



UNIVERSITI PUTRA MALAYSIA

***DESIGN AND ANALYSIS OF WAVELENGTH
INTERROGATION-BASED SURFACE PLASMON
RESONANCE SYSTEM***

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**DESIGN AND ANALYSIS OF WAVELENGTH
INTERROGATION-BASED SURFACE PLASMON
RESONANCE SYSTEM**

By

ALIUDDIN BIN ANWAR

199158

**Thesis Submitted to the Department of Physics, Universiti Putra Malaysia, in partial
Fulfilment of the Requirements for the Degree of Bachelor of Science in
Instrumentation Science with Honours**

January 2022

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DEDICATION

To my beloved parents Anwar Bin Guntur and Hayani Abdullah @ Manyie Ak Abon

For their unstoppable love and support

To my precious siblings and family

For their support and make my life full of happiness

To all my very cheerful friends

For the memories that we had been created together and make my life cheerful

To all my lectures

For helping me in this journey thoroughly and the knowledge given to me

Thank you all

ABSTRACT

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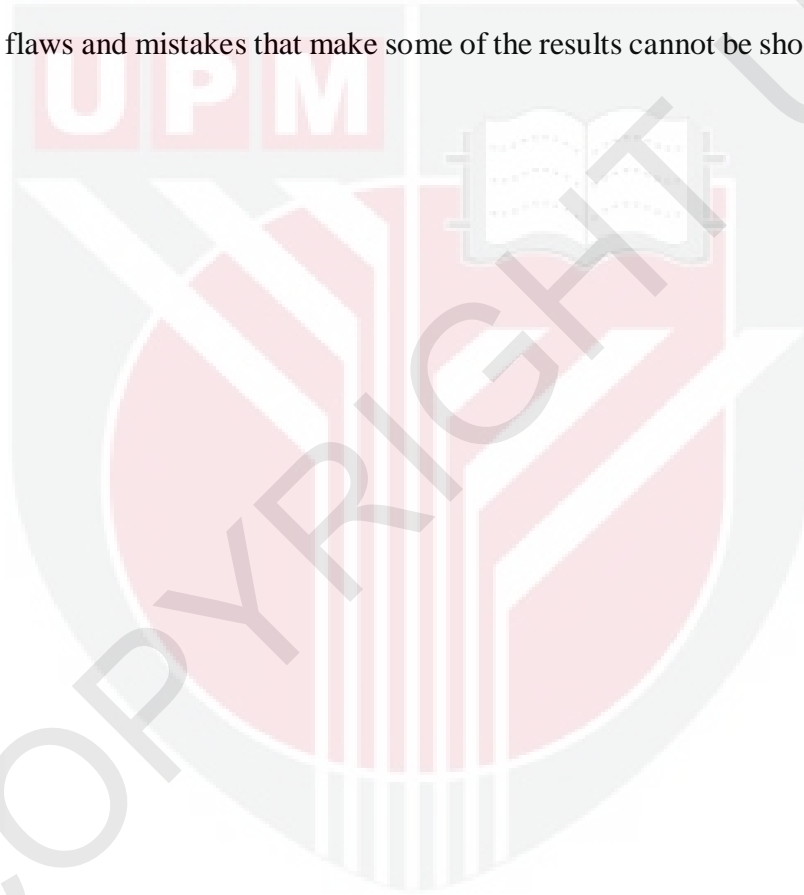
January 2022

Supervisor: Dr. Nizam Bin Tamchek (PhD)

Department: Department of Physics, Faculty of Science.

Surface Plasmon Resonance (SPR) has become an important optical biosensing technology in the areas of biochemistry, biology, and medical sciences because of its real-time, label-free, and noninvasive nature. SPR has not been implemented in undergraduate laboratories due to the high cost commercial instruments and consumables. There are many types of SPR systems that had been created and still in research such as angular interrogation based SPR system, phase interrogation based SPR system and wavelength interrogation based SPR system. In this study, a wavelength interrogation SPR system is designed and analyzed. We develop a specially designed SPR system that will show how the light will move and behave along the system and instruments that used for the design. Most of the instruments are available on the lab and some are homemade. Through the study, there are many parameters that

need to be measured and the system need to be made perfectly with given instruments. The instruments need to be placed at the right position and the right range between them. We also need to have skill on how to use the optical instruments so the material that used in this experiment being put at the right place and position. This system is able to detect metal ion at certain concentrations and show the difference of the light spectrum on different medium. There are also some problems that need consideration because of some flaws and mistakes that make some of the results cannot be shown and measured.



ABSTRAK

REKABENTUK DAN ANALISIS SISTEM RESONANS PERMUKAAN PLASMON BERASASKAN PANJANG GELOMBANG

Oleh

ALIUDDIN BIN ANWAR

Januari 2022

Penyelia: Dr. Nizam Bin Tamchek (PhD)

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Resonans permukaan plasmon (SPR) telah menjadi teknologi optic biopenderiaan yang penting dalam bidang biokimia, biologi dan sains perubatan kerana sifat masa nyata, bebas label dan ketidakinvasifnya. SPR belum dilaksanakan di makmal prasiswa kerana kos peralatan komersial dan bahan habis pakai yang tinggi. Terdapat banyak jenis system SPR yang telah dicipta dan masih dalam kajian seperti sistem SPR berasaskan sudut, SPR berasaskan fasa dan SPR berasaskan panjang gelombang. Dalam kajian ini, sistem SPR berasaskan panjang gelombang direka bentuk dan dianalisis. Kami membangunkan sistem SPR yang direka khas dengan menunjukkan cara Cahaya akan bergerak dan berkelakuan di sepanjang sistem dan instrument yang digunakan untuk reka bentuk ini. Kebanyakan instrument boleh didapati di makmal dan ada yang dibuat sendiri. Melalui kajian ini, terdapat banyak

parameter yang perlu diukur dan sistem perlu dibuat dengan sempurna dengan menggunakan instrument yang sedia ada. Instrumen perlu diletakkan pada kedudukan dan julat yang betul di antara instrument-instrumen tersebut. Kami juga perlu mempunyai kemahiran menggunakan alat optik supaya bahan yang digunakan dalam eksperimen ini diletakkan pada tempat dan kedudukan yang betul. Sistem ini mampu untuk mengesan ion logam pada kepekatan tertentu dan menunjukkan perbezaan spektrum cahaya pada medium yang berbeza. Terdapat juga beberapa masalah yang perlu dipertimbangkan kerana beberapa kelemahan dan kesilapan yang menyebabkan beberapa keputusan tidak dapat ditunjukkan dan diukur.

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May Allah bless all of us.

APPROVAL

This thesis entitled “Design and Analysis of wavelength-interrogation based Surface Plasmon Resonance System” by Aliuddin Bin Anwar (Matric No.: 199158), was submitted to the Department of Physics, Faculty of Science, Universiti Putra Malaysia and has been accepted as partial fulfilment of the requirement for the degree of Bachelor of Science in Instrumentation Science with Honours.

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TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	v
ACKNOWLEDGEMENT	vii
APPROVAL	viii
DELARATION	ix
TABLE OF CONTENTS	x
LIST OF FIGURES	xii

CHAPTER

1 INTRODUCTION

1.1 Introduction	1
1.2 Motivation of studies	1
1.3 Problem statement	2
1.4 Objectives	3
1.5 Outline of Thesis	4

2 LITERATURE REVIEW

2.1 Introduction	5
2.2 Light Source Technology	6
2.3 Kretschmann Configuration	7
2.4 Surface Plasmon Resonance as Biosensor and Chemical Sensor	9

3 Methodology of Wavelength Interrogation-based SPR System

3.1 Introduction	12
3.2 Experiment design and setup	14
3.3 Procedure	15
3.4 Method of data interpretation	16

4 Results and Discussion

4.1	Introducton	18
4.2	Surface Plasmon Resonance Setup	18
4.2.1	Total Internal Refletion in Prism Coupler	19
4.2.2	Resonance effect while prism coupler coated with gold	21
4.3	Response of the SPR to alcohol	22
4.4	Surface Plasmon Resonance Spectrum	23
5	Conclusion	
5.1	Outcome of the project	25
5.2	Project related problem	25
	REFERENCES	27
	VITAE	28

