI water, and then sonicated three times for 3-5 minutes each by replacing contents with fresh ethanol. The cleaned sample was air dried with a nitrogen gun and then stored in a clean beaker at 60°C overnight [5].

The slides were then immersed in an APTES ethanolic solution with ratio of 1:10 (V_{APTES} : V_{EtOH} = 1 : 10) for 5 hours. The recommended time was (V_{APTES} : V_{EtOH} = 1 : 10) for 15 minutes. However no APTES deposition was achieved at this rate. Tests were also done at (V_{APTES} : V_{EtOH} = 1 : 5) for 3 hours and deposition was achieved, but it was not very well achieved under 3 hours. One reason for this could be due to older APTES solution used that could have lost its properties and taken longer to deposit on the glass surface. However enabling deposition at (V_{APTES} : V_{EtOH} = 1 : 10) for 5 hours and (V_{APTES} : V_{EtOH} = 1 : 5) for 3 hours, we obtained the best results.
For this process, we adopted the procedure of Nidhi Nath and Ashutosh Chilkoti. After soaking in APTES, the glass sample was rinsed with DI water, sonicated three times in ethanol, and dried at 120 °C for 3 h. “Extensive rinsing of silanized glass and baking at 120 °C is necessary to prevent the formation of multilayers. Silane multilayers, which are easily formed during silanization of glass … [must be avoided, because they cause] aggregation of gold nanoparticles … [2].

The APTES-functionalized glass slide was placed in a small clean beaker and completely immersed in the colloidal gold nanoparticles. The sample was immersed for a period of 24 hours in the colloidal gold solution.

A graphical representation of the process is represented in Figure 4-10.

![Figure 4-10 Schematic illustration of a APTES functionalized nanostructured multilayer system for gold on glass deposition.](image)

Figure 4-10 Schematic illustration of a APTES functionalized nanostructured multilayer system for gold on glass deposition.
4.3.4. Optical characterization of gold nanospheres on APTES coated glass

The amine bonds proved to be a good linker and performed a good job of generating attraction between the AuNS and the APTES coated glass. This section discusses the waveforms derived from the spectrometer and images captured by the SEM.

4.3.4.1. APTES spectrum through spectrometer

The immobilized AuNS on the glass substrate gave a yellowish orange tint to the sample, because the visible light spectrum for yellow and orange falls within this spectrum [15]. AuNP on glass show a spectrum at 520 nm to 560 nm close to the spectrum for orange which is from 590 nm to 620 nm. See range of wavelengths in Table 1.

Table 1 shows the visible light spectrum and where the AuNP would fit in at approx. 520 nm [15]

<table>
<thead>
<tr>
<th>Color</th>
<th>Wavelength</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>violet</td>
<td>380–450 nm</td>
<td>668–789 THz</td>
</tr>
<tr>
<td>indigo</td>
<td>420–450 nm</td>
<td>668–714 THz</td>
</tr>
<tr>
<td>blue</td>
<td>450–495 nm</td>
<td>606–668 THz</td>
</tr>
<tr>
<td>green</td>
<td>495–570 nm</td>
<td>526–606 THz</td>
</tr>
<tr>
<td>yellow</td>
<td>570–590 nm</td>
<td>508–526 THz</td>
</tr>
<tr>
<td>orange</td>
<td>590–620 nm</td>
<td>484–508 THz</td>
</tr>
<tr>
<td>red</td>
<td>620–750 nm</td>
<td>400–484 THz</td>
</tr>
</tbody>
</table>

Waveforms were taken for absorption vs. wavelength at different points on the samples, and the best waveforms captured are represented in Figure 4-11. Observe that the maximum peak falls between 520 nm and 540 nm. These all represent strong single mode spectrums for AuNS.
Figure 4-11 Examples of the AuNS spectrums for APTES silanized glass samples in air.

4.3.4.2. APTES deposition topography in SEM (Scanning Electron Microscope)

After a few good samples were created with the APTES AuNS deposition technique, pictures were taken in the SEM to see the alignment and orientation of the AuNS. The sets of pictures represent the samples created using the above procedures. Initially the
depositions of the AuNS on the glass sample were very scattered and low in density. Density is very important because a good density gold deposition would give a much stronger spectrum; as opposed to maybe a very thinly deposited and scattered layer which will have a very faint or almost undetectable spectrum. Eventually, we were able to consistently achieve good density gold deposition on the glass substrate. The samples presented are AuNS at 20 nm – 30 nm diameter. Figure 4-12 show some images captured from the SEM that have AuNS deposited on the glass substrate.

Figure 4-12 SEM images showing dense patterns of AuNS deposition due to APTES.
4.3.5. Results for APTES use

APTES worked well to immobilize AuNS on the glass substrate. Combinations of solvents such as methanol and ethanol were used to determine a change in refractive index. All absorbance vs. wavelength plots were taken using the flow cell structure and the presence of bubbles during measurements were minimized to the fullest extent, and in most instances avoided completely with repeated sessions and a lot of practice. The discrepancies caused due to bubbles have been explained in the 3.2.1 section earlier.

All AuNS immobilized on the samples were 20 nm diameter for the plots discussed in the following section. The specifications set in the spectrometer were the same for all measurements and shown below in Table 2.

