Analysis of Surface Plasmon Resonance Biosensors for Protein Detection

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Thesis Submitted To
The Department of Electrical and Electronics Engineering of BRAC University in Partial Fulfillment of Bachelor of Science Degree

Thesis Supervised By
Avijit Das
LETTER OF TRANSMITTAL

14th December, 2016
To,
Avijit Das
Department of Electrical and Electronics Engineering
BRAC University.

Subject: Submission of thesis report for completing our graduation.

Dear Sir,

With high reverence I want to state that we have finished our thesis report on the “Analysis of Surface Plasmon Resonance biosensors for protein detection”. We did some elaborate research to compile our report in a comprehensive manner. In this thesis, all the team members contributed equally with sheer hard work. We faced some challenges in working as a team and to put people’s perception. I will like to take this platform to thank you.

To end, I would like you to kindly accept our thesis report and to acknowledge our devotion and efforts.

Thanking you in anticipation.

Yours Sincerely,

Jannatul Maoya
On behalf of
Kazi Diganta Bisher
Abdullah Al Faruque
DECLARATION

We hereby declare that this report is based on the results that we have done in our thesis work. Contents of work found by other researchers are mentioned by references. This thesis has never been previously submitted for any degree, neither in whole nor in part.
Acknowledgement

First and foremost, we would like to express deepest gratitude to Almighty, for always having made us feel so blessed, and for always being greatest strength. This is a respect for us to thank the individuals who have made this proposal conceivable. To start with of all we might want to pay our most profound appreciation to our administrator, Avijit Das for giving us the chance to deal with this venture under his watch. His support, direction and consolation from the underlying stage to the end has empowered us to comprehend the idea driving this proposition work. We are likewise appreciative to all the employees and lab specialized officers for their direction and support. At long last, all the on account of All-powerful Allah that we have resulted in these present circumstances organize in this way.
The Surface Plasmon Resonance (SPR) biosensor method has emerged as a very flexible and powerful approach for detecting a wide diversity of bio-molecular interactions. This Surface Plasmon Resonance (SPR) for biosensing was demonstrated 40 years ago. In the present contribution, its general background is described together with the necessary developments both in instrumentation and surface chemistry, the high cost of commercial medical devices and consumable has prevented SPR from being introduced in the undergraduate laboratory. The object is to analyze surface plasmon resonance biosensor for protein detection to improve the sensitivity of medical devices and to ease disease detection. Modelling of the SPR response by using a computer program which calculates reflection and transmission of polarized light in a satisfied structure (stack of parallel layers) sandwiched between semi-infinite substrate (prism) and ambient (sample solution) media is also used.
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Chapter 1

1.1 Introduction

The importance of rapid and exquisite detection of chemical and biological analytes increases significantly for many sensitive and sensational areas like medical diagnostics, food control, and environmental monitoring which leads to the optical biosensors based on surface plasmon resonance (SPR) and optical waveguide spectroscopy to push forward in these fields extensively. Surface Plasmon Resonance (SPR) is a very recent technique and provides a significant opportunity to measure biomolecular interactions in real-time in a label-free environment. Surface plasmon resonance (SPR) is a unique analysis to monitor the changes in refractive index in the vicinity of the metal surface. This physical phenomenon of SPR was first observed by Wood in 1902 which has found its application in sensitive detection in sub-molecular coverage. The physical meaning of surface plasmon is known to be created by collective oscillation of conduction of free electron at the surface of the metal film which is the solution to Maxwell’s Equation for some certain metal-dielectric geometries which keeps SPR from not being described by quantum physics in spite of being a part of nanophotonics. As a part of our project of detecting different bio-molecular proteins based on the application of surface plasmon.

1.1.1 Background

Surface plasmons are quanta of plasma, a surface electromagnetic wave whose propagation is confined to the metal–dielectric layer or metal-vacuum. Surface plasmons (SPs) or surface plasmon polaritons (SPP) propagate electromagnetic wave in the metal-dielectric package. This surface plasmon is created in a parallel direction along with metal. An American scientist Robert W. Wood first observed this incident \(^{[1]}\). He found some dark and light band pattern in reflected light of metal by using a different wavelength of light, but he didn’t explain properly that time. After 62 years, in 1968 scientist Otto \(^{[2]}\) and almost on the same time another group of German scientist, Kretschmann, and Raether also got some remarkable result on this SPR assay. This SPR was demonstrated in an acceptable method in 1982 to detect gas and bimolecular and commercially
available for biomolecular interaction applications from 1990 under BIACORE, a subsidiary company of General Electric. Those primary bacterial pathogen distinguished toward SPR biosensor might have been Escherichia coli. Scientist Fratamico et al. (45) utilized an angular modulation SPR biosensor developed in BIAcore by a sandwich test on identifying E. coli. To use this SPR practically, we need a clearer image which was introduced by Knoll and Rothenhausler [3] who showed us slight different method where increased the throughput but decreased the sensitivity of traditional SPR method. In this advanced microfabrication development, it plays a role to sample more data in a single chip. Another advance work was done by Ouellet et al. he demonstrated another method where multiple analytes can be monitored simultaneously with multiple ligands. [4]

1.1.2 Present Scenario
In a very short term, surface plasmon resonance (SPR) has become one of the significant fields for study which motivates to determine the behavior of biologically active materials which exhibit strong affinity interactions on the label-free excitation of the electron. SPR biosensors are increasingly used in biochemistry and bioanalytical chemistry to investigate antibody-antigen interactions, DNA hybridization, to diagnose bacteria- and virus-induced diseases, to identify hormones, steroids, and immunoglobulins, blood plasma condensation. Using SPR biosensors, it is possible to analyze the mixtures of substances keeping a very similar chemical structure because SPR allows identifying only those molecules that specifically interact with biologically active substance immobilized on the surface of SPR biosensor. SPR biosensors are applied to monitor interactions between immobilized biologically active substance and analyte in real-time without labeling. SPR biosensors in many cases may be used to perform the measurements with an immobilized biological recognition element. Therefore, at present SPR is one of the most promising methods for determining the interactions between ligand and receptor- the organs that respond to light, heat and transmit the signal to the sensory nerve, antigen, and antibody, thus being widely used in diagnostics and biomedical research.