LIST OF FIGURES

Figure

- 1(A) Schematic diagram of self-constructed wavelength interrogation-based SPR system base on Kretschmann configuration in the experiment of analysis of protein interaction by a wavelength interrogation-based SPR sensor
- (B) Schematic diagram of how the material put on the gold thin film to gain the resonance wavelength
- 2 Schematic design of the wavelength interrogation-based SPR sensor
- 3 Prism coupler and light beam
- 4 Procedure
- 5 Schematic representation of surface plasmon resonance system based wavelength interrogation
- 6 Schematic diagram on how to analyze on the directed light on a display whether it is refracted or already reach the critical angle and reflected
- 7 Optical design of the light beam inside the prism coupler undergoes total internal reflection
- 8 Coated prism coupler with gold
- 9 The depth from the resonance effect shift a bit to the right along with the intensity of the light while a few drops of alcohol dropped on the gold thin film
- 10 SPR curve signal of the gold thin film in contact with deionized water
- 11 SPR curve signal of the gold-air and gold deionized water

Chapter 1

Introduction

1.1 Introduction

Surface Plasmon Resonance (SPR) is the resonant oscillation of conduction electrons at the interface between negative and positive permittivity material stimulated by incident light. It is a well-established, label-free biosensor technology or a chemical sensor for researching real-time interactions between various biomolecules and biochemical mechanisms. Basically, SPR refers to optical phenomena that use a quantum mechanical principle to monitor changes in refractive index. In most experiments, the SPR had worked by immobilized or captured a target which is the molecule onto a surface known as a sensor chip. We use an optical measurement system to captures changes occurring on the surface of the sensor chip.

Since SPR used in a real-time analysis of a biological systems since 1990s, it has become an important optical biosensing technology in the areas of biochemistry, biology and medical sciences because of its real time, label-free, and noninvasive nature. There are two types of SPR system that commonly used for biosensing or chemical sensor which is angular interrogation and wavelength interrogation. Both of this types of sensor can be developed based on the attenuated total reflection (ATR) configuration. Usually, the sensors system are constructed based on Kretschmann configuration that include a prism coupler to make the light perform total internal reflection (TIR) wave phenomena along the waveguide layer. In the principle of prism coupler, the planar waveguide configuration is unable to interrogate the incident angle scanning. As a consequence, the wavelength interrogation method is the only option for the signal acquisition technique.

The wavelength interrogation-based SPR system, the SPR spectral profile can be obtained by either scanning the incidence wavelength or using a spectrometer for analyzing the reflected beam. It provides a wide dynamic range, and its detection sensitivity could be based SPR sensors are much more flexible in terms of optimization because the operation wavelength range can be easily tuned to achieve the best SPR excitation.

1.2 Motivation of studies

In these studies, it is about designing a self-constructed system that will be in a very good use in the future. SPR that has been used in many experiments as a technology in biosensors still have many undiscovered applications and its capabilities in other fields. Since it has shown a very good performance but still have some weaknesses, I believed that by studying more in this research those flaws could be overcome easily and make this system ideal to be used in related field. There are numerous challenges that need to be solved through repeated attempts and understanding so that I can find ways to get near the problems and solved it.

In recent years, the development of wavelength interrogation-based SPR system has not create many attentions from researchers. Most of the advancement and applications were used on angular interrogation SPR system because of it got many tests from the research conducted before so they know how to execute the experiments with many references (JOUR, 2019). In this situation researchers only could conduct an experiment with what the SPR system capable of analyze. By involving myself in this research, I can determine the capabilities of wavelength interrogation-based SPR system and then relate its characteristics in another studies. There will be more specific insights that I can see in these studies based on what the wavelength interrogation-based SPR system potential in science.

1.3 Problem statements

Nowadays, there are many researches and studies have been conducted related to the SPR system. The analysis of wavelength interrogation-based SPR system has been approach by many researchers because of the proven properties and characteristics are useful in biosensor and chemical sensor field (Homola, Jiri., 2008). It also widely spread in many application as they are specific, sensitive, quantitative and do not require labeling of the molecules. This optical sensors are then considered as good candidates for the measurement or analyzing small molecules. However, in constructing the wavelength interrogation SPR system requires time and the construction need to be executed accurately. In this study the wavelength interrogation-based SPR

system will be constructed based on previous experiments designed by other researchers that use the SPR system in biosensing.

Recently, there are many studies related to the SPR system have been developed with their own variations of types and advancement made regarding the situation of the experiment. As wavelength interrogation proves properties and characteristics, it is believed that wavelength interrogation have the ability to be significantly useful in optical and sensing field. Yuk et al. introduced an SPR imaging (SPRi) sensor for the investigation of protein interactions on arrays, where, in addition to the calculation of the resonance wavelength for the SPR reflectivity spectra, the authors also incorporated the position control (Zeng et al., 2017). With these arrangements, they could quantitatively analyze the specific binding of anti-Rac1 and anti-RhoA to RaC1 and RhoA on the protein arrays. Nevertheless, there were still some limitations of the works toward its optical and structural properties. Therefore, it is important to put efforts in improving the analysis and properties of wavelength interrogation-based SPR system in order to completely understand the characteristics of wavelength interrogation-based SPR system.

Furthermore, wavelength interrogation-based SPR system has a lot of advantages as it is low cost and only require simple preparations. The SPR system usually have high sensitivity and very common in achieving real-time monitoring of biological interactions. Even though the characteristics and properties of this wavelength interrogation-based SPR system have been determine as well as proved by researchers, there were still small amount of work and experiment been done regarding the system. It is considered a common type of SPR system as well as the angular interrogation technique but most of the time the angular interrogation shown a great characterizations in their experiments. Thus, this is a new approach regarding wavelength interrogation-based SPR system in designing and analyzing molecules for biosensor and chemical sensing.

1.4 Objectives

The objectives of this studies are:

- Design a wavelength interrogation-based SPR system.
- To detect and determine the limit of the proposed SPR system.

- To study the change of resonance wavelength on the interface of gold-air and gold deionized water

1.5 Outline of Thesis

This dissertation is organized as follows.

- Chapter 2 discusses previous works the areas of SPR system, theory proposed before and the studies of the construction of SPR system.
- Chapter 3 discusses the method that will be used in constructing the proposed SPR system along with it's databases implementations and demonstrates the methodology for wavelength interrogation-based SPR system.
- Chapter 4 discusses the results and discussion from the wavelength interrogation SPR setup.
- Chapter 5 conclude this thesis from chapter 1 until chapter 4.

Chapter 2

Literature Review

2.1 Introduction

Each type of SPR system has a few key components, method, apparatus used, configuration, characteristics and applications. Method is one of the important aspect in SPR systems as it will identify the type of SPR system used. It has a strong influence on the classifying the SPR. The major importance of method is to make the SPR system the most effective in getting the results on analyzing the material in this studies.

In conventional research, which the majority of this literature review, the apparatus used will affect the method. For example, an angular interrogation-based SPR system would need a monochromatic lights while a wavelength interrogation-based SPR system use polychromatic as the light source. From there, we can differentiate the two SPR system but in terms of their configuration still use the Kretschmann configuration that include a prism coupler in the development of both SPR systems. Indeed, wavelength interrogation-based SPR system is one of the most difficult to construct because of minimal resources by researchers. The wavelength interrogation-based SPR system still remains the most useful to analyze biomolecules just like angular interrogation-based SPR. One way to confirm this by looking at the studies before this where the wavelength interrogation-based had contributed in many research such as in analyzing protein arrays, silicon and other biomolecule that need a high sensitivity sensor system.

The characteristics of SPR system plays essential roles for material analyzer development and evaluation. Therefore, the applications of the SPR system could be determine easily and meet the needs of the analyzer. Moreover, by simply applying the characteristics of SPR system some advancement could be made just by modifying or add some material that could enhance the sensitivity, detection limit and frequency of the sensor. For example, an experiment conducted by applying Sol-gel copolymer-templated mesoporous silica films with a thickness of 70nm and interpore spacing of 4.34 nm were fabricated on gold layer covered glass substrates for application as a wavelength-interrogated SPR sensor. A comparison between the experimental data obtained with the coated and uncoated SPR chips demonstrated that the mesoporous silica film effectively

enhanced the sensor response to individual adsorption of cysteamine molecules and lead(II) ions (Zhang et al., 2012) .