Table 2 Spectrometer specifications.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Integration Time</td>
<td>10 msec</td>
</tr>
<tr>
<td>Average</td>
<td>5</td>
</tr>
<tr>
<td>Boxcar</td>
<td>10</td>
</tr>
<tr>
<td>Strobe Frequency</td>
<td>10 msec</td>
</tr>
</tbody>
</table>

The refractive index values change for different solvents and solutions. These changes in refractive index values were used to compare the solvent interactions with AuNP, between ethanol and methanol. Temperature is also an important factor and could lead to varied refractive indices for the same solvent in different environments. In our experiments, all temperatures were considered room temperature at 25°C or 298.15ºK.
Results are described in the next section first for ethanol for a specific densely populated AuNS sample, and then with methanol with the same AuNS sample. All instances for solvent characterizations are compared with air, to use as a reference comparison before and after solvents are introduced.

4.3.5.1. Ethanol absorbance vs. wavelength referenced with air

The refractive index shift due to introduction of ethanol is discussed in this section. The refractive index (nD) for ethanol is 1.35931 [16].

Figure 4-13 Absorbance vs. Wavelength plots for 20 nm AuNS in Air vs. Ethanol
4.3.5.2. Methanol absorbance vs. wavelength referenced with air

The refractive index shift due to introduction of methanol is discussed in this section. The refractive index (nD) for methanol is 1.32715 [16].

![Absorbance vs. Wavelength plot](image)

Figure 4-14 Absorbance vs. Wavelength plots for 20 nm AuNS in Air vs. Methanol.

If there are discrepancies in the waveforms, they are likely due to occurrence of bubbles, in spite of the care and precautions taken to avoid them. With repeated sessions and a lot of practice, bubbles were largely eliminated, but few remnant bubbles could not be avoided. However too much time was not spent to perfect this technique, because the primary consideration here was to deposit AuNR for obtaining a dual mode spectrum.
4.3.6. APTES immobilization technique repeated with AuNR for dual mode

After the success we encountered with APTES, the same procedure was used for immobilizing AuNR on glass substrate. After repeated attempts, variations in silanization of glass, and different concentrations of APTES, the immobilizing technique was unsuccessful. These same procedures were also tried with MPTMS, but that too produced negative results. Further study was performed on the chemistry of how APTES and the AuNR worked individually, and why it worked well for AuNS in APTES, but showed no deposition on glass substrate for AuNR.

4.3.6.1. Nanorod growth solution Cetyltrimethyl-Ammonium Bromide (CTAB)

After careful analysis and study of the AuNR, we confirmed that the individual nanorods that we have been using are encapsulated in surfactant cetyltrimethyl-ammonium bromide (CTAB) solution. During preparation phase, citrate stabilized gold nanoparticle seeds are treated with the nanorods growth solution containing CTAB (Ted Pella, Inc.). Gold nanoparticles stay separated from each other because they are stabilized by citrate anions [18]. The citrate anions repel the particles from one another and prevent them from aggregation. This happens because citrates have negative surface charge repulsion, which keeps the gold nanoparticles apart. However in such a process, the nanorods stabilized by the CTAB bi-layer give them a net positive charge [19]. This positive charge is repelled with the positively charged APTES layer and the AuNR are not deposited on the surface, but instead repelled. For this reason, we had to pick some chemically silanized glass layer that would attract the CTAB encapsulation around the AuNR. One such chemical use is discussed in the next section which attracts to the CTAB around the nanorods.
4.4. 16-Mercaptohexadecanoic acid (16-MHA)

Immobilization of AuNR onto a SAM of 16-mercaptophexadecanoic acid (16-MHA) is discussed in this section. The procedure involves formation of a SAM of 16-MHA molecules onto gold coated glass slide and then introduce in the AuNR solution for immobilization. It was discussed earlier how the nanorods are stabilized in solution due to surface modification by the surfactant CTAB. “Attractive electrostatic interactions between the [negatively charged] carboxylic acid group on the SAM and the positively charged CTAB molecules are likely responsible for the nanorod immobilization” [19]. Furthermore, a big advantage of using electrostatic interactions is that the density of immobilized rods on the surface can be controlled by simple pH variation. Illustration below shows a graphical representation of how the 16-MHA acid terminated gold layered glass substrate is attached with CTAB encapsulated AuNR.

Figure 4-15 Immobilization of CTAB-Modified Gold Nanorods onto SAM of 16-MHA.
In our experiments, we performed procedures on AuNR with an aspect ratio of value ‘3’.

We used nanorods features with range at length of 30 nm and width of 10 nm, giving us an aspect ratio of approx. 3.

\[
\text{Aspect Ratio} = \frac{\text{Length of major axis}}{\text{Width of rod diameter}} = \frac{30\text{nm}}{10\text{nm}} = 3.
\]

The plasmon resonance absorption band splits into two bands as the particles become more elongated along one axis.

4.4.1. Diluted AuNR solutions used with CTAB solution

Many trial and error procedures were performed with AuNS in earlier processes since availability of AuNS was easier and it was less expensive. AuNR on the other hand was a much more expensive commodity, so after a few trials, decision was made to dilute the AuNR solution to have more quantity for testing. The AuNR were shipped with CTAB concentration of 0.1 M. If we had to dilute the AuNR solution, it would need to be in a CTAB solution with the same concentration. CTAB was in powdered form, so to determine how much was needed; we performed the following calculations:

Molecular Weight of CTAB in use = 364.45 g/mol

Molarity = \frac{\text{# of moles of solute}}{1 \text{ liter of solution}}

\[364.45 \text{ g mol}^{-1} \times 0.1 \text{ M} = 36.445 \text{ g} \times \frac{1}{1000 \text{ ml}} = 36.445 \text{ mg ml}^{-1}\]
Based on the calculations, 36.445 mg of CTAB was taken for every 1 ml of DI water. This ratio added up because based on the MSDS sheet provided with the CTAB solution, the solubility of CTAB in water is at $50 \frac{\text{mg}}{\text{ml}}$.