1.1.3 The motivation for the Thesis
Surface Plasmon Resonance (SPR) is the future leader of medical science, biochemistry, and biological research. Although it has been hundred years passed since the discovery of SPR but the practical application of surface plasmon has enormously increased after 1990’s. Now surface plasmon resonance has an extremely wide range of research areas involving the drug discovery, solar sensor, examining the physical properties of metal, biosensors, bio-molecular detection and much more. Plasmonics creates a new field in Silicon Photonic Platform like Plasmonic Optical Modulator, Plasmonic Photo detector etc. and it is an
interesting research area for a wide range of application like a nano-optical tip or antenna, spectroscopy which draws our interest in surface plasmon resonance and we did a small research on protein detection using surface plasmon resonance spectroscopy.

1.2 Overview of the thesis

1.2.1 Theoretical overview
Surface plasmon polaritons is the electromagnetic excitation of the electron plasma that propagates through the interface between a dielectric and a conductor confined in the perpendicular direction which can be characterized in terms of the dispersion and spatial profile of metal/dielectric structure. Although term plasmons deals with nanoparticles, it involves the theory of Maxwell’s equation rather than quantum physics which provides an advantage to describe the SPR phenomena using the wave equation on a single interface like metal/dielectric formation which leads our investigation on SPR to our expected results.

1.2.2 Analyzed System
Advance research on surface plasmon resonance (SPR) has introduced a few couples of system that has been proposed and developed by the intellectuals. Although there is some configuration that has been widely used for the detection of SPR, we have done our work following the prism coupled formation proposed for the physical explanation of the SPR phenomena initiated by Lord Rayleigh and further Otto and Kretschmann made the complete explanation on the application of SPR based sensor to biomolecular interaction. Our research on the SPR based response of protein molecules is completed by following the Kretschmann defined system that includes a prism for the high refractive index that attenuates the distraction and noble metal films for generating the plasmon on its surface and a monochromatic light has been used as the source of our system to accumulate the total internal reflection.

1.2.3 FDTD Simulation
As the experimental part of our thesis, we use the Lumerical Suite 2013b software for simulating the result of our work. We made the FDTD simulation by using the software in which a fixed region is required to do the 2D/3D simulation and we used only the 2D simulation configuring a siO₂ glass prism coupler a metal film layer and a layer for the analytes. The plane wave source is being used at a visible light frequency and to capture the response DFT monitor is being used.
1.2.4 Project analysis

The last and most important part of our thesis includes four types of variation in examining the characteristic behavior of five types of bio-protein – Fibrinogen, Immunoglobin G, Human serum Albumin (HSA), Adenomatous polyposis Coli (APC) and Lysozyme. Our thesis investigates the responsive behavior of those proteins in presence of the surface plasmon created by plane wave source at a visible wavelength of light at the surface of the metal film. To monitor the response we make four variations in the proposed system.

**Variation in the wavelength of light**

The first observance of our thesis is by investigating the response of the proteins in presence of variation in the wavelength of light and for this purpose, we fixed two wavelengths at visible range, one is 630nm and another one is 740nm. By using that two wavelengths we measure the resonance angle shift and calculate the shift of the angle.

**Variation in the width of the metal**

This part of our thesis includes the variation in the width of the metal film, Silver (Ag) a noble metal that has been used for creating the surface plasmon at metal-dielectric (protein) medium to observe the variation in the response of the protein molecules at different evanescent field produced by the plasmon. Here we also observe the protein response by measuring the resonance angle shift to get the overview of the response of the protein.

**Variation in the metal film**

This section of the thesis involves our variation in the metal film on which the plasmon is created. In this section, we configure the metal films into three separate subsections involving the only silver (50nm) and the other two is gold/silver and silver/gold combination and observe the response in the form of a shift in the resonance angle and measure the sensitivity towards the plasmon produced on their surface.

**Variation of Background Environment**

In this section of our research, we made a variation in the physical or the background environment by adding a chaotropic agent with the regular buffer solution to observe characteristic of HSA. We use only the water as an ideal buffer solution to complete all the examination and in this section, we add Urea to manipulate the refractive index of water and its corresponding response of HSA is being observed.
Chapter 2

2.1 Theory

The name Surface plasmon resonance has three keywords, Surface, Plasmon, and Resonance. To explain this SPR we need to focus the middle word Plasmon. Creating plasmon in a surface is actually surface plasmon. Additionally, there also creates oscillations so then it is surface plasmon resonance.

Plasma is the fourth state of matter which is actually ionized gas where an equal number of positively charged ions and negatively charged electrons consist. This ions and electrons got free but coexist when we can provide enough energy. Plasma also creates magnetic fields along with electric fields.

Plasmon is the special state of Plasma. It is basically the oscillation state of plasma. The quantization of the collective longitudinal excitation of a conductive electron gas in a metal is known as Plasmon. When an electron passes through a thin metal or reflecting electron or a prot from a metallic film like gold or silver or combination of both gold and silver, then Plasmon excites. In a short way, the plasma oscillation of a quantum is known as Plasmon.

2.1.1 Principle of SPR

SPR system can be described with an optical phenomenon of total internal reflection (TIR). Total internal reflection occurs when the light travels from higher refractive index (n1) medium to lower refractive index (n2) medium as long as the incident angle $\theta$ is greater than the critical angle $\theta_c$.

The equation of critical angle is, $\sin (\theta_c) = \frac{n_2}{n_1}$ [6]
Based on this equation, the TIR happens if $\theta > \theta_c$.

![Total Internal Reflection Diagram](image)

**Fig 2.1.1: Total Internal Reflection Diagram**

Under the condition of TIR, evanescent waves are formed.