2.2 Light Source Technology

From the first observed events in 1902 to the first introduced concepts for gas sensing and biosensing in 1983, SPR sensor research has spanned more than eight decades back. There are many sensing technologies that contribute to substantial progress in SPR sensor evolution such as nanostructuring, optical technology, fluidic technology and light source technology. The trend of SPR sensor configurations, as well as its methodology and optical designs are strongly influence by the development of light source technology as a critical component (Prabowo et al., 2018). The fundamental principle of SPR sensor construction layed on the resonance of a strong electromagnetic field oscillation at the interface of nanometal film and a dielectric medium with p-polarized incident light resulting in a dark band profile in the light reflectivity at a specific wavelength and incident angle (Prabowo et al., 2018). This shown that the light source is a crucial component for an SPR system so it can reach the ultimate sensing performance, configuration, optical design and real-time data acquisition methodology.

Since the introduction of incandescent light and a halogen lamp, light technology has undergone a long evolution. The evolution had made technologies that include light source configuration such as SPR sensor system as well evolve in terms of its sensing principles, configurations, miniaturization and portability designs. In the surface plasmon generation, a particular wavelength requires a specific configuration to match the light propagation constant of the incident light as a precise angle of incidence (Prabowo et al., 2018) . This is because the priority of investigation and change of the entire sensor configuration is determined by the light source preference. For example, by using the polychromatic light source, the wavelength interrogation is necessary to obtain the SPR reflectivity (Prabowo et al., 2018) . It explains that the light source are playing essential roles in SPR system development where we use a polychromatic light for the wavelength interrogation and it is different light source for different SPR system such as the angular interrogation type that use monochromatic light.

On the other hand, the halogen lamp that been categorized as the incandescent lamp with a tungsten filament is the light source that used for SPR system with wavelength interrogation sensor because it can provide polychromatic wavelength source in the SPR sensing configuration. It shown that for the wavelength interrogation methods, the light source with polychromatic wavelength is suitable for it. Hastings et al. proposed a halogen lamp guided by optical fiber to couple the SPR using the Kretschmann configuration to enhance the long and short range SPR mode due to the broadband wavelength of the incident light. Then, Kazuma et al. explored the use of halogen light for localized surface plasmon resonance (LSPR) coupling by high refractive index prism. The halogen lamp is used as the excitation source of the SPR sensor.

Lastly, in the SPR sensor the spectral color from the light source holds an important role in determining the detection method. The wavelength interrogation method uses polychromatic light sources because it contains several wavelength values, many incident angles to obtain resonance condition. For example, the research to analysis protein arrays was performed by the line-scanning mode of a self-developed wavelength interrogation-based SPR sensor, configured by the Kretschmann geometry of the attenuated total reflection method (Jong et al., 2004) . A 20 W quartz tungsten halogen lamp was used as the light source due to its stable output (Oriel, Stratford, CT, USA). A polarizer was positioned at the input light path to obtain transverse magnetic polarized light. (Jong et al., 2004). The incidence angle was adjusted by a beam steering device to obtain the resonance wavelength and the incidence angle was set at 48° (Jong et al., 2004). Protein arrays were coupled with a fused silican prism *via* an index matching fluid and mounted on an x-y linear stage (Jong et al., 2004). Then, the protein arrays were automatically scanned by the line-scanning mode (Jong et al., 2004). From the research, it is crucial that we need to adjust the settings of the incident angle for it to give the right wavelength so the sensor can work properly in resonance condition.

2.3 Kretschmann Configuration

From the beginning of the discoveries and research of the SPR sensor system, the Kretschmann configuration is used to construct the SPR system. Even though there are already

some advancements been made nowadays regarding the SPR system, scientists and researchers still use the Kretschmann configuration in terms of its method, design, geometry and other that related to constructing the SPR system. In general, there are two common detection methods used for SPR sensors based on Kretschmann configuration, including wavelength interrogation and angle interrogation (Wang et al., 2017) . Both sensors have high values of detection accuracy and sensitivity. While Kretschmann configuration SPR systems can implement various chemical or biological species and events thus structure-based SPR systems are usually equipped with complicated optical system to obtain spectral information change.

In recent research, there are many ways were developed to achieve SPR effects, such as prism-coupled structures, modification of metallic nanoparticles and fabrication of nanostructures (Jana, Jayasmita & Ganguly, Mainak & Pal, Tarasankar., 2016). Among all those ways, the prism coupler structure is the most widely adopted in commercial SPR systems especially the Kretschmann structure which consists of a high refractive index prism. In 1968, the surface plasmon excitation using prism coupler was introduced by Kretschmann and Otto. The prism coupling based on Kretschmann configuration has become a standard technique to excite the SP in regards to its alignment simplicity leading to easily controlled parameter and variable (Prabowo et al., 2018). Thus, sensors with prism coupler with Kretschmann configuration can be easily integrated with any sort of interrogation including angular, wavelength, intensity, or phase modulation. From the previous research, the prism coupler will be attenuated total reflection for the lights that came from the light source. In the experiment of analysis of protein interaction on protein arrays by a wavelength interrogation-based surface plasmon resonance the collimated white light enters a polarizer and passes through prism that in contact with a protein chip at the fixed incidence angle. The reflected light is collected into an optical fiber and analyzed by a spectrometer.

Besides that, the Kretschmann configuration also include a thin gold film. A conventional SPR sensor composed of metal film and substrate, the metal film is basically noble metal such as gold or silver etc. The gold film is the best choice because of its strong oxidation and corrosion resistance in all kinds of environments (Zhao et al., 2019) but it possesses weak adsorption and binding ability to molecules and also got low detection sensitivity. However, the sensitivity can get enhanced just like the theory proposed before this where they cover the gold thin film with

topological insulator (Bi_2Te_3) on the gold film. It was demonstrated the presented sensor exhibits undergoes a great sensitivity enhancement on both angular and wavelength interrogation modes compared to conventional SPR sensor. In particular, the maximum sensitivity of wavelength-interrogation sensor can be further increased above 25% with 3 QL (Bi_2Te_3) sheet (Zhao et al., 2019). Besides, there were reports stated that gold shows larger shift in the resonance angle corresponding to the changes in refractive index of the sensing medium, which provides a high sensitivity than other metal. For example, Silver thin film show better performance than gold in sensitivity but the weak chemical stability of silver impedes its further development (Kooyman 2008).

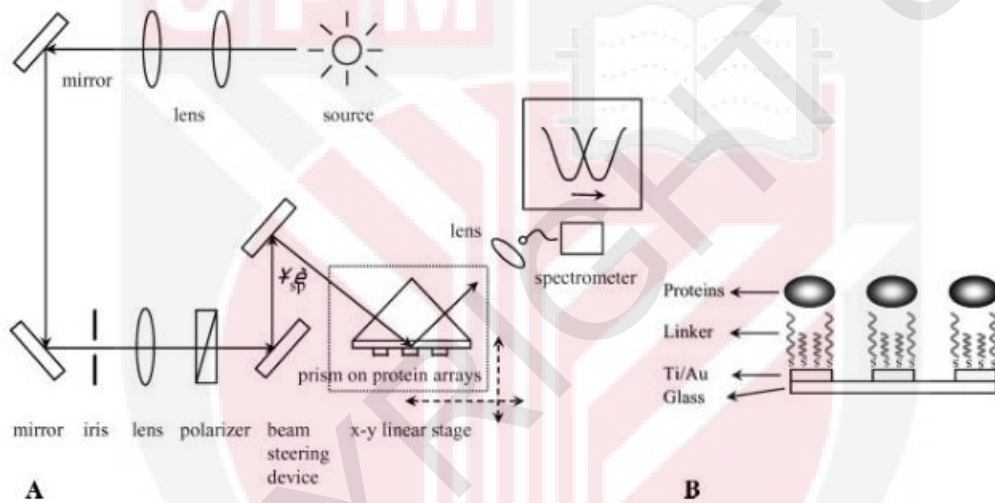


Figure 1 : (A) Schematic diagram of self-constructed wavelength interrogation-based SPR system base on Kretschmann configuration in the experiment of analysis of protein interaction by a wavelength interrogation-based SPR biosensor.

(B) Schematic diagram of how the material put on the gold think film to gain the resonance wavelength.

(Jong et al., 2004)

2.4 Surface Plasmon Resonance as Biosensor and Chemical Sensor

The surface plasmon resonance could be used in many applications for quantitative detection of chemical and biological species. These include food quality or safety, medical diagnostics, biological studies, biomolecule interactions, drug discovery and environmental monitoring. The SPR are simply called as biosensor or chemical sensor because of its real-time detection, smooth handling, high sensitivity, simple fabrication and stable structure. It is suitable to be used to analyze small matter or particles that can't be seen with naked eyes. Biosensor is a short for "biological sensor" that allow the quantitative development of some complex biochemical parameter and a chemical sensor is a device that measures and detects chemical qualities in analyte and converts the sensed chemical data into electronic data.