![Absorption spectra of CTAB stabilized gold nanorods in increasing order of (CTAB : DI water) ratio (5 : 1), (5 : 2), and (5 : 3) respectively.](image)

From the above illustration, it can be observed that diluting the AuNR solution changes the absorbance spectrum slightly, but has very little or no effect on the wavelength. This would not affect the final outcome due to diluting AuNR. As a result (5 : 3) ratio of (CTAB : DI) was chosen to be used to soak the acid terminated glass substrate.
4.4.2. Reagents and materials

Glass slides (dimensions – 25 mm x 25 mm x 0.17-0.25 mm) were purchased from Fisher Scientific - Fisher brand. In chemicals, 16-mercaptohexadecanoic acid (Sigma-Aldrich), cetyltrimethyl-ammonium bromide (Sigma-Aldrich), ethanol (200 proof), sulfuric acid (H₂SO₄) conc., and hydrogen peroxide (H₂O₂) at 30% aqueous grade were used. DI water was used from the filtration system and the AuNR with aspect ratio of ‘3’ [length of 30 nm and width of 10 nm] were used (Ted Pella, Inc.)

4.4.3. Procedure for depositing 16-MHA

For this process, we adopted the procedure of Murphy et al,

Glass slides … used for immobilization were cleaned thoroughly using piranha solution (H₂SO₄ : H₂O₂ = 3:1), rinsed in deionized water and dried [with a nitrogen gun]. After initially depositing a layer of [5-10 nm titanium, a 20-25 nm] thick gold film was sputter-coated onto [the cleaned glass slide]. These slides were immersed in 0.001 Molar (1 mM) ethanolic solution of … 16-MHA for a period of 36 hours [with occasional stirring] [19].

16-MHA was in powdered form, so to determine how much was needed, we performed the following calculations,

\[
\text{Molecular Weight of 16-MHA in use} = 288.49 \ \text{g mol}^{-1}
\]

\[
\text{Molarity} = \frac{\# \ of \ moles \ of \ solute}{\text{1 liter of solution}}
\]
Based on the calculations, 0.289 mg of 16-MHA was taken for every 1 ml of ethanol.

Tests were also done with a slightly higher concentration of 16-MHA at 0.01 Molar (10mM),

\[
\frac{288.49 \text{ g}}{\text{mol}} \times 1 \text{ mM} = 0.28849 \text{ g} \times \frac{1}{1000 \text{ ml}} = 0.28849 \frac{\text{mg}}{\text{ml}}
\]

Based on the above calculations, 2.885 mg of 16-MHA was taken for every 1 ml of ethanol.

The results used for our discussions are taken with 1 mM ethanolic solution of 16-MHA. After letting set for 36 hours, the films were rinsed in ethanol and dried with a nitrogen gun.

4.4.4. Discussions for 16-MHA use

4.4.4.1. Discussion for acidity of AuNR solution and soaking time dependence criterion for 16-MHA

For this process, we adopted the procedure of Murphy et al. "The SAM-coated glass slides were immersed into the [AuNR] solution held at pH 6.5 for a period of 4 h [during immobilization]" [19]. Holding the pH level at 6.5 is very critical. Controlled experiments were performed by Murphy et al. that demonstrated the electrostatic interactions used for nanorod assembly. “Immobilization of the nanorods onto 16-MHA SAM was studied at 5 different pH values (pH of 1, 3.3, 6.5, 8, and 11)” [19]. At lower
pH values, fewer rods on the surface were visible, because of the protonation of acid groups. In chemistry, protonation is the addition of a proton ($H^+$) to an atom, molecule, or ion [20]. At higher pH values, the acid groups of the SAM would become highly deprotonated leading to a strong attractive force on the AuNR surface. At very high pH values, the number of rods is considerably reduced due to aggregation of the rods on the layer. “The gold nanorods were found to aggregate or “crash out of solution” at high pH (>10)” [19]. Immobilization on the surface was at its optimal level at pH 6.5 and considerably reduced at other pH values. Thus, the choice of pH 6.5 was made because of optimum conditions prevailing at this pH, resulting in a high density of well distributed nanorods.

It is also important to note that the 4 hour duration has been carefully monitored with controlled experiments performed by Gole, Orendorff, and Murphy [19]. In their work, samples were taken at various time intervals to measure depletion of AuNR from the solution, and complete adsorption was achieved in 4 hours.

Unfortunately, in our experiments, the soak time was monitored, but acidity was not monitored and not held constant at pH 6.5. This will explain the reason why we encountered an overall scanty deposition as explained in the next section, since we must have been working at a lower pH levels by default since it remained unmonitored.
4.4.4.2. 16-MHA deposition patterns in EBL (Electron Beam Lithography)

The surface morphology of the immobilized rods is studied in the EBL using e-LiNE software provided by Raith USA Inc. Deposition of the AuNR on the sample is low in density and assembled in clusters. Density is very important because a good density gold deposition would give a much stronger spectrum; as opposed to maybe a very scantily deposited and scattered layer which will have a very faint or almost undetectable spectrum. Below are some pictures from the EBL that show AuNR deposited features on the thin gold layer substrate. There are many AuNS mixed in with the clustered AuNR. These are the AuNS that did not form into rods during the preparation phase with the introduction of the growth solution. Big AuNR with aspect ratio of ‘3’ were selected to easily detect them in the EBL and study their deposition pattern better.