The evanescent wave is one kind of wave where an oscillating electric or magnetic field does not propagate as an electromagnetic wave.[7] Using monochromatic light and plane polarized and a thin noble metal film (gold or silver) coated with two different value of refractive index the evanescent wave is observed with free oscillating electrons called plasmon. We can measure the declining angle value of the intensity of reflected light which is called resonance angle and that must be greater than the critical angle (theta c) because of the resonance energy transfer from evanescent wave to surface plasmon. The refractive index of the medium close to the metal controls the resonance angle. Using different types of biomolecules presents us the different value of refractive angle which causes for resonance angle change. This angle change has a linear relationship with the concentration or refractive index of target biomolecule analyte on the surface of metal. Harmonizing this linear relationship, the analyte and ligand association and dissociation can be observed. [8]
2.1.2 Mathematical part

However, to understand SPR deeply, we have to go back in Maxwell’s equation. Maxwell’s equation have a basic explanation of incident of metals with electromagnetic fields. We know that reflecting lights is a property of metal and it goes high when the wavelength is invisible spectrum (390nm-700nm) [9]. At this situation, electromagnetic waves do not propagate through metal. A little higher from visual range wavelength metals behaves as dielectric. It also allows electromagnetic propagation after getting dielectric function. Noble metals have more absorption in this area. If we want to know why this phenomenon depends on high frequency then the relation of induced current and electron relaxation time (τ) can describe it.

Now Maxwell’s electromagnetic equation

\[ \nabla \cdot D = \rho_{\text{ext}} \]  
\[ \text{...............(1)} \]

Here, D is for dielectric displacement

\[ \nabla \cdot E = -\frac{\partial B}{\partial t} \]  
\[ \text{...............(2)} \]

Here, E is the electromagnetic field and B for magnetic induction.

Now to introduce polarization the equation will be,

\[ \mathbf{D} = \varepsilon_0 \mathbf{E} + \mathbf{P} \]  
\[ \text{...............(3)} \]

And for magnetization M,

\[ \mathbf{H} = \frac{1}{\mu_0} \mathbf{B} - \mathbf{M} \]  
\[ \text{...............(4)} \]

Now the transverse wave,

\[ \mathbf{K}.\mathbf{E} = 0 \]  
\[ \text{...............(5)} \]

In gas mode, if plasma frequency (ωₚ) is less than metal frequency (ω) then the transverse waves propagate. This transvers waves are two types, one is transvers magnetic (TM or p) and another is transverse electric (TE or s). However only in TE polarization mode SPR occurs. Surface plasmon doesn’t create in TE polarization mode. Adding in TM mode only Ex, Ez and Hy value cannot be zero, corresponding in Hx, Hz, and Ey cannot be zero in TE mode. As our focus in TM mode because of SPR creation so here we introduce TM polarization equation,
$$E_x = -i \frac{1}{\omega \varepsilon_0 \varepsilon} \frac{\partial H_y}{\partial z} \quad (6)$$

$$E_z = - \frac{\beta}{\omega \varepsilon_0 \varepsilon} H_y \quad (7)$$

And the wave equation for \( z < 0 \) is,

$$H_y(z) = A_2 e^{i\beta x} e^{-k_2 z} \quad (8)$$

And for \( z > 0 \),

$$H_y(z) = A_1 e^{i\beta x} e^{-k_1 z} \quad (9)$$

Now from this wave equation of \( H_y \) we get,

$$\frac{K_2}{K_1} = -\frac{\varepsilon_2}{\varepsilon_1} \quad (10)$$

The surface wave happens only at the interface between analyte and metals of opposite sign of real part of their dielectric permittivity. To complete the wave equation we need the expression of \( H_y \) resulting,

$$K_1^2 = \beta^2 - K_0^2 \varepsilon_1 \quad (11)$$

$$K_2^2 = \beta^2 - K_0^2 \varepsilon_2 \quad (12)$$

According to all these equations, we get the main equation of SPR propagation,

$$\beta = k_0 \sqrt{\frac{\varepsilon_1 \varepsilon_2}{\varepsilon_1 + \varepsilon_2}} \quad (13)$$

Here \( \beta \) is for propagation, \( k_0 \) is constant and \( \varepsilon \) is dielectric permittivity and noble metal permittivity. To simplify we can use the constant \( K_0 \) equation,

$$K_0 = \frac{\omega}{c} \quad (14)$$

Where \( c \) = speed of light in vacuum

Both real and complex condition, this equation is acceptable and responsible for the evanescent field. Notwithstanding, the EM field of SPP decays into metal and a dielectric medium, most of the field is present in that dielectric medium. For this reason, the real part of the function is highly sensitive to changing refractive index. [10]
This SPR based biosensor has label-free detection along with biomolecules which can be used in drug detection, biomedical research, medical science, pathology test etc. There are some professional use of SPR like sensing different kinds of protein [11], biomolecules, blood sampling. Though there are some different kinds of biosensor method, the SPR is used most for its sensitivity capacity.
Chapter 3

3.1 Systems used to study SPR

Though this SPR biosensor system is quite new in modern science, there are already established several configurations that are capable generating also capturing surface plasmon resonance. Among them these are the leading systems:

- Optical waveguide system
- Grating coupled system
- Optical fibers
- Prism coupled (TIR)

Our proposed system is based on the prism coupled Kretschmann configuration system. We will discuss in another subsection later.

3.1.1 Optical waveguide system

Those optical waveguide frameworks bring a few engaging offers. They offer a basic manner on control the optical path, which is more they would tiny and wavy. Eventually, Tom's perusing fluctuating the plot of the frequency of the light, a light wave will be guided by that waveguide. Once entering that locale with a grating (2400 lines/mm) Furthermore an over thin metal layer, it evanescently penetrates through the metal layer. In those wind of the waveguide, the out-coming light is distinguished Eventually Tom's perusing photodiodes. An algorithm is used to model that adsorbed material linearly to surface focuses. [12]

![Fig 3.1.1: Optical Waveguide System](image)
3.1.2 Grating coupled system

The grating coupled system was first used in 1983. [13] This system uses the physics of diffraction grating. In traditional SPR we use glass prism but here this grating replaces this glass prism. Here this grating also made by gold. This grating is more like sinusoidal grating which is optimal grating. Furthermore, the incident angle of light depends on those grating and the wavelength of resonance represents the period (top to top) and amplitude (top to through). Like traditional system by varying the incident angle and taking values of reflected light intensity a binding curve is created.