On the previous research, a gold-silver alloy film based SPR sensor with wavelength interrogation was developed to detect cancer antigen 125 (CA125) using a sandwich immunoassay. They used a new type of AuAg-SPR sensor to detect CA125 because of SPR properties that measures molecular interactions in real-time without labels so it is suitable for biomarker detection. The idea is they simulated the sensitivity of gold film based SPR (Au-SPR) sensor and AuAg-SPR sensor, and then investigated the AuAg-SPR's sensitive characteristics, including the angle and the RI sensitivity (Yi et al., 2020) . Because of gold films are preferentially used due to their high chemical stability, significant biocompatibility and affinity but not the best to achieve high sensitivity while silver has poor chemical stability that yields increased sensitivity and has a sharper angular resonance than gold the SPR system improve the detection sensitivity to some extent but they are often time-consuming, costly and complicate the overall metal film fabrication process(Yi et al., 2020) . The theoretical simulation results illustrated that the AuAg-SPR sensor had higher sensitivity than Au-SPR sensor. The experiment results in the detection of the CA125 at a concentration of 0.1 U/mL (0.8 ng/mL) by using the AuAg-SPR.

The other experiment related with medical fields is the SPR act as biosensors in cancer detection. The focus is placed on applications of SPR biosensors in testing the effectiveness of cancer markers and in the discovery of new cancer markers. Generally, biosensors are used with one of three SPR techniques including fluidic version, nonfluidic imaging and localized. In this case, the SPR biosensors used for cancer detection is the fluidic version (Falkowski et al., 2021). The method that used is just simply directing the analyte to the biosensor and then the entrapment

of the analyte by the antibody that covered by the linker on the 50 nm gold film causes changes in the SPR signal and creates an analytical signal. On the other hand, the discovery of new cancer markers is still a shortage of biosensors offering near 100% specificity. In the recent research, eight new markers for different types of cancer have been proposed in combination with related analytical methods for marker determination. In most of these reports the specificity are not ideal. The SPR that used as biosensors in clinical and diagnostic applications are ready to use but such factors like availability of suitable instruments, the possibility of creating a portable device, the cost of a single measurement, conditions of biosensor storage, etc. should also be taken into consideration (Falkowski et al., 2021).

Furthermore, another experiment was carried out for analysis of microarrays by using wavelength-interrogation SPR imaging. An approach to microarray analysis in a label-free and real time manner by using the SPR method. There are three main configurations used in SPR sensors which is amplitude measurement, phase modulation and spectroscopy-based measurement (Liu et al., 2012). The link between the change in reflection light intensity and the refractive index of bound analyte is used to calculate amplitude. Amplitude measurements are susceptible to stray light and spatial inhomogeneity in the light source because they are reliant on direct intensity fluctuations. When SPR occur, phase modulation-based SPR sensors make use of the phase shift. In this experiment, they introduced a new technique called polarization interferometry (PI) method to overcome some problems related to this SPR system such as errors from the optimum thickness occur in the prepared metal films by magnetron sputtering and the immobilized biomolecular layer on the metal film that change the optimum thickness. Finally, they confirmed the SPR results by fluorescence imaging that identifies bacteria in this microarray experiment.

As the conclusion, the SPR system is well-known in biosensor and chemical sensing field as a device for analysis small matter. We can see that the experiment are very full of enhancement and improvement since the SPR had get many attention in the recent years because of its presented characterization. Even though there is an experiment was carried out by an angular interrogation SPR system it is still comparable with wavelength interrogation mode eventhough it is an understudied method that have lack of information and research on it.

Chapter 3

Methodology

3.1 Introduction

Principle of the experiment is based on the optical properties itself. The theories are related to the surface plasmons excitation in designing and analyzing wavelength interrogation-based SPR system. The most important theories including the total internal reflection (TIR), surface plasmons, evanescent wave, light wave and SPR dependencies.

The wavelength interrogation-based SPR system is flexible when it comes to optimization because the operation wavelength range can be easily tuned to achieve the best surface plasmon excitation, have a wide dynamic range and its detection sensitivity could be as high as that offered by an angular interrogation-based SPR. In the wavelength interrogation mode, the angle of incidence is fixed while the SPR spectral profile is continuously obtained to monitor the SPR dark band. The SPR spectral profile can be obtained in wavelength interrogation mode by scanning the incident wavelength or evaluating the reflected beam with a spectrometer.

In general, SPR is a physical process that can occur when plane-polarized light hits a thin metal film under total internal reflection conditions then, then, the electromagnetic wave propagates. The electro-magnetic wave propagating along the surface of a thin metal layer is what called surface plasmon. When the light beam makes contacts with the prism while travelling from a denser medium to a less dense one, the light is bent towards the plane of interface. The incidence angle in the prism can be adjusted until it reaches the critical angle. All incoming light is reflected within the circular prism at this point makes the light in TIR condition. Although there is no light coming out of the prism when in TIR condition, the electrical field of the photons will extend about a quarter of a wavelength beyond the reflection surface. By constructing the SPR system based on Kretschmann configuration, where p-polarised, collimated light beam undergoes TIR at a thin metal film interface the optical excitation of the surface plasmon can be achieved.

In most SPR types, gold is one of the common thin film of a noble metal on the reflection site because it gives a SPR signal at convenient combinations of reflectance angle and wavelength. Furthermore, gold is chemically inert to solutions and solutes typically used in biochemical

contexts. The electrical field energy of the photon can interact with electron constellation in the gold surface when it is at the right position. The energy from the incident light photons is transmitted to the electrons, which then turn into surface plasmons. Based on quantum theory, a plasmon is the particle name of the electron density wave. It is the photon and electron behavior that can only be described when they have both wave and particle properties. Simply put, when in TIR situation the quantum energy of the photon is accurate, the photons convert to plasmons leaving a gap in the reflected light intensity.

When in TIR, the opposite side of the interface produce an electric field when the photons are reflected. On both sides of the film, the plasmons for a similar field that extends into the medium. This field is called the evanescent wave because the amplitude of the wave decreases exponentially with increasing distance from the interface surface, decaying over about one light wavelength. The wavelength of the evanescent field wave is usually just the same as that of the incident light and the energy is dissipated by heat. This phrase refers to the refractive index of light because photons have different speeds in different material so light seem to be refracted. In the same way, the velocity as well as the momentum of the plasmons is changed when the composition of the medium changes. Because of the change in momentum, the angle of incident light at which the resonance occurs changes.

The coupling of the light wave to the surface plasmons can be monitored in several ways. The most common method used in angular interrogation SPR is by fixing the angle of incident light and then the wavelength can be varied until resonance occurs. For the wavelength interrogation, the phase shift of the light is detected when the coupling between light and plasmons occur. This method uses a reference beam and has a high sensitivity but requires a more complex setup than other methods.

The characteristics of the metal film, the wavelength of the incident light and the refractive index of the medium on either side of the metal film are all influencing the SPR angle. Because of the refractive index is sensitive to temperature (H. Nilshans et. Al., 1998), it is important to perform the measurements at defined temperatures. In some cases, this dependency can be exploited. The refractive index is not dependent on the density of the media. The metal requires conduction band electrons capable of resonating with the incoming light at a suitable wavelength. Metals that satisfy this condition are silver, gold, copper, aluminum, sodium and

indium. In addition, the metal on the sensor surface must be free of oxides sulfides and it must not react with other molecules on exposure to the atmosphere or liquid. Since indium is too expensive, sodium is too reactive, copper and aluminum too broad in their SPR response and silver too susceptible to oxidation, gold is considered as the most practical metal. Gold is very resistant to oxidation and other atmospheric contaminants and compatible with many chemical modification systems. The thickness of the metal layer is important. Above an optimum thickness the dark band profile in reflective light becomes shallow while below the optimum thickness the dark band becomes broader. From the previous research, the optimal thickness of the gold layer is ± 50 nm (Rakesh S., 2018). The light source should be polychromatic and p-polarized to obtain the suitable effects in this wavelength interrogation. Since the metal film, the incident light and temperature are constant, the SPR signal is directly dependent on the change of the refractive index of the medium on the sensor side of the SPR surface.