Figure 4-17 EBL images for 16-MHA AuNR deposition on thin gold substrate. (A – H) show the deposition of AuNR and AuNS on the 16-MHA terminated surface. (A – F) have legend showing distance at 100 nm and (G, H) have legend showing distance at 20 nm on the region.
Figure 4-17, continued

(C)  (D)

(E)  (F)

(G)  (H)
4.4.4.3. 16-MHA use disadvantages

It is evident from the discussions and EBL images in the previous sections that surface deposition density varied in different places of the sample. This was one issue with the process. This could be because we did not monitor the acidity level of the AuNR solution and it could have been at a low acidity level throughout the immobilization period. Certain spots were so scattered and scanty that we had to trace over a considerable distance in the EBL to get to another populated region of the AuNR deposition. Another issue was the unfortunate high presence of AuNS deposition amid other deposited AuNR particles. One good example of this phenomenon is shown in figure below.

![Image](image_url)

Figure 4-18 More AuNS than AuNR deposited in what is supposed to be a primarily AuNR region.
Here there is almost an overrun of AuNS over AuNR in the visible region. This infiltration of AuNS can cause a problem because when the incident light is directed over this region, we would get a very faint dual mode response. Chances are that we may get only a single mode response which would be expected from an AuNS deposition layer. This would defeat the purpose of getting a dual mode with AuNR.

In conclusion, the lack of monitoring for the acidity of AuNR solution, coupled with too much AuNS contamination in the AuNR solution, led to a more mediocre deposition than expected. Even if the expected density of AuNR deposition would be achieved by controlling the pH of the AuNR solution, and experimentally determined the right soaking time; the issue still remained about knowing which parts of the sample were more useable than others. To overcome this, we decided to change our immobilizing technique from chemically depositing AuNR on the surface, to creating carefully aligned patterns on the glass surface with AuNR in carefully designated, pre determined, and calculated regions. One big advantage with this would be that we would know exactly where to set the incident light onto the setup and get the expected response. The next chapter explains in detail the procedures used to achieve this selective patterning.
5. CHAPTER 5 – Selectively patterned sensor fabrication using the Electron Beam Lithography (EBL) method

In this section, the fabrication, measurement technique, and dual mode output plots from custom created and exquisitely patterned AuNR are discussed. The advantages of using the selective patterning method as opposed to a generalized chemically immobilized technique are also discussed. Very similar to 16-MHA, a clean glass sample was coated with a very thin layer of Titanium (Ti), then with a thin layer of Gold (Au). Ti was first deposited to enhance subsequent Au deposition. A high resolution positive photo resist PMMA (polymethyl methacrylate) is then spin coated on the substrate. Patterns were created on this PMMA with an EBL exposure. After the PMMA was developed, gold was sputter coated and then PMMA was lifted off to create a pattern of the desired gold array. At the end, the results are discussed with pictures and plots derived from the spectrometer. In this chapter, the fabrication steps are explained in detail.

5.1. Base sample preparation with Ti-Au deposition

The base glass substrate layer was similar to the ones used in chemical immobilization techniques, (dimensions – 25 mm x 25 mm x 0.17-0.25 mm) purchased from Fisher Scientific - Fisher brand. The substrate was cleansed free of oil or organic material by sonication with fresh ethanol. After cleansing the sample, it was air dried with a nitrogen gun and then stored in a clean beaker at 60°C for 1 hour to dehydrate the sample.
The patterned gold had to be attached to the glass substrate in some way. It is extremely clear by now that gold on glass deposition is not a simple process, however gold on gold deposition is not a tedious process since metals bind together with ease. To facilitate this process, initially a very thin layer of Ti was deposited on the glass surface. An E-Beam Evaporator was used to deposit an approx. 10-15 nm Ti layer on the cleaned glass substrate. The Ti metal would create a uniformly deposited pattern with the SiO₂ surface. After this, approx. 15-20 nm thin layer of gold was immediately deposited by E-Beam Evaporation to prevent Ti layer from absorbing O₂ from the surrounding area and forming TiO₂. The Ti and Au deposition layer thicknesses were confirmed with an Ellipsometer (J A Woollam Co., Inc. Spectroscopic Ellipsometer: EC-400). Au on Ti deposition is a strong bond since it is a metal-metal bond. At this point, we have a sensor substrate structure as shown below.

![Diagram of Si layer with Ti and Au layers](image)

Figure 5-1 Si layer with an approx. 10 nm Ti layer and 15 nm Au layer

5.2. Spin coating PMMA and Soft Baking

5.2.1. PMMA spin coating details

For our fabrication process, we used single layer 950K PMMA 2% solids in Anisole. After a pre bake of the sample at 150°C for 30 min [22], PMMA layer was spin coated on the cleaned substrate at 1600 rpm for 30 sec to get a thickness layer of approx. 100 nm ± 5 nm [21, 22].
5.2.2. Soft Baking

The PMMA coated substrate was soft baked at a temperature of 180°C for 2 min. The recommended temperature and time was 180°C at 60 – 90 sec. However, it was also mentioned that process variables such as soft bake, exposure conditions, choice of resist and developer could be optimized to achieve desired results [21]. For our purposes, to achieve best results, we used 120 sec (2 min) baking time. This soft bake step would remove solvents in the photo resist and dry the top surface of the substrate. It is crucial to perform soft-baking in imaging because the photo resist layer become photosensitive only after soft baking. Over soft-baking degrades the photosensitivity of resists by either reducing solubility, or degrading a section of the sensitive layer. Under soft-baking would prevent light from reaching the sensitive layer [9]. The soft baking needed to be at just the right temperature and time. PMMA thickness measurements were carried out using the ellipsometer.