The grating formula is

\[ n_{\text{eff}} - n_a \sin \alpha = \frac{k\lambda_o}{\Lambda} \ ] \hspace{1cm} (15)

![Grating Coupled System](image)

Fig 3.1.2: Grating Coupled System

There would two basic optical configurations for grating configure sensors: input and output grating couplers. An information grating coupler necessities a light source with a chance to be shone through that substrate onto the grating coupler. The test influences the coupling angle, what’s more, an identifier on the end of the waveguide faculties the vicinity about light. On the other hand, a yield grating coupler employments a pre-coupled light source. When that light achieves the grating coupler, it may be uncoupled during a point subject to those \( n_{\text{eff}} \). The detector, which may be not appended of the grating coupler, observes this reflection.

3.1.3 Optical fiber

Passing lights through a glass tunnel in TIR mode is known as optical fiber technology. In spite of the fact that modes are actually a greater amount of a vitality dissemination in the fiber, they could make considered perfect likewise separate angles about aggregate inner reflection concerning illustration the light bounces over and over again along that fiber. Low-order models enter those fiber center at a shallow angle, What's more in ricochet over and over again gradually. That vitality
has a tendency will make dispersed essential in the fiber center. Higher-order modes, on the different hand, enter at a soak point What's more thusly ricochet over and over again thick, as quickly. Their vitality spreads more under those cladding, also because of that transient wave, past the limit of the waveguide itself.

The fiber permits us on clear through a number of coupling wavelengths. These wavelengths could make transformed at the same time toward utilizing a broadband, multi-wavelength wellspring, for example, white light or monochromatic light. Toward measuring those power about every wavelength abandoning those fiber, a spectrophotometer camwood figure out which wavelength coupled for those surface plasmon Furthermore hence upon what amount of analyte is introduced. The fiber optic SPR sensor will be constructed utilizing an expansive breadth – as a rule, 400 nm – multimode fiber. A specific period of the fiber will be totally stripped about its cladding What's more a surface plasmon metal, for example, silver will be kept in its spot. This length relies on the breadth of the fiber and determines those number about reflections happening during the surface plasmon metal interface. Whether the period will be excessively awful short, insufflate coupling will happen. Assuming that the period will be excessively long, coupling will be extremely solid and the least coupling power will a chance to be challenging to determine.[14]

![Fig 3.1.3: Optical Fiber System](image)
3.1.4 Prism coupled system

The prism-based system is quite similar to grating and fiber optics system. There used a prism instead of grating and glass tunnel.

However, this system has two configuration

- Otto configuration
- Kretschmann configuration

The difference between this two configurations is the air gap.

Otto configuration

In the Otto setup the sensing guideline is a variety of the air gap thickness $d$ throughout the reflection of the incident wave at the metal/air layer. The sensitivity configured from the optical reflectance on an air gap thickness $d$ and angle of incident (theta) of the light beam. The reflectance and the affectability need aid determinedly dependent on those starting air gap thickness.

Not only those amplitude, as well as those stage of the p-polarized part from a light source reflected from a metal film under SPR states, is reliant on encompassing states that need aid transformed toward an evanescent wave. Those s-polarized part may be very much unaesthetic. Which results in the phase shift between p-polarization and s-polarization. [15] this configuration is preferable when we don’t want direct contact with the metal surface and analyte, for example for studies of surface quality.
Kretschmann configuration

The Kretschmann configuration is the traditional way of SPR system. It also depends on prism coupled system. But the main difference between this two is the air gap between metal film and analyte. Here, there is no air gap between metal film and analyte. In this system SPR occurs between metal and analyte layer. As there is no air gap mainly another material of different refractive index, the SPR generate more and results will be more accurate.

3.2 Analyzed System

This configuration is much sensitive for detecting different types of protein and biomolecules. [16]. so we decided to continue on the basis of this configuration in our angle shifting of different protein layer research. It is also prism coupled system but here we don’t use any air gap between metal layers. In this configuration, we need Prism, noble metal, protein for test and monochromatic light source. As it is not possible for us to go to hardware operation for technical limitations we do our job in software named FDTD which is specially designed for this type of Plasma resonance experiments.

However, the Kretschmann configuration follows the rule of total internal reflection (TIR). As we know that to perform TIR we must pass a light beam through different refractive index property elements. Here we use prism as primary refractive index substance which index is 1.52 (crown glass). Then we use a noble metal layer which is very thin, like gold, silver, and copper. This metal must have dielectric functionality. Actually, it is not much important to be noble metal but it must
have fulfilled some conditions. Here it is $\varepsilon_{np} = -2 \varepsilon_b$ here b stands for background environment. According to law only some metals can create SPR like Au, Ag, Cu, Pt, and Pd. Among them Cu is unstable and Au and Ag has powerful resonance where other two has less resonance. So, in our work we decided to use silver. Moreover silver/gold gold/silver dual layer is used also. This layer is so thin like 50nm~80nm. There is no air gap between prism and metal. So the SPR will create on the opposite surface of incident light. And it will be perpendicular with electromagnetic wave. Equally important, we also use monochromatic light source as incident light. It must be remembered that the light will inject across prism. There must not any gap between prism and light source, because if there is any gap, in that gap there may be different refractive index as we know in vacuum it is 1.00 and air ~1.0. This gap may put disturbance and change our results. As our principle is based on TIR so incident angle is also important here. To get perfect result we use sweep option of software it cover with light beam a big amount of area of metal plate with wide an angle. Another important component of our configuration is analyte, here we use protein layer. Actually it is that layer what we want to test. It will vary for different types of testing materials. To compare the result we first use water as analyte as base information. Then we use different types of protein with different types of refractive index. In our experiment we use IgG, Lysozyme, and HSA. Finally a monitor is need to capture reflected light from metal. With this reflected light we get graph which helps to reach a result.

