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Chemical Sensing Using Transmission Surface Plasmon Resonance Spectroscopy (T-SPR)

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คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

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โดย

นางสาวประภัสสร สืบสาย

รายงานชิ้นนี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตร
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Chemical Sensing Using Transmission Surface Plasmon Resonance Spectroscopy (T-SPR)

Miss Prapussorn Suebsay

In partially fulfillment for the degree of Bachelor of Science
Department of Chemistry, Faculty of Science
Chulalongkorn University
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Project title	Chemical Sensing Using Transmission Surface Plasmon Resonance Spectroscopy (T-SPR)
Student Name	Miss Prapussorn Suebsay
Field of study	Chemistry
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Accepted by Department of Chemistry, Faculty of Science, Chulalongkorn University in Partial fulfillment of the requirement for the Degree of Bachelor of Science

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ชื่อโครงการ การตรวจวัดสารเคมีเป้าหมายด้วยเทคนิคการสั่นของพลาสมอนบนพื้นผิวแบบส่องผ่าน
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บทคัดย่อ

เทคนิคการสั่นของพลาสมอนเป็นเทคนิคที่ได้รับความนิยมอย่างมากในการใช้เพื่อตรวจวัดสารเคมีเป้าหมายที่มีปริมาณน้อย ๆ โดยใช้หลักการสะท้อนกลับของแสงบนพื้นผิวที่มุมวิกฤต ซึ่งต้องอาศัยเครื่องมือสำเร็จรูป และมีความซับซ้อนในการติดตั้งอุปกรณ์เชิงแสงจำนวนมาก เพื่อให้สามารถตรวจวัดสัญญาณได้อย่างถูกต้องและแม่นยำ งานวิจัยนี้จึงมุ่งเน้นไปที่การพัฒนาเครื่องตรวจวัดสารเคมีเป้าหมายด้วยเทคนิคการสั่นของพลาสมอนบนพื้นผิวแบบส่องผ่าน ซึ่งสามารถตรวจวัดได้โดยใช้เครื่องยูวี วิสิเบิลสเปคโตรมิเตอร์แบบทั่วไป การติดตั้งเครื่องมือไม่ซับซ้อน และสัญญาณที่ได้มีคุณภาพดีไม่แตกต่างจากการวัดแบบสะท้อนกลับ ในงานวิจัยนี้แผ่นเกรตติงจากซีดี ดีวีดี และบีดีอาร์ ที่เคลือบด้วยทองคำหนา 40 นาโนเมตร ถูกนำมาใช้เป็นอุปกรณ์เสริมในการตรวจวัดสัญญาณ ลักษณะพื้นผิวและค่าพารามิเตอร์ของเกรตติงถูกตรวจสอบและแปรผลด้วยกล้องจุลทรรศน์แรงอะตอม เมื่อทำการทดลองด้วยเทคนิคการสั่นของพลาสมอนบนพื้นผิวแบบส่องผ่านในช่วงวิสิเบิลที่ 450-750 นาโนเมตร พบว่าสเปกตรัมการสั่นของพลาสมอนที่ได้จากแผ่นเกรตติงบีดีอาร์มีสัญญาณที่ชัดและมีแนวโน้มในการเปลี่ยนความยาวคล่นแสงที่เป็นเส้นตรงตามองศาจากแกนหมุน โดยมีค่า R^2 อยู่ที่ 0.9989 และ 0.9978 เมื่อทดลองโดยใช้อากาศและน้ำเป็นตัวกลางตามลำดับ ด้วยเหตุนี้เฉพาะแผ่นเกรตติงจากบีดีอาร์จึงถูกนำมาใช้ศึกษาผลการเปลี่ยนแปลงการสั่นของ พลาสมอนจากค่าดัชนีหักเหของตัวกลาง ในการศึกษานี้ใช้เอทิลีนไกลคอลที่ความเข้มข้นร้อยละ 10-50 โดยมวลเป็นตัวทดสอบ จากการทดลองพบว่าเครื่องตรวจวัดและอุปกรณ์เสริมนี้สามารถตรวจวัดความเข้มที่เพิ่มขึ้นของสเปกตรัมจากการสั่นของพลาสมอนเมื่อค่าดัชนีหักเหเพิ่มขึ้นได้จริงเมื่อทำการตรวจวัดที่องศาการหมุนเดียวกัน โดยได้ความสัมพันธ์เป็นเส้นตรงที่มี R^2 = 0.8962

คำสำคัญ : เทคนิคการสั่นของพลาสมอนบนพื้นผิวแบบส่องผ่าน, เกรตติง, ค่าดัชนีหักเห

Project Title Chemical Sensing Using Transmission Surface Plasmon Resonance Spectroscopy (T-SPR)

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Advisor Name Associate Professor Kanet Wongravee, Ph.D.