5.3. EBL imaging, exposure parameters and selective patterning details

Before the final sample fabrication step, many trial runs were performed to predict how the EBL would create patterns based on PMMA thickness, exposure levels, dose considerations, resolution levels, and voltage levels. All steps were performed in the EBL station using the e-LiNE software provided by Raith, USA Inc Figure 5-2.
Square patterns were created with a few miscellaneous features shown in Table 3, over a wide range of dose factors starting at 0.5, in increments of 0.5, till about 4. This procedure was performed to determine the exact exposure parameters to be used for writing the nanorod array patterns. Initially $900 \, \text{pC/cm}$ was used to make 20 nm lines in 100 nm PMMA at 30 keV beam energy. Later, the beam energy was reduced to 10 keV to give a good undercut profile to make it better conducive to the liftoff process. After trying some more line doses, optimum voltage and currents were chosen at $300 \, \text{pC/cm}$ to make 20 nm lines in 100 nm PMMA at 10 keV.
5.3.1. Exposure parameters

Calculation of the dose was based on the exposure parameters with three different kinds of doses: area dose, line dose, and dot dose [23]. Which type of dose is chosen depends on the pattern. Dose can be defined as follows:

\[
\text{Area Dose} = \frac{I_{\text{beam}} \cdot T_{\text{dwell}}}{S^2} \quad \text{Unit is } \frac{\mu \text{As}}{\text{cm}^2}
\]

\[
\text{Line Dose} = \frac{I_{\text{beam}} \cdot T_{\text{dwell}}}{S} \quad \text{Unit is } \frac{\text{pAs}}{\text{cm}}
\]

\[
\text{Dot Dose} = I_{\text{beam}} \cdot T_{\text{dwell}} \quad \text{Unit is } \text{pAs}
\]

Equation 6 Formulas for Area Dose, Line Dose, and Dot Dose [23].

\( I_{\text{beam}} \) [nA]: is beam current defined by column e.g. filament, aperture, beam energy.

\( T_{\text{dwell}} \) [µs]: is the dwell time, i.e. the amount of time the electrons are directed at a particular position in the pattern.

\( S \) (Step size) [cm]: is defined by our needs like how we want to move the beam from one point to another, e.g. accuracy, throughput.

The required exposure dose can vary depending on PMMA thickness, the beam energy, and development conditions (concentration, temperature, etc.).

5.3.2. Exposure parameter settings

The following exposure parameters were used:

- Beam Energy: 10 keV.
- Dose Factor Increments: start: 1.01, Increments: 0.2, end: 4.01 (16 total columns).
- Aperture Size: 30 µm.
- Write Field Size: 100 µm.
- Working Distance: 10 mm.
Beam Current \( (I_{\text{beam}}) \): .20 nA.

Beam Speed: 10.288 \( \frac{\text{mm}}{\text{sec}} \).

Line Speed: 6.9 \( \frac{\text{mm}}{\text{sec}} \).

**Area Exposure Parameters:**

Area Step Size (S): 20 nm.

Area Dwell Time (\( T_{\text{dwell}} \)): 1.944 \( \mu \text{s} \).

Area Dose: 100 \( \frac{\mu \text{C}}{\text{cm}^2} \).

**Line Exposure Parameters:**

Area Step Size (S): 10 nm.

Area Dwell Time (\( T_{\text{dwell}} \)): 1.458 \( \mu \text{s} \).

Area Dose: 300 \( \frac{\text{pC}}{\text{cm}} \).

5.3.3. **Nanorod array patterned structure, distribution and organization**

It is also important to note that certain rows had more densely populated (exactly double) nanorod distributions than their counterparts. These densely populated regions will be termed as ‘more dense’ (MD) and its counterpart as ‘less dense’ (LD). The setup for the nanorod array was arranged in an increasing order starting with 60 nm x 20 nm and ending at 150 nm x 50 nm. The rows started at 60 nm x 20 nm, 90 nm x 30 nm (LD), 90 nm x 30 nm (MD), 120 nm x 40 nm (LD), 120 nm x 40 nm (MD), 150 nm x 50 nm Lines (L), 150 nm x 50 nm Boxes (B), and C-structure 90 nm x 30 nm. All dimensions for nanorods are described in the form of length (nm) x width (nm). The distribution looked like the matrix represented in Table 3.
Table 3 Representation of the nanorod array distribution with a 10 row x 16 column array.