Fig 3.2: Analyzed System (Kretschmann Configuration)
3.3 Detection System

The SPR has three types of detection system.

3.3.1 Angular detection

The most widely recognized method measures the point at which the best loss of light intensity, known as the SPR least, happens. This permits coordinate estimation of changes in the refractive index as biomolecules tie to the sensor surface. After reflecting from metal layer lights caught by monitor and feedback is generated by angle value instead of refractive index. Using different refractive materials results different angle value. And we come to a result by doing difference of those reflected light intensity angle value.

3.3.2 Intensity Detection

A less complex technique includes measuring the intensity of the reflected light at a settled point. As the surface refractive index changes, the intensity angle will move, prompting to an adjustment with intensity at this settled edge. This method requires less exactness in the component itself as is appropriate for more straightforward, less expensive sensors.

3.3.3 Wavelength Detection

The third detection mode is SPR wavelength detection mode which illuminates the sensor with White light and inspecting the spectra which are returned. Tying occasions will prompt progressions in the wavelength toward which resonance occurs, and this canwood a chance to be distinguished through transforms in the reflected light intensity level crosswise over that range. In the dominant part about situations, angular detection stays the gold standard detection mode. Intensity furthermore wavelength identification have their uses, especially previously, a waveguide coupled systems.
In our work we used this angle detection method because of its high detection ability.

### 3.4 Protein as a Biomolecule

#### 3.4.1 Fibrinogen

Fibrinogen is a kind of glycoprotein produced by liver that helps to blood clots formation. At the point when there is damage and draining happens, the body frames blood coagulation through a progression of steps. In one of the last strides, solvent fibrinogen is changed over into insoluble fibrin strings that crosslink together to frame a net that balances out and follows at the harm site until the territory has mended.

Usually these elevations in the fibrinogen level are temporary, returning to normal after the underlying condition has been resolved. Elevated levels may be seen with Acute infections, Cancer, Coronary heart disease, myocardial infarction, Stroke, Inflammatory disorders (like rheumatoid arthritis and glomerulonephritis, a form of kidney disease), Trauma, Cigarette smoking, Pregnancy, Peripheral artery disease

While fibrinogen levels are elevated, a person's risk of developing a blood clot may be increased and, over time, they could contribute to an increased risk for developing cardiovascular disease. So, using SPR to sense this protein agent may advance medical science by detecting those symptoms. [25]
3.4.2 Immunoglobulin G

Immunoglobulin is a special kind of protein made by the human immune system to defend bacteria, virus, and toxins. Among them, immunoglobulin G is the most common type of antibody found in blood, mucus fluid, cerebrospinal fluid, and peritoneal fluid. So detecting this protein will help doctors diagnose immunodeficiencies. This protein test helps demonstrate serologic immunity to measles, mumps, and rubella (MMR), hepatitis B virus, and varicella (chickenpox), among others. [26]

3.4.3 Human Serum Albumin (HSA)

Human serum albumin (HSA) is the most plentiful protein in human plasma with an atomic weight of 66,437 Da (in view of amino acid structure). The basic works of this serum are transporting hormones, fatty acids, and other compounds, buffering pH, and maintaining oncotic pressure, among other functions.

3.4.4 Adenomatous Polyposis Coli (APC)

Adenomatous Polyposis Coli is classified as a tumor suppressor gene. This APC protein helps control how often a body cell divides. As a result, mutation of this gene can cause cancer. This protein also helps to ensure the correct number of chromosomes in the nucleolus. So by detecting the change of character of this protein helps us detect cancer.

3.4.5 Lysozyme

It is a kind of attacking enzyme cell which attacks bacteria cell walls by catalyzing hydrolysis. This enzyme generally found in tears, saliva, human milk, and mucus. [27] The functions of this enzyme are attacking bacteria cell walls (especially in gram positive bacteria). So it is very important enzyme for human for their defense system.
Chapter 4

4.1 FDTD Simulations

4.1.1 FDTD Region

FDTD region is required to determine the simulation area. Utilizing this area we could make any types of changes what we required, for example, an altered range done which main vital waves need aid used to get palatable outcome avoiding unwanted waves or using the desired background like water or biomolecule agents, in which platform we want out the outcome. FDTD result calculates the effect utilizing every last one of qualities for this district just. Same time figuring result, it overlooks other waves of the hotspot. Similarly, it only calculates giving background environment. It is so sensitive that a single change of background environment or light source or material change (various types of glass prism or gold layer with different refractive index).

The geometry of FDTD region can work both 3D and 2D what user wants. In our simulations, we use the 2D surface to test. The FDTD approach depends on a direct numerical arrangement of the time-subordinate Maxwell's twist conditions. The photonic gadget is laid out in the X-Z plane. The engendering is along Z. The Y-course is accepted to be vast. This supposition evacuates all the $\partial/\partial y$ subsidiaries from Maxwell's conditions and parts them into two (TE and TM) free arrangements of conditions. Furthermore, in 3D reproductions, the recreation area is a cubic box, the space steps are $Dx$, $Dy$, and $Dz$ in x, y, and z headings separately. Every field segments is displayed by a 3D exhibit — $Ex(i,j,k)$, $Ey(i,j,k)$, $Ez(i,j,k)$, $Hx(i,j,k)$, $Hy(i,j,k)$, $Hz(i,j,k)$. These positions and the documentation demonstrate that the E and H segments are interleaved at interims of $1/2Dh$ in space and $1/2Dt$ with the end goal of executing a jump calculation. Lastly, it also calculates the simulation time. Depending this simulation time the accuracy of simulation results rely on. [18]

So this simulation region has three changeable area:

- Dimension (2D or 3D)
- Background Environment (different types of refractive index representing different analytes)
- Simulation Time (duration of time)[19]

Another important part of FDTD region is mesh. There are some helpful rules for the mesh.
• The largest mesh cell must not be bigger than about a tenth of the smallest wavelength.
• Make the mesh as coarse as possible and as fine as needed
• Keep an adequate distance to an absorbing boundary (Try to keep a distance of a quarter wavelength to a PML)
• Create a smooth mesh: Neighboring cell sizes should not exceed a factor of ~2 [20]

### 4.1.2 Prism

The principle purpose behind utilizing the prism here is to give the evanescent field (by means of the total internal reflection, TIR) that energizes plasmons in a metal film that is covered on a base of the demure. In our system, we use most common triangular shape prism. As prism material, we used glass ($SiO_2$). This glass prism ($SiO_2$) has ~1.50 refractive index.