3.2 Experiment design and setup

Schematic design of the wavelength interrogation-based SPR sensor

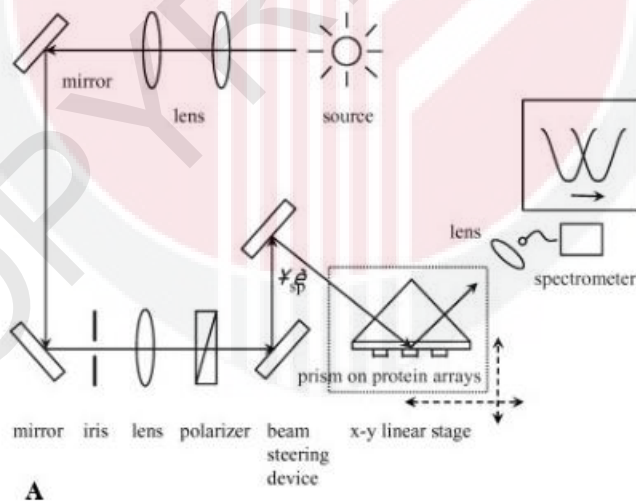


Figure 2 Wavelength interrogation-based SPR system setup

(Jong et al., 2004)

Prism coupler

When zoomed in the prism coupler on the setup direct the light at certain angle to reach TIR.

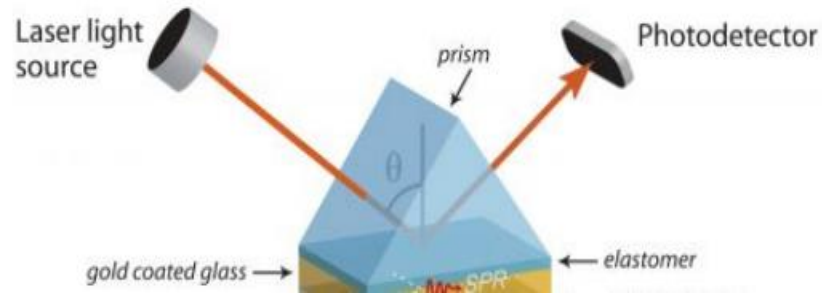


Figure 3 Prism coupler and light beam

(Bionavis, 2014)

3.3 Procedure

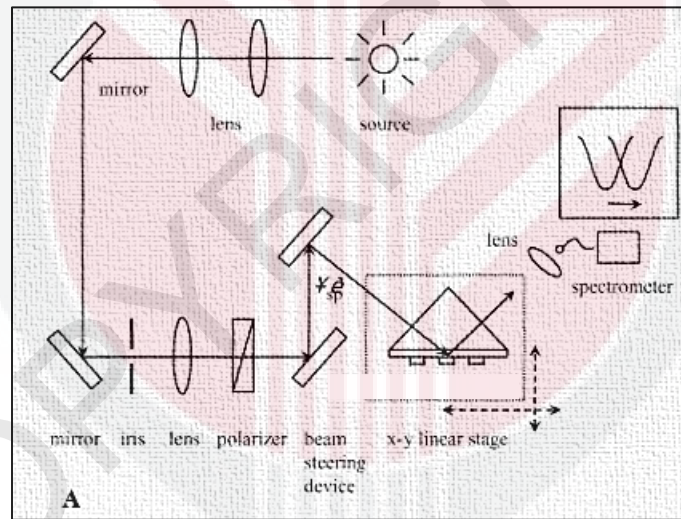


Figure 4

1. The wavelength interrogation-based SPR system is constructed based on the schematic diagram in Figure 4.
2. The lenses are placed right before it enters an instrument to make sure it does not spread.
3. Place the polarizer at the input light.
4. Adjust the light pointing so it will go through the polarizer.

5. Steer the polarized light from the polarizer so it will enter the prism coupler
6. Place the gold thin film under the prism coupler
7. Under the coated glass of the prism coupler with gold insert a sensor chip.
8. Adjust the incidence angle to obtain the resonance wavelength at the right incidence angle.
9. The reflected light will go through the lens then enter the spectrometer to be examined.

3.4 Method for data interpretation

In this work, the SPR sensor using the attenuated total reflection geometry is used in Kretschmann configuration. In this configuration a spectrometer is also included because most of the analysis will occur there by showing the behavior of the light on the computer screen which is connected to a PC that has software to interpret the data. The spectrometer is an instrument used to probe a property of light as a function of its portion of the electromagnetic spectrum, typically its wavelength, frequency or energy. In most cases, the property being measured is light intensity, however other variables such as polarization can also be assessed. A spectrometer can operate over any range of light theory, but most do so in a specific portion of the electromagnetic spectrum. For accurate wavelength selection and the generation of spectra, a dispersive element that separates light into its constituent wavelengths is required. This dispersive element is a prism coupler where constructive and destructive interference is used to spatially separate polychromatic light that is incident on the prism.

A typical system includes a prism coupler coated with a thin metal film isolating the sensing medium from the prism. The analysis on the prism coupler started when the metal layer is directly on top of the TIR surface enabling a more efficient plasmon generation. When the light travels from the higher refractive index medium to the lower refractive index medium, the TIR can take place within the medium as long as the incident angle is greater than the critical angle calculated from the expression $\sin(\theta_c) = n_2/n_1$. Evanescent waves are formed in the lower refractive index medium under the distance to the interface of the mediums. When the prism is coated with gold film with suitable thickness is placed at the interface, the evanescent wave is

enhanced, penetrating the gold film and existing in the medium with lower refractive index. The magnitude of the parallel wave vector of the evanescent wave

$$k_{evan,||} = \frac{2\pi}{\lambda} n_1 \sin(\theta)$$

where λ is the wavelength of the incident light, n_1 is the refractive index medium, and θ is the incident angle.

Besides that, the setup include polarizers to polarized the polychromatic light in this system. The polarizers are the devices that allow one polarization state to be transmitted while blocking all others. Polarized light can have linear, circular or elliptical polarization. To select a specific polarization of light, polarizers are used. The light is called unpolarized if the direction of this electric field fluctuates randomly in time. Unpolarized light can be considered a rapidly varying random combination of p- and s-polarized light. An ideal linear polarizer will only transmit one of the two linear polarizations, reducing the initial unpolarized intensity I_0 by half.

$$I = \frac{I_0}{2}$$

While for linearly polarized light with intensity I_0 the intensity transmitted through an ideal polarizer, I , can be described by Malus' law,

$$I = I_0 \cos^2 \theta$$

For the wavelength interrogation-based SPR system, the p-polarized light is transmitted from the polarizer because it has an electric field that is parallel to the plane of incidence.

Furthermore, the analysis of biomolecules will need the gold film coated on a glass substrate. It will immobilize one of the binding partners onto the surface of the sensor. The light source will illuminate the gold film, and the detector measures the unique optical spectrum produced by the SPR phenomenon. When the TIR condition occurs, there will be a plasmonic wave generated on the gold surface, with the electrical field of this wave extending hundred of nanometers into the space above the surface of the sensor. The reflected light from prism coupler will have a dark band intensity at a certain angle due to the plasmonic wave. When the molecule binds to the surface of the sensor chip, the refractive index of the space in which the plasmonic wave is propagating through changes and the reflectance angle will change. The amount it change

depends on how heavy the material bound onto the surface and the shift can be measured in real time.



Chapter 4

Results and Discussion

4.1 Introduction

In this section, the SPR system is designed with the mechanical and optical units. and presented. The applicability of this SPR system and the light behavior showing parameter that could be observed will be further analyzed.

4.2 Surface Plasmon Resonance Setup

In the figure 5 shows the schematic diagram of the Surface Plasmon Resonance (SPR) system based on wavelength interrogation based design. As introduced on the previous chapter, a polarizer is placed to allows light waves of one polarization pass through while blocking light waves of other light source in the room such as the sun light from outside. In this system, the light source that used is a laser with a power of 100 W. The high power laser will provide a high intensity light at high speed optical scanning which in turn will show more resonance measurements. Although laser is monochromatic light, it still can show the right behavior of a wavelength interrogation based SPR system while we can not use halogen lamp as the light source due to the lack of instrument in the lab and Malaysia movement control order still ongoing so any posted items from outside the country border will took a long time. The broadband light can be directed from the light source since the laser direct its light in a straight line then enter the polarizer. In this system, the SPR is configured in a Kretschmann configuration with an attenuated total internal reflection method where a gold film is placed on the surface of the prism coupler using a droplet of refractive index matching liquid to avoid entrapment of air-bubbles. The gold film act as the sensing layer where arrays of genetic elements are shown if the system used as a sensor. The prism is fixed on a stage that enables a one-dimensional parallel scan. At the reflected light beam part, the light beam is directed to the slit of a spectrometer.