<table>
<thead>
<tr>
<th>C - structure</th>
<th>90 x 30</th>
<th>90 x 30</th>
<th>90 x 30</th>
<th></th>
<th>90 x 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>150 x 50 (Boxes)</td>
<td>150 x 50 B</td>
<td>150 x 50 B</td>
<td>150 x 50 B</td>
<td>→</td>
<td>→</td>
</tr>
<tr>
<td>150 x 50 (Lines)</td>
<td>150 x 50 L</td>
<td>150 x 50 L</td>
<td>150 x 50 L</td>
<td>→</td>
<td>→</td>
</tr>
<tr>
<td>120 x 40 more dense</td>
<td>120 x 40 MD</td>
<td>120 x 40 MD</td>
<td>120 x 40 MD</td>
<td>→</td>
<td>→</td>
</tr>
<tr>
<td>120 x 40 less dense</td>
<td>120 x 40 LD</td>
<td>120 x 40 LD</td>
<td>120 x 40 LD</td>
<td>→</td>
<td>→</td>
</tr>
<tr>
<td>90 x 30 more dense</td>
<td>90 x 30 MD</td>
<td>90 x 30 MD</td>
<td>90 x 30 MD</td>
<td>→</td>
<td>→</td>
</tr>
<tr>
<td>90 x 30 less dense</td>
<td>90 x 30 LD</td>
<td>90 x 30 LD</td>
<td>90 x 30 LD</td>
<td>→</td>
<td>→</td>
</tr>
<tr>
<td>60 x 20 Std</td>
<td>60 x 20</td>
<td>60 x 20</td>
<td>60 x 20</td>
<td>→</td>
<td>→</td>
</tr>
<tr>
<td>Dose Factor</td>
<td>1.01</td>
<td>1.21</td>
<td>1.41</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each cell distribution had nanorods arranged in equal number of distributed rows and columns with dimensions of each cell being approx. 50 µm x 50 µm. Density for cells with LD had standard distributions and cells with MD had almost twice the nanorod distribution. Detailed pictures of the final product are shown in Figure 5-9. Each individual array distribution is explained in Figure 5-3.
Figure 5-3 Nanorod array distribution within an individual cell explained

5.4. PMMA Development

After the exposure process, the next step was to develop the PMMA and remove the regions exposed by the EBL, and prepare it for further gold deposition. Since the resist here is PMMA which is a positive resist, the exposed regions would be etched away in the developer solution. Methyl isobutyl ketone (MIBK) \([(CH_3)_2CHCH_2C(O)CH_3]\) and Isopropanol (IPA) were used for the development process. The sample was agitated in a ratio of 1:3 (MIBK : IPA) for 1 min 30 sec, which leads to slight overdevelopment and an undercut profile [24]. One advantage for using an EBL process is that simply using the EBL technique leads to an automatic undercut in the features since electrons have a tendency of scattering. The recommended ratio for (MIBK : IPA) is 1:3 at 30 sec [22], however, we needed to develop for a longer time to create a better undercut. The undercut profile is suitable for the liftoff process. After completion, the glass sample was rinsed in DI water and dried with a nitrogen gun. The fabrication process steps achieved till this point is represented in Figure 5-4. [Procedural assistance provided by Dr. Chandan Samantaray].
5.5. Deposition of gold layer on developed PMMA

The next step after PMMA development was to deposit a 40 nm – 50 nm thick gold layer. This could be performed either with the e-beam evaporation system or sputtering system. The next section will discuss the advantages and disadvantages for the use of one or both the systems.

5.5.1. Discussion for use of Sputtering system vs. E-Beam Evaporation system

Sputtering has an advantage for gold film deposition because the thickness of the sputtered gold film is uniform when compared to that of the E-Beam Evaporated gold film. Uniform deposition is good, but one problem with sputtering is that the PMMA walls will also be coated which makes liftoff difficult. This is one reason why it is good to slightly overdevelop the PMMA layer and use EBL since they make a prominent undercut which facilitate in liftoff. Another advantage of sputtering over e-beam evaporation is that there is no concern of having the heat source very close to the sample. In the University of Kentucky e-beam evaporation system, the heat source is very close to the sample during evaporation, which can damage or peel off some PMMA deposited
layer. This can affect deposited gold even before liftoff. PMMA heats up a lot which is not recommended since PMMA flows at \( \approx 200^\circ\text{C} \). The sputtering process does not melt the underlying PMMA layers which leads to high resolution patterns after liftoff.

5.5.2. Effects of using E-Beam Evaporation for gold layer deposition

In our first fabricated sensor, e-beam evaporation was used for the 40 nm – 50 nm gold deposition process. During liftoff, in N-methylpyrrolidone (NMP) at 50°C - 60°C and 5 min agitation, there was no sign of liftoff. Agitation was performed at the same temperature for 30 min, but still there was no liftoff. With a little additional semi-abrasive agitation, liftoff began around the sample. This was not an issue when samples were fabricated with Sputtered gold. Figure 5-5 shows a few pictures from the sample where liftoff has been performed and PMMA has peeled off rather than lifted off.

Figure 5-5 Patterned structures uneven liftoff due to E-Beam Evaporation. Dark regions on samples show areas where liftoff has been successfully performed. (A - C) No liftoff was observed in surrounding gold region. (D – H) Partial or peeled PMMA giving partial liftoff or torn regions in some instances.
5.5.3. Sputter coating gold layer for deposition

Keeping the advantages of sputtering technique in view from our previous discussions, it was the best choice versus e-beam evaporation at this particular step. Gold was deposited over the developed PMMA layer using sputtering. 99.9% pure gold targets were used [Kurt J. Lesker, Inc]. Base pressure was held at 0.20 mTorr and Argon gas was used to flow into the chamber at a 15 scc/m flow rate. This single target sputtering system was supplied with a constant power of 70W. The deposition was carried at a pressure of 3.5 mTorr and at a rate of $4.6 \text{ Å sec}^{-1}$ [procedural assistance provided by Donnie Keathley]. The
next step was the liftoff process where it leaves behind the desired features of gold on the substrate. Before liftoff, some pictures were taken in a camera through a microscope. Figure 5-6 shows a few pictures indicating the array described in Table 33 and how they were visible with surrounding gold deposited in the background.