### 4.1.3 Source

There are different kinds of sources available in FDTD solutions. Their fields and resultant are also different. However some kinds of sources are described here,

Point sources (dipole): Oscillating dipoles act as sources in Maxwell's equation to produce electromagnetic fields. Their position and direction are specified in terms of the center position and their orientation through angles theta, phi.

Gaussian and Cauchy/Lorentzian beam sources: A Gaussian source characterizes a light emission radiation proliferating in a particular bearing, with the abundance characterized by a Gaussian cross-segment of a given width. Of course, the Gaussian sources utilize a scalar pillar guess for the electric field which is legitimate the length of the abdomen bar distance across is much bigger than as far as possible. The scalar estimate expects that the fields toward spread are zero. For a profoundly engaged pillar, there is additionally a thin focal point source that will infuse a completely vectorial bar. The cross segment of this pillar will be a Gaussian if the focal point is not filled, and will be a sync work if the focal point is filled. For every situation, the pillars are infused along a line opposite to the proliferation course and are cut at the edges of the source. For more data on the use of this source, visit the plane wave and shaft sources page.

Plane wave sources: Plane wave sources are utilized to infuse along the side uniform electromagnetic vitality from one side of the source area. In two-dimensional reproductions, the
plane wave source infuses along a line, while in three-dimensional recreations the plane wave source infuses along a plane. It is likewise conceivable to infuse a plane wave at an edge. The plane wave source is really an indistinguishable question from the Gaussian source, with the main distinction being the SOURCE SHAPE setting. Intermittent or Bloch limit conditions ought to be utilized with Bloch/occasional short plane wave source. Diffracting plane wave source can be utilized with PML as a part of all headings. At the point when a broadband outcome at calculated plane wave frequency is sought after with one reproduction without utilizing Bloch BCs, the FAST source system ought to be utilized. We also used this plane wave source in our simulation.

We chose y-axis as the infusion pivot since the biomolecule layer was kept parallel with the source and the wave will proliferate towards y-axis having a forward course. Point theta and edge phi were set to -25 degree and 0 degree individually. As we are doing two-dimensional recreation, just point theta is required which sets the edge regarding the infusion hub. While doing three-dimensional recreations point theta and edge phi both are required. Wavelength and recurrence are another essential parameters for a specific wave source. We used 630nm and 740nm wavelengths for the simulations. We got 475.861 THz and 405.125THz frequencies for 630nm and 740nm wavelengths respectively. By and large, SPR makes in visual range extend which is about from 390nm to 700nm. As far as recurrence which compares to a band in the region of 430-770THz. There are also some other kinds of sources namely Total-field scattered-field sources, Mode sources.

4.1.4 Metals

As we need a metal layer in our Kretschmann configuration this software has a vast index and database of metals. But we only need noble metals because of their strong resonance capacity. We selected silver and gold as the metal film. That metal has a different refractive index which works as a secondary medium of the light source. After propagating light the SPR creates on the metal surface. Generally, there are no criteria of change as those properties are fixed. But we can add different metal properties manually.

Firstly, we used a silver film having a width of 50nm. We also used the combination of these noble metals 50nm in total. Where the silver film was 25nm and the gold film was also 25nm. Once we injected the light to the silver where the gold film was just on the silver and SPR created on the gold surface. In contrast, we injected the light to the gold where the silver film was just on the gold
and SPR created on the silver surface. Moreover, better SPR depends on the width of this metal film. If the film is too thick, SPR cannot be created as desired. We also studied the result for different width of the metal film from 50nm to 70nm which will be discussed later.

4.1.5 Physical Environment

It is important to test SPR in an environment. Without any environment means the system is in vacuum medium or air as the refractive index of vacuum and the air is very close and it is ~1.000. The background environment is important because the different medium has a different refractive index and our result is very much dependable on it. To make it realistic we use water as background index which refractive index is 1.33. This background environment works only in FDTD region. It has effects in results also. We compared our angle shifting with respect to background index. In another section, we varied the background index using Urea.

4.1.6 Analytes

Here we use different types of biomolecules as an analyte. Analyte cum biomolecules are used for as test materials. As we want to show the difference of human body change like different kinds of a disease condition such as diabetics percentage. A biomolecule layer is located on the metal layer where we put the sample of the proteins. The SPR creates between the metal layer and biomolecule layer. We used five proteins of the human body as a sample like Fibrinogen (Fb), Immunoglobulin (IgG), Human Serum Albumin (HSA), Adenomatous Polyposis Coil (APC), Lysozyme. The refractive index of these proteins were used to denote them in FDTD as below:
<table>
<thead>
<tr>
<th>Biomolecules</th>
<th>Refractive Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen (Fb)</td>
<td>1.39</td>
</tr>
<tr>
<td>Immunoglobulin G (IgG)</td>
<td>1.41</td>
</tr>
<tr>
<td>Human Serum Albumin (HSA)</td>
<td>1.45</td>
</tr>
<tr>
<td>Adenomatous Polyposis Coli (APC)</td>
<td>1.46</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>1.495</td>
</tr>
</tbody>
</table>

Table 4.1.6: Refractive Index of Biomolecules

We used the property of biomolecules’ refractive index. Depending on different types of protein, we got different results. These proteins absorb light waves differently according to the incident light, width of the metal layer, the material of metal layer and also background index and produce different SPR profile. We varied incident light, the width of the metal layer, the material of metal layer and the background to compare the results for each protein.