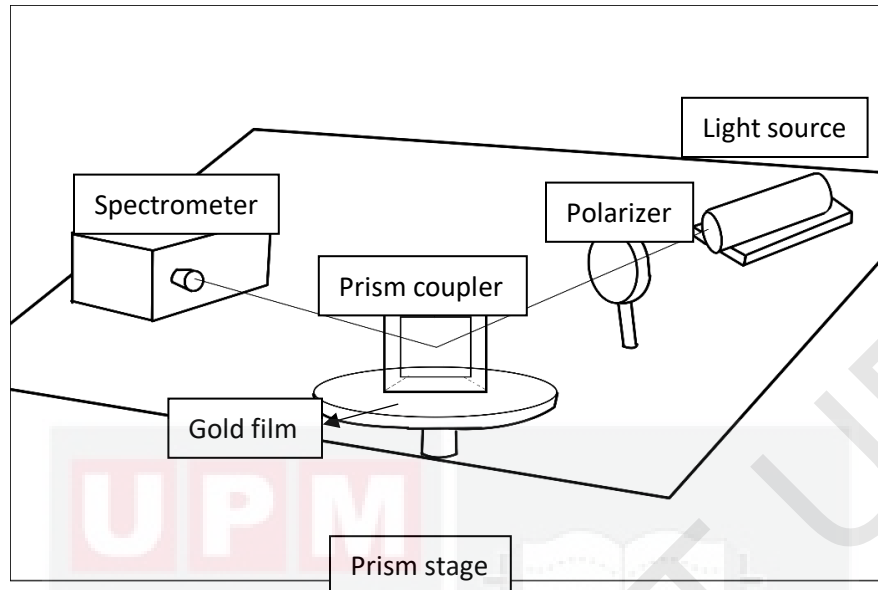


Fig. 5 Schematic representation of surface plasmon resonance system based wavelength-interrogation SPR.

The instruments are mounted on an optical breadboard built from steel. The top surface has a rigid of equally spaced threaded holes for mounting equipment and optical components. Based on the schematic diagram above there are lack of equipment and instrument in the lab that made the project could not finish perfectly which is a few lenses and a polarizer.

In the previous chapter, the light source for a wavelength-interrogation based SPR system should be a halogen lamp with white light that need to be focus by small lenses so the light could get direct easily into the spectrometer. The need of a second polarizer that placed after the light beam reflected out from the prism coupler is based on polarization interferometry (PI) techniques, which the technique will let the SPR performance improve and reduce restrictions on allowable parameters of SPR-supporting metal films. The system still can be finish with available equipment as the crucial part which is prism coupler and the light source are present that allows the system show the behavior of an SPR system. By using the halogen lamp, the directed light usually spread widely so the a few lenses are needed to focus the light so it moves in one straight line. Because of a laser is used as the light source for this SPR system the light already moving in a straight line which already focus and easily can be seen where it moves even thought it gets reflected and refracted by the prism coupler. By using a monochromatic light in this setup it is an issue in this wavelength interrogation based SPR system since the reflectivity profile plot is more critical.

4.2.1 Total Internal Reflection in Prism Coupler

In prism coupler, total internal reflection refers to the complete reflection of light from the light source where the light was directed to and then goes back out of the prism coupler after being refracted and reflected. When the angle of incidence exceeds a particular limiting angle, known as the critical angle, the phenomenon happens. Changing the incidence angle (θ) will also changes the out-coming light until it reaches a critical angle. At that point, all the incoming light reflects within the triangular prism. In Figure 6, shows how the critical angle could be determined just by looking at the light behavior after being directed through the prism coupler. On the left side of the setup the light usually only refracts within the triangular prism coupler.

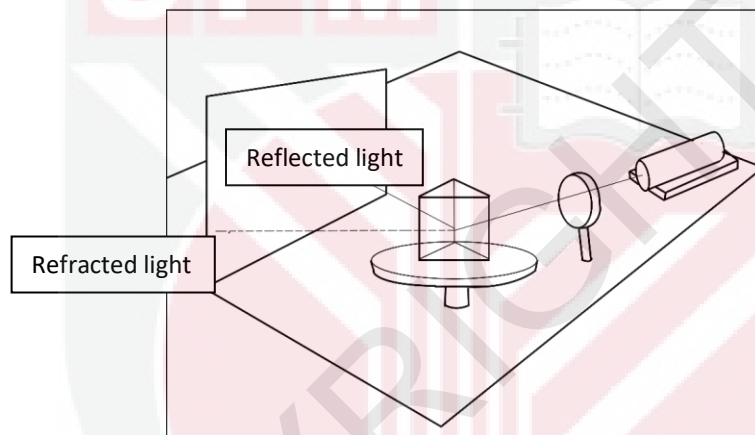


Fig 6. Schematic diagram on how to analyze on the directed light on a display whether it is refracted or already reach the critical angle and reflected

The prism coupler needs to be adjusted until the refracted light on the display disappear. When the refracted light had disappeared on the display there would be a new directed light on the other side of the prism coupler so it is already reflected because it is on the critical angle of the prism coupler.

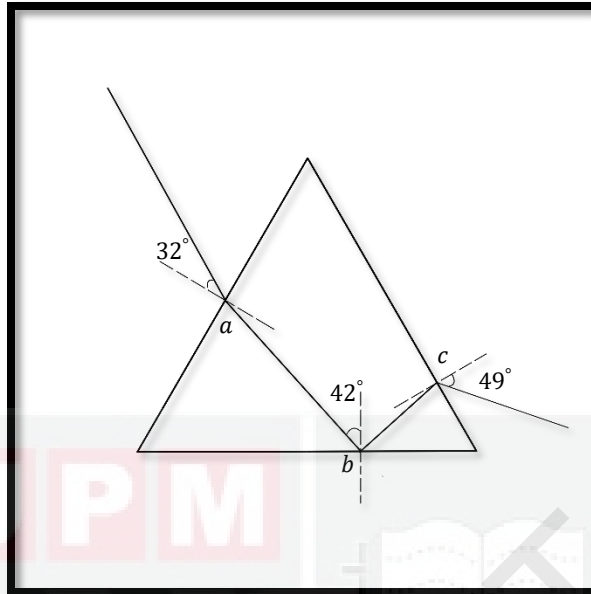


Fig 7 Optical design of the light beam inside the prism coupler displays total internal reflection

Based on figure 2.1, there are three crucial point in the prism coupler where the light beam behavior changed due to different medium and condition where air refractive index is 1.00 while 1.50 for the prism coupler. At first at point *a*, the polarized light enters the prism coupler at the 32° angle and being refracted inside the glass due to different refractive index. At point *b*, the light supposed to be refracted out of the glass but after a few adjustments where the directed light to the point *b* reach 42° the refracted light disappear and it reach 90° with the normal line and there is a new light appear directed to the point *c*. This can be calculated based on Snell's law.

Snell's law state that :

$$n_1 \sin \theta_1 = n_2 \sin \theta_2$$

$$\text{where } n_1 > n_2$$

$$\sin \theta_1 = \frac{n_2 \sin \theta_2}{n_1}$$

$$\theta_1 = \sin^{-1} \left(\frac{n_2 \sin \theta_2}{n_1} \right)$$

$$\theta_1 = \sin^{-1} \left(\frac{1.0 \sin 90^\circ}{1.5} \right)$$

$$\theta_1 = 41.8^\circ$$

At point c, the light appears and refracted at 49° angle from its normal line. The total internal reflection in this prism coupler seems to appear at the edge of the prism coupler. Even with naked eyes, the light also could be seen to appear at that point and can be measured by using an angle measurement tool that mount on the prism coupler stage.

4.2.2 Resonance effect while prism coupler coated with gold

Now the prism is coated with a thin gold film on the reflection site of the prism. In this case, gold is used just like mentioned on the previous chapter because it gives an SPR signal at convenient combinations of reflectance angle and wavelength. If the SPR system is made to function as a sensor to chemical substance gold is chemically inert to solutions and solutes typically used in biochemical contexts. Total internal reflection of the beam at the interface between the glass substrate and the gold layer of the SPR chip creates an evanescent field that penetrates the gold layer to excite surface plasmon polariton at the gold layer interface at a wavelength that satisfies the phase-matching condition when the gold coats the prism coupler.