Figure 5-6 Features after sputter deposited gold onto substrate and before liftoff.

The fabrication process steps till this point is illustrated below,

Figure 5-7 Sputtering gold on substrate creates regions with gold deposited in the EBL patterned area and gold deposited on the undeveloped PMMA.
5.6. Liftoff process

The liftoff process will leave behind the desired features on the substrate. The PMMA photo resist film is soluble in N-methylpyrrolidinone (NMP) [PMMA stripping solution]. To facilitate liftoff, NMP was heated to a temperature of 60°C and the sample was introduced into this solution. With a mild agitation for about 5 minutes, gold metal liftoff could be observed over the sections where undeveloped PMMA was present. After most of the metal had been lifted off, the gold deposition on the regions patterned by the EBL was observed. The fabrication process at this final step is shown below.

![Figure 5-8 Final fabrication step after liftoff, with gold thickness layer of about 50 nm.](image)

The sample was sprayed with NMP to peel out the remaining gold and then blown dry with a N₂ gun. Because of a good undercut profile due to EBL, high dose, and long development time, the liftoff process cleared the entire unwanted metal layer.

5.7. Final gold pattern deposition thickness

The final gold thickness was measured using the DEKTAK surface Profilometer (Veeco, Inc.). The difference between the previously deposited thin gold layer, and the newly deposited gold layer on the patterned region was taken. The thickness of the AuNR was
determined to be at approx. 45 nm – 55 nm. We have assumed the layer thickness at 50 nm as an average for all experimental purposes.

5.8. Discussion for patterned structures in the EBL

The pattern distributions were discussed in Table 3 as rows starting at 60 nm x 20 nm, and ending in C-structure 90 nm x 30 nm. The C-type structure was created to generate a possibly triple mode absorbance vs. wavelength feedback. The description of each slide is explained below the figures at Figure 5-9.

Figure 5-9 Close-up of AuNR patterned sensor structure cells after liftoff.
Figure 5-9, continued

Following are the specifications for the AuNR from Figure 5-9 with dimensions, density, and dose factors shown all values are in units of nanometers: (A) 90x30 LD dose - 1.61, (B) 90x30 LD dose - 1.61, (C) 90x30 LD dose - 1.8, (D) 90x30 LD dose - 1.81, (E) Interlocked, (F) 120x40 LD dose 1.81, (G) 60x20 dose – 4.01, (H) 60x20 dose – 2.81,
5.8.1. EBL patterned structure observations

Dose factors were one of the important criterions for a good resolution rod creation. After reviewing all the slides, it can be gathered that the best resolution rods are created with dose factors ranging between [1.5 … 3], with the best resolutions observed at around 2.0. The range of dose factors we used started at 1.01 and ended at 4.01 in increments of 0.2. As the dose factors went really high or really low in value, the formations of rods were reduced to nonaligned patterns and began losing the geometric shape of a rod. Also the best rod creation from an accuracy aspect had to be the 90 nm x 30 nm and 120 nm x 40 nm features. Most 60 nm x 20 nm rods ranged slightly higher than the expected size, and larger rods at 150 nm x 50 nm were more rectangular than rod elliptical shaped. C-type structures unfortunately turned out really bad and looked more like incomplete spheres than C-type structures.

5.9. Experimental Setup

This section explains the experimental setup used for the LSPR sensor. The basis of the setup is to introduce white light from source, on to the array of nanorods on the LSPR sensor, collect the transmitted spectrum, and plot the absorbance vs. wavelength. The setup was designed to work for a non flow cell type structure. Our aim initially was to
locate the dual (transverse and longitudinal) modes for the different array structures, then move onto creating a setup for a flow cell structure. Figure 5-10 shows a block diagram of the entire experimental setup used for our testing.

Figure 5-10 Block diagram of EBL patterned AuNR experimental setup.

5.9.1. Input handling

Light from a halogen lamp was routed to a collimating lens through an optical fiber. This was used to focus light from the fiber through a polarizer (ThorLabs, Inc.) through an objective lens, and onto the sensor. The collimating lenses convert divergent beams of light into a parallel beam, which provide a more streamlined incidence of the rays [25]. This is very important and is illustrated in Figure 5-11.

Figure 5-11 How a collimating lens affects a stream of light rays. Top: without a collimator. Bottom: with a collimator.
The polarizer can be adjusted such that either transverse or longitudinal polarized wave data can be collected. “In optics, an objective is the lens or mirror in a microscope or other optical instrument that gathers the light coming from the object being observed, and focuses the rays to produce a real image” [26]. This was also very important to our experiment because care had to be taken to confirm that the diameter of the light spot from the fiber, focused onto the sample, was smaller than that of the dimensions of the square nanorod array. The objective lenses used in our experiment were 10x and 5x microscope objectives (Newport, Inc.) which focused the light on the sample.

5.9.2. Output handling

The real, sharp, and focused light was passed through the sensor sample and introduced onto a splitter that split the sensor output into (5.9.2.1) the spectrometer input, and (5.9.2.2) WinTV input (Hauppauge, Inc.).