4.1.7 Monitor

FDTD solution has multiple monitors to observe the reflection of injected light waves. Such as, index monitors, effective index monitors, time-domain monitors, movie monitors, frequency-domain field monitors, mode expansion monitors. We used frequency-domain field monitor while running the simulation.

Frequency-domain field monitors collect the field profile in the frequency domain from simulation results across some spatial region within the simulation in the FDTD specified by us. FDTD uses standard Fourier transform to get the result on the monitor. The monitor observes the reflection of the injected light we created. Using this monitor we can get the amount of reflected light and the rate of propagation of electromagnetic waves. Through the amount of reflected light, the absorption of a light wave can be found. Depending on the absorption, we can observe how much SPR creates. Several graphs can be found from the monitor as result such as electric field graph,
magnetic field graph, pointing vector graph etc. We used wavelength vs. angle theta graph to observe our desired result using the angle shifting for different parameters. [21]
Chapter 5

5.1 Experimental Report

In our project, we practice surface plasmon resonance (SPR) sensing system constructing with a bilayer configuration formed by a glass prism coated with a thin metal film and used bio-protein layers working as the analytes for the experiment to calculate and compare the SP resonance angle of the proteins with respect to the angle of the metal film based on their absorption of surface plasmon by calculating the reflectance against the incident angle for a fixed uniform wavelength of light. In this project, we followed the Kretschmann developed configuration called the attenuated total internal reflection (ATR) method considering p-polarized laser beam.

The absorption characteristics of the bio-proteins were simulated by following the Fresnel theory for wave propagation analysis solving for the amplitudes of the electric and magnetic fields for any isotropic multilayered media. [22]

In fig-5.1 (a) we see the angle shifting based on the amount of SPR creation. This change occurs for the change of refractive index in comparison with default background index.

Again, in fig 5.1 (b) we see the electric field profile of SPR on metal film using biomolecule agent. Here red color indicates that the intensity of SPR. At left corner intensity of SPR is high and it decrease gradually to right side. Finally at right side there is no SPR detected.
Our experiment of the transducing layers, three different types of configuration were used to investigate the SPR absorption property of the protein analytes with a variation in the wavelength of the incident light, changing the width of the thin metal film containing a noble metal, three different configurations in the variation of the metal film involving silver(Ag) only, a silver(Ag)_gold(Au) and gold(Au)_silver(Ag) configuration surrounded by an environment filled up with water with a refractive index of 1.33 and finally adding a Chaotropic Agent to study the behavioral change of refractive index of Human Serum Albumin (HSA). The optimized thickness used in this experiment for the transducing layers are a 50nm-silver and for the silver(Ag)_gold(Au) and gold(Au)_silver(Ag) configurations, both metals share the 50% of the total width to study the improvement in sensitivity. A SiO$_2$ glass prism is being used for the higher refractive index that provides a comparatively larger shift in wavelength resulting in better sensitivity in the SPR.
5.1.1 Variation of Incident Light

Plasmons are the collective oscillation in the free electrons of metals and wavelength of light has a huge role in SPR. For metal nanoparticles, SPR response is enhanced at certain frequencies of light. For noble metals, a good SPR response can be achieved using the visible lights, which gives the particles a strong color proving the existence of SPR. Our experiment deals with the two variations of incident light at the visible range with a wavelength of 630nm and 740nm to investigate the changes in the SPR response. The result of varying the wavelength attached to the fig -5.1.1 and it states the behavior of the SPR response in terms of angle differences with respect to the 50nm silver at a background index of 1.33. According to the graph, we got the result for the five bio-protein molecules as follows- the angle difference is 1.0056 for Fibrinogen (Fb), 1.2079 for Immunoglobulin G (IgG), 1.4010 Human Serum Albumin (HSA), 1.5096 for the Adenomatous polyposis Coli (APC) and 1.6571 for Lysozyme using the wavelength of 740nm which is a positive response to an increase in the differences according to the increase in the refractive index of 1.39(Fb),1.41(IgG),1.45(HSA),1.46(APC) & 1.495(Lysozyme). Similarly, using the wavelength of 630 nm, we got the difference of 0.1407 for Fb, 0.2410 for IgG, 0.3406 for HSA, 0.3571 for APC and 0.5919 for Lysozyme which is also increasing in amount which explains the impact of wavelength variation in the response of SPR absorption and surface plasmon shows a better characteristics for the higher wavelength or the higher frequency of light following the relationship equation between the wavelength and frequency –

\[ \lambda = \frac{v}{f} \]  

\( \lambda \) is the wavelength  
\( v \) is the velocity of the wave (default is the velocity of light in vacuum: 300.000 Km/s)  
\( f \) is the frequency
Fig 5.1.1: Variation of Wavelength of Incident Light, (A) 630nm (Blue), (B) 740nm (Green)

The graph containing the data response for the protein molecules shows the better response towards the higher wavelength and to compare from our report we get the better response for the wavelength of 740nm.

5.1.2 Variation of Width

Surface plasmon resonance (SPR) spectroscopy has a strong affinity for the quantitative analysis of interactions at the surface of the noble metal film. The technique is used in the experiment to exploit the surface plasmon wave propagating along the surface of the thin metal film to probe the refractive index by varying the width of silver. As SPR has a highly surface sensitive nature of creating a strong evanescent field at the metal/dielectric interface under the total internal reflection condition. The physicochemical phenomena at the metal surface induce dielectric constant and thickness changes of the functionalized dielectric film. The changes shift the resonance condition of the surface plasmon wave, which can be observed experimentally as SPR curve shifts. The evanescent field in the metal film has two origins: a weak ATR-generated evanescent field at the
prism/metal interface and a strong SPR-generated evanescent field at the metal/dielectric interface. [23]

The dielectric constant of the material film changes with its thickness due to electron scattering at the film boundary. For simplicity, we kept the optical constant of the metal film is assumed constant and is independent of the metal film thickness. We changed the width of our metal film (Ag) from a range of 50nm to 70nm to understand the SP response. Although the ATR-generated evanescent field is very weak and does not change significantly as the thickness of the metal film increases absorption of the silver film can be noticed as per increasing the width less plasmon is created or absorbed by the dielectric and the graph shows a decay in the resonance angle shift proving less efficiency.