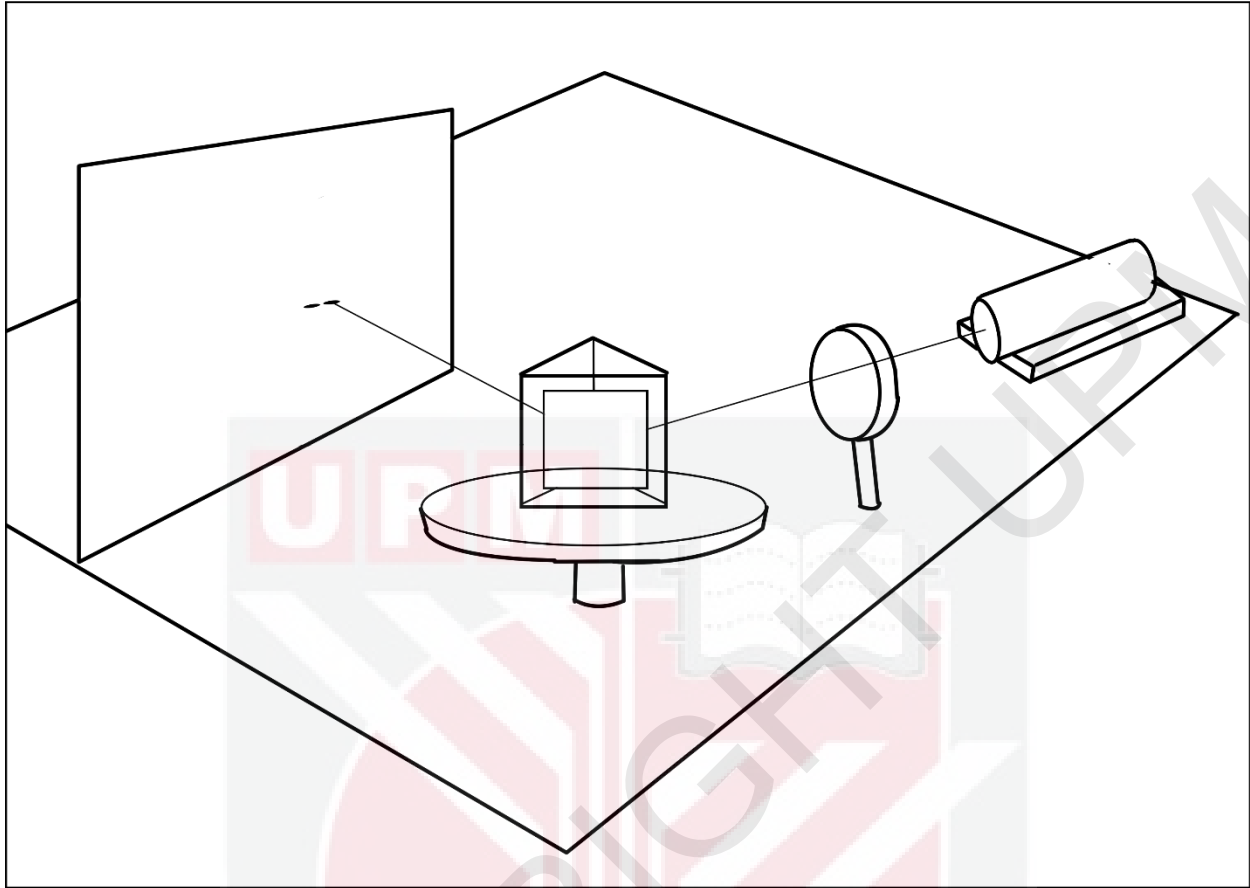


Fig 8 Coated prism coupler with gold thin film

Before the light is in total internal reflection, there are two beams beyond the prism coupler which is the reflected light and the transmitted light that disappear at 42° angle. When the gold is coating the prism coupler the plasma and resonance effect take place which essentially just means the light is going to disappear but not going to be reflected or transmitted either. It is going to go completely into the oscillation of the electrons in the gold foil. After a few adjustments of the prism coupler back and forth so at that particular angle is constant for that particular piece of metal so that means that gold is at its suitable thickness. There is one directed light on the cardboard from the prism coupler and the light appear to have a dark band in the middle of it.

It's crucial to validate the characteristic of light propagation in the p-polarization state before observing the SPR event. The light reflectance behavior of a bare gold thin film and a prism employing a p-polarized laser shows great agreement with the theoretical conclusions. For the laser and prism coupler, the critical angles are 42° . I found that the location of the SPR deep is

beyond the critical angles and it shows resonance effect with just a bit rotation from the critical angle.

4.3 Response of the SPR to alcohol

To test the applicability of this SPR system for the detection of metal ion, I prepared a piece of gold film and a few drops of alcohol to examine the change that would occur on the directed light properties. Figure 4 shows the setup on where and how to put the substance which is alcohol at the prism coupler coated with gold thin film. In the figure also shows how the light would change in terms of its position, intensity and movement that pointed on the cardboard.

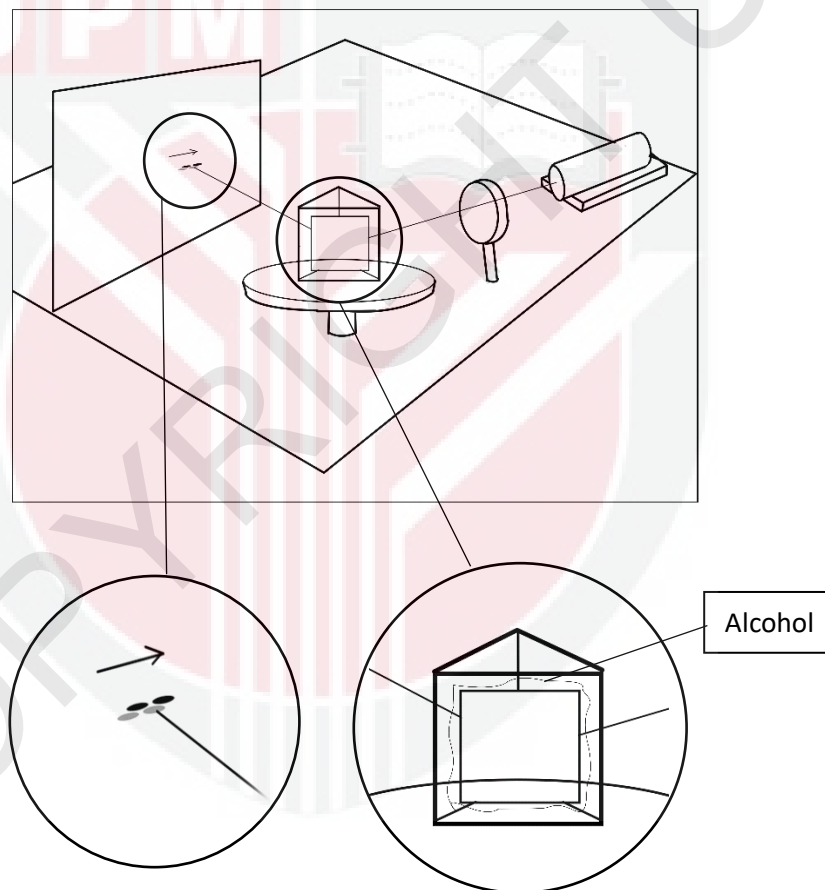


Fig 9 The depth from the resonance effect shift a bit to the right along with the intensity of the light while a few drops of alcohol dropped on the gold thin film

4.4 Surface Plasmon Resonance Light Behaviour

Deionized water with specific concentrations was injected before the SPR signal was recorded. The reflectance curve of the gold thin film in contact with deionized water is following the theoretical data from previous study. The obtained reflectance curve was fitted as shown in figure 5.