5.9.2.1. Spectrometer input

The primary output from the splitter was sent to the spectrometer. This spectrometer was interfaced to a computer to plot the absorbance spectrum. This was the basis of our sensing device and the step where we generated absorbance vs. wavelength plots.

5.9.2.2. Win TV input

The secondary output from the splitter was sent to the WinTV (Hauppauge, Inc.) device. We had to locate the individual array of AuNR based on the structure and dose factor, where the white light was intended to be incident. This location was located through a microscopic video lens which had an output to a WinTV (Hauppauge, Inc.) hardware on
the computer. Below are a few captured pictures of the array located through one such measurement period to use as a tool to incident the white light through a specific region.

![Image](image.png)

(A) Very faint golden squares indicate each region of the AuNR array. (B) Is an example of the array of AuNR for 60 x 20 nm dimensions for a 1.0 dose factor.

Once the region was located, the setup was guaranteed to have the light spot pass through the chosen array and the signal generated would be a real response.

### 5.10. Results for patterned sensor with EBL

The sensor response is consistent with what is to be expected from an AuNR structure giving dual (transverse and longitudinal) modes. The sensor absorbance becomes much stronger as the size of nanorods goes up, e.g. from 60 nm x 30 nm to 150 nm x 50 nm (L). The charts in Figure 5-13 give a good representation of what is observed.
Figure 5-13 Numerous sensor dual mode responses to light through different patterned arrays and dose levels as absorbance vs. wavelength spectrums.
Figure 5-13, continued

Absorbance vs. Wavelength
90 x 30 Dose 1.41

Absorbance vs. Wavelength
90 x 30 LD Dose 2.01

Absorbance vs. Wavelength
90 x 30 LD Dose 3.01
Figure 5-13, continued

Absorbance vs. Wavelength
90 x 30 MD Dose 3.21

Absorbance vs. Wavelength
90 x 30 MD Dose 3.41

Absorbance vs. Wavelength
120 x 40 MD Dose 3.21

Wavelength

Transverse Spectrum
Longitudinal Spectrum
5.11. Observations for patterned sensor with EBL

Finally we have documented that we can achieve dual mode outputs for patterned AuNR on a thin gold film. The plots in the above figures are consistent with our discussion in section 2.4.2 where it was discussed that the geometrical factor $L$ is dependent on the transverse and longitudinal lengths of the nanorods, and that as value for $L$ increases and becomes more elliptical, the absorbance band shifts more towards the infrared and a big
variation between the longitudinal modes can be observed. The geometry in our observations changed from 60 nm x 20 nm to 150 nm x 50 nm. In contrast, the transverse mode stays mostly unchanged. For the transverse mode, the peak absorption falls at around 540 nm to 555 nm averaging at 550 nm and dependent on geometry. The wavelength for the longitudinal mode ranged from 550 nm to 800 nm, with its peak at about 600 nm to 750 nm depending on geometry.
6. CHAPTER 6 – CONCLUSION

The intended purpose of this research was to create a sensor device that could produce a dual mode spectrum to detect changes in one or more stimuli, and be able to use it in a laboratory environment. It has been demonstrated clearly from the results of the selectively patterned and fabricated dual mode sensor, that we were able to generate AuNR on the surface of a glass substrate, and get the expected spectrum for different size nanorods. Starting out with using chemically terminated SAM layers and immobilizing AuNS, advances were made, and finally AuNR deposition was achieved even with the chemically silanized method. However, the new EBL fabricated method was not only more precise, but was also more viable from a practicality perspective. This is because the user now has the capability to test numerous different sizes, types, shapes, and possible aspect ratio variations on one single glass sample and only a few hundred nanometers away from each other.

The sensor needs further optimization to be usable with a flow cell type of attachment to the current setup, so that refractive index variations can be detected due to introduction of various liquids. The hope is that a bulk of the work has been done by providing a tabletop device that can have multiple detection uses. For practical purposes, it might be a little cumbersome at first because optimizations might need to be done each time before use, depending on the purpose and object of sensing. However this can also be viewed as strength, because a few minor optimizations can increase the versatility of this sensor by a tremendous extent.
CHAPTER 7 - FUTURE EXPERIMENTAL WORK AND USAGE TRENDS

SPR techniques have been successfully applied in various fields, and used in chemical and biochemical sensing. The detection and analysis of chemical and biochemical substances is needed in many important areas including medicine, environmental monitoring, biotechnology, food and drug monitoring [27]. SPR sensor technology holds potential for applications in such areas.

Chemical SPR sensors are based on the measurement of SPR variations due to surface changes due to different solutions or a chemical reaction of an analyte which results in changes in its optical properties. These applications rely primarily on changes in the refractive index on surface layer induced by the variation of analyte molecules which can be detected as a change in the spectrum. Such sensors could be used in a water purification plant, or bio-medical settings.

Due to high sensitivity to the refractive index change in the surrounding environment, the SPR technique receives attention in the application of biotechnological detection [10]. SPR sensors can also be used for observation of bio-molecular interactions. This can be achieved by functionalizing the immobilized AuNR and monitoring changes in refractive index due to receptor-ligand interactions. SPR sensors can be used for such monitoring of the ligand-analyte (most often antigen - antibody) binding to determine the affinity of the molecules [28]. These applications with further modifications can be important to scientists for studies in the fields of immunology, molecular biology, cell biology and biochemistry.
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