![Variation of width of Metal Film](image)

Fig 5.1.2: Variation of Width of Silver in the Range from 50~70nm
5.1.3 Variation of Metal film

In our project observing the SPR behavior, we put a variation in the metal film with three different configurations including silver (50nm) only, silver/gold and gold/silver sharing the half of the width. The wavelength for the SPR dip set to 740 nm with an incident angle of -25 and calculated the SPR for our experimental protein molecules by the terms of resonance angle difference with respect to the physical environment using no analyte. The resonance angle difference for the configuration of only silver (Ag) is as follows – 1.0856 for Fb, 1.2697 for IgG, 1.5010 for HSA, 1.610 for APC and 1.7601 for the Lysozyme. Similarly, for the configuration of silver/gold, the result is as follows- 0.8683 for Fb, 1.0547 for IgG, 1.3075 for HSA, 1.4407 for APC and for lysozyme the difference is 1.6102. Again for the third configuration of gold/silver the resonance angle differences with respect to no protein analyte is like- 1.0020 for Fb, 1.1594 for IgG, 1.4351 for HSA, 1.5352 for APC and 1.7612 for Lysozyme.

Fig 5.1.3: Variation of Metal Film (a) Silver (b) Gold/Silver (c) Silver/Gold
Table 5.1.3: Comparative Response of the Protein Molecules for the three variations of Metal Film

<table>
<thead>
<tr>
<th>Protein</th>
<th>Fb</th>
<th>IgG</th>
<th>HSA</th>
<th>APC</th>
<th>Lysozyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silver</td>
<td>1.0856</td>
<td>1.2697</td>
<td>1.5010</td>
<td>1.6100</td>
<td>1.7601</td>
</tr>
<tr>
<td>Silver/Gold</td>
<td>0.8683</td>
<td>1.0547</td>
<td>1.3075</td>
<td>1.4407</td>
<td>1.6102</td>
</tr>
<tr>
<td>Gold/Silver</td>
<td>1.0020</td>
<td>1.1594</td>
<td>1.4351</td>
<td>1.5352</td>
<td>1.6712</td>
</tr>
</tbody>
</table>

Fig 5.1.3: Variation of the metal films- Silver (blue), Silver/Gold (green), Gold/Silver (red)

From the three configurations used for the experiment shows that almost all three has shown a close response to SPR sensing and only the bilayer configuration used by a 50nm silver has shown higher differences whereas the other two has a very keen gap to each other for all the five proteins.[24]
5.1.4 Variation of Background Environment

In our experiment, we generally used water as our background environment which refractive index is 1.33. In this part of our experiment, we used chaotropic agent along with background environment. The main idea of using a chaotropic agent in background environment is to study the change of refractive index of the environment that has been used previously. After injecting chaotropic agent, the refractive index of background environment changes with time. Here we used Urea as a chaotropic agent with water and as a biomolecule, we used Human Serum Albumin (HSA). After a certain time when the index of background environment increased to 1.3448 and 1.3848 we did the experiment. Because of using Urea as a chaotropic agent, the refractive of water increased significantly. On the other hand, chaotropic agent manipulates the biomolecule to lose its characteristics. As a result, the refractive index of HSA decreases simultaneously. We took four decreasing values of refractive index of HSA like 1.45, 1.44, 1.42, and 1.40.

When the background environment was only water, we got the best resonance angle difference for different analytes as follows - 1.3336 for analyte 1.45, 1.2708 for analyte 1.44, 1.1451 for analyte 1.42 and 1.0567 for analyte 1.40. As the background index increased gradually, the resonance angle difference decreased. When the background index increased to 1.3448, we got the resonance angle difference as follows - 1.0824 for analyte 1.45, 1.0296 for analyte 1.44, 0.8311 for analyte 1.42 and 0.7055 for analyte 1.40. Again, when the background index increased to 1.3848 after 30 min of injecting Urea in water, we studied the resonance angle difference as follows - 0.5170 for analyte 1.45, 0.4914 for analyte 1.44, 0.2657 for analyte 1.42 and 0.0774 for analyte 1.40.
Fig 5.1.4: Variation of the background environment- Background Index-1.33 (blue), Background Index-1.3448 (green), Background Index-1.3848 (red)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Background index-1.33</th>
<th>Background index-1.3448</th>
<th>Background index-1.3848</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.45</td>
<td>1.3336</td>
<td>1.0824</td>
<td>0.5170</td>
</tr>
<tr>
<td>1.44</td>
<td>1.2708</td>
<td>1.0296</td>
<td>0.4914</td>
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<tr>
<td>1.42</td>
<td>1.1451</td>
<td>0.8311</td>
<td>0.2657</td>
</tr>
<tr>
<td>1.40</td>
<td>1.0567</td>
<td>0.7055</td>
<td>0.0774</td>
</tr>
</tbody>
</table>

Table Fig 5.1.4: Comparative Response of Refractive index of HSA in Presence of 6M Urea

After 30 minutes of injecting 6M Urea, the refractive index of biomolecule gradually decreased. As a result, the incident angle difference between the refractive index of background and the biomolecule also decreased gradually.
Chapter 6
6.1 Conclusion

The target of our thesis on the analysis of protein detection using the surface plasmon resonance technique leads to the result which shows the density changes in the adsorbing layers of the proteins (Fb, IgG, HSA, APC, Lysozyme) in terms of the resonance angle shift using the total internal reflection method. From the research, we could come to a decision that the change in refractive index of the analytes during the adsorbing process varies in accordance with the wavelength of incident lights, the thickness of the metal film, the size of the analytes and the buffer solution. In addition to that, we made a comparison of the sensitivity of the proteins towards the temporary field created on the surface of the metal. Besides the consequences in the quantitative analysis of the protein adsorption by measuring the change of refractive index, it also helps to understand the role of adsorption process based on the plasma frequency, noble metals and the influences of the physical environment.
Chapter 7

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