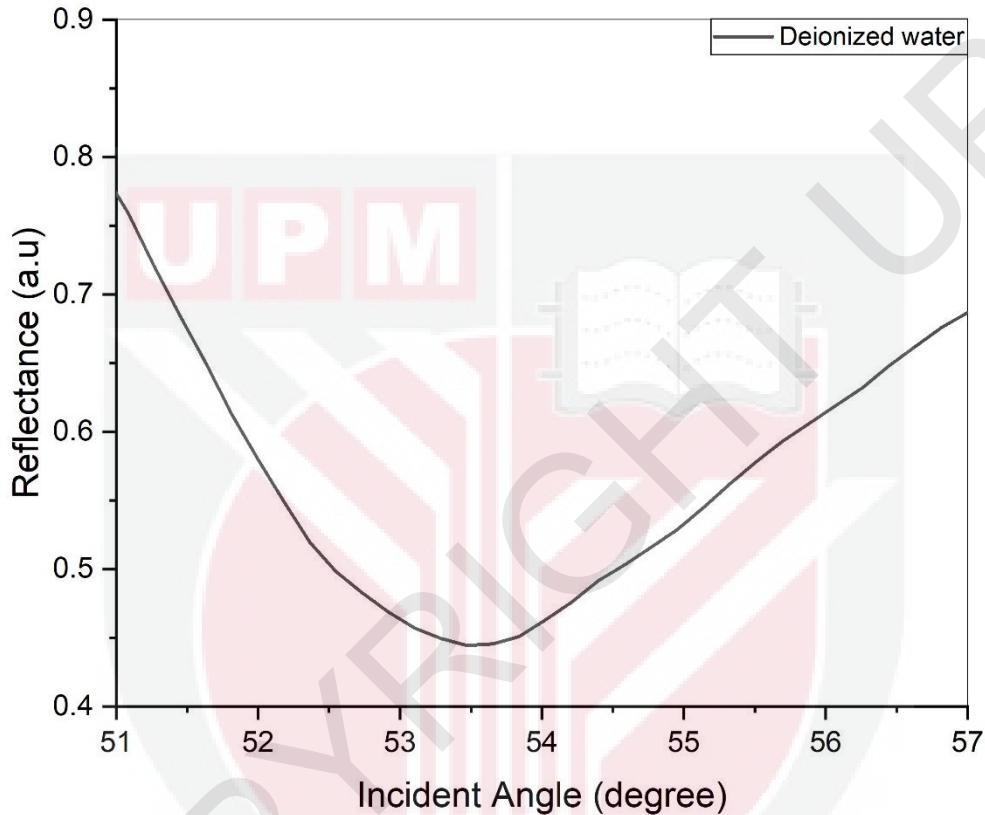


Fig 10 SPR curve signal of the gold thin film in contact with deionized water.

According to the graph in figure 10, the SPR curve can be formed because of the different intensity of the directed light and a depth in the middle of the graph showing that the intensity decrease at that angle. Just like shown on the previous part, where the effect with alcohol the intensity along with its incident angle shifts and move right or left depend on the type of the solution and the thickness of the metal dielectric. For an SPR system, it works by showing the characteristics of the final point of the reflected light such as the intensity, depth from the light intensity, position of the depth from x and y direction and the light beam positioning.

Because of the SPR curve, incident angle and light position varies with different condition. We can compare the curved and depth in two situation. Figure 11 shows both curved

of gold-air and gold-deionized water.

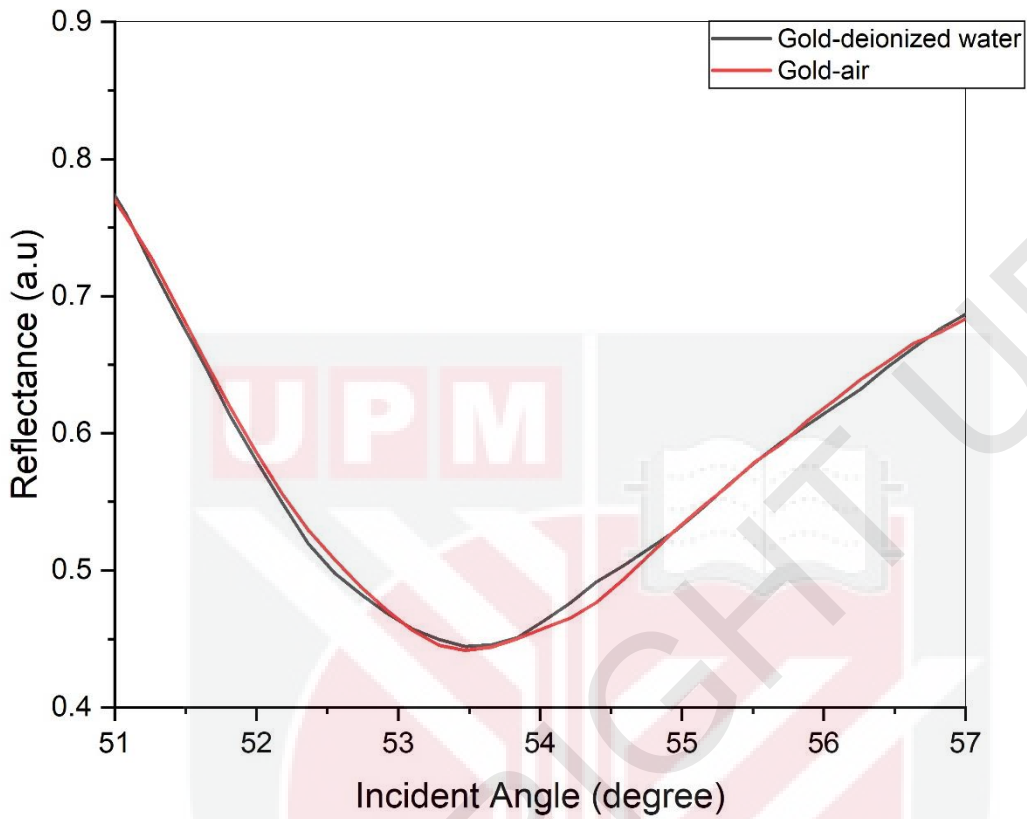


Fig 11 SPR curve signal of the gold-air and gold deionized water.

From the curve, there can be seen that the position is slightly different. The curved can also be seen different from the way it shifts its intensity and the lowest intensity they reach. Because of the instruments in the lab had been use for a long time or already old the graph show very little difference and its not very accurate when this two substance added on the system.

Chapter 5

Conclusion

5.1 Outcome of the project

In this work, a wavelength interrogation based SPR system was experimentally and theoretically demonstrated. This SPR system was established with a prism coupler on a rotating stage with angle measurement tools at the top made the platform has flexible angle adjustment and ease the operation. The gold thin film was coating the prism coupler. A series of experiments were carried out using lab instruments and some home made apparatus and the following conclusions were drawn based on experimental and simulation results. The design of a wavelength interrogation-based SPR system that is angle-dependent allows the system's dynamic range to be widened by adjusting the angle using the rotating stage of the prism coupler. Even though the design is incomplete because of the lack of instrument and equipment needed to execute a wavelength-interrogation SPR system, the behavior of the system still shows the right practice that an SPR system will show. For example, we cannot use a halogen lamp as the light source since it is important for it to have a few lenses and second polarizer otherwise the light from the light source could not focus in one straight line and will not demonstrate SPR spectrum such as the depth of the light and the resonance effect that we got from the system. Besides that, the resonance effect of the SPR spectrum can be seen clearly that made that SPR is functioning properly, so it gives some results that explained how the SPR system works along with the sensor system in the SPR system. In this study, the change of resonance wavelength on the interface of gold-air and gold deionized water can be seen from the graph. The graph show that the reflectance and incident angle of the depth is at the different position and is not the same due to different medium and refractive index of both conditions. Then, the system response to alcohol that act as metal ion in this experiment mainly results in the change of reflectance and incident angle on the display. Due to time constraints, we can not observe the light spectrum using spectrometer to see more clear of the light behavior. To the best of our knowledge, this work demonstrates the application of wavelength-interrogation based SPR system for detection of metal ion and the change of resonance wavelength on different medium.

5.2 Project related problem

From the experiment, there are some problem that need to be solved and paid attention to. Firstly, there are a few parameters that need to measure so that any behavior from the system could be explained easily. Because of there are some parameters such as wavelength, frequency and light intensity that we did not measure, we cannot calculate by using the formula derived from previous studies and get a few important data. Then, the lack of instrument made the wavelength-interrogation SPR system could not be execute but still show the right behavior. In order to use halogen lamp as the light source, the second polarizer on the reflectance arm of the system. Besides that, some missing lenses is needed to focus the light if we are going to use the halogen lamp. This is due the light from the halogen is spreading instead of in one straight line like laser.

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