Department of Chemistry, Faculty of Science, Chulalongkorn University, Academic Year 2019

Abstract

Surface plasmon resonance (SPR) technique has become widely used in chemical sensing. Generally, the surface plasmon resonance is measured by the incident light reflected from the surface of the metal at the critical angle, this involves “reflection detection mode”. To determine SPR signal, the instrument with high complexity of several optical accessories is required in order to obtain the accurate information. In this study, the development of transmission surface plasmon resonance spectroscopy (T-SPR) is proposed as the instrument installation is uncomplicated and it provides good quality of plasmon signals. The gratings made from optical storage discs such as CD-R, DVD and BD-R coated with 40nm gold were used as important substrates to acquire T-SPR signal. Their surface morphologies and grating parameters were investigated by Atomic Force Microscopy (AFM). The T-SPR signals of the prepared grating were acquired at wavelength of 450-750 nm. It was found that the BD-R grating provided the distinguished SPR spectra with the correlation of incident angle and λ_{max} of T-SPR peak with R^2 of 0.9989 and 0.9978 using air and water medium respectively. Therefore, the BD-R grating was used in the further experiments to monitor the effect of reflective index on T-SPR spectra. Mixture of ethylene glycol solutions with 10-50 % w/w were used as a validation condition. The detected T-SPR peak is sensitive to the reflective index of the medium with R^2 = 0.8962

Keywords: transmission surface plasmon resonance, grating, reflective index

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Prapussorn Suebsay

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MPBA	4-Mercaptophenylboronic acid
SPR	surface plasmon resonance
T-SPR	transmission surface plasmon resonance

Chapter 1

INTRODUCTION

1.1. Introduction

Nowadays, surface plasmon resonance (SPR) on the surface of metals is widely used as the technique for chemical sensing for the target chemicals [1], in trace amount, by investigating the movement of the electron on the surface of the metal and the changing of the refractive index when the light passes through the chemical and react with the surface metal [2]. This technique can be used as a sensor to determine and quantify the target molecules through labeling detection [3]. However, the chemical sensing by using SPR had to monitor the reflective light from the metal surface at the critical angle (reflection mode). Commercial SPR devices are extremely expensive and cannot be easily installed in the laboratory as several optical accessories are required to align and install in order to obtain the best performance of the instrument and obtain the precise data. Moreover, reflection-type surface plasmon resonance could not be easily developed or adapted into optical sensing with simplicity. Therefore, the conventional method is preserved only for hi-end research laboratories. This study was aimed to develop a surface plasmon resonance technique using transmission mode (T-SPR) in which the incident light can transmit through the metal surface. In this case, the incident light which passed through the grating substrate was collected and analyzed as shown in figure 1.1. This technique can be applied and used UV-visible spectroscopy which is a simple instrument available in many laboratories leading to the cost-saving and complicated optical accessories are not required in order to maintain the best performance of the detection. All optical accessories using in this technique, for example, the wire grid polarizer, collimator, iris diaphragm, etc. were generally easy to buy or make. Moreover, the experimental setup of transmission mode was easier than the reflection mode as the light source, sample holder and detector were aligned in the straight line without precise angular alignment and it provided strong spectroscopic signals [4]. Furthermore, the chemical sensing from the transmission mode was able to be measured a broader range of the target chemical including macromolecule or cell [5]. In addition, this technique was able to use with other accessories like the grating to enhance the signal. As the SPR phenomenon can occur when the polarized light hits the metal film on the surface of grating or prism, the gratings using in this experiment were made from the optical storage discs like CD-R discs, DVD discs and BD-R discs, as the grating template, in order to save the cost on the imported materials and find the way to change unwanted components into a useful optical accessory.

In the last decade, transmission surface plasmon resonance have been used and published. It was mostly used as a chemical sensor as it has a wide range of detection. In 2009, Changkui. et. al[2]. reported the use of T-SPR as a sensor using a dielectric grating on a silver film. In 2010, W.H. Yeh. et. al[5]. reported the development of a substrate material created by PDMS instead of the glass prism. In 2017, C. Lertvachirapaiboon. et. al[4]. reported the use of T-SPR techniques as a potential biosensor applications. The authors reported that this technique is useful as it can provide a simple and flexible optical configuration, strong spectroscopic signal and allow for the real-time monitoring of data. Although this technique has been widely used, the preparation of the substrate was expensive and the alternative substrate was complicated and took a long time to make. Therefore, in this experiment, new discs were used to make the grating and preliminarily studied morphology in order to control the quality of the data. All gratings in the experiment were coated with a 40 nm-thick gold film before studied their morphology by using atomic force microscopy. As the parameters of the grating played an important role in the SPR spectra, therefore, it was necessary to distinguish the differences among three types of grating and compare the differences with the SPR data for a better understanding of this project. The T-SPR spectroscopy in this experiment has the rotatable base of the sample holder in order to experiment in many angles and to find the angle that provided the highest intensity of SPR. The effect of the reflective index of the medium was later studied by the experiment using five different concentrations of ethylene glycol as medium. It is important to investigate the sensitivity of the SPR spectra from the T-SPR spectroscopy as developing the T-SPR spectroscopy to be a chemical sensing meant it should be able to detect the target chemical in many different mediums. Therefore, the T-SPR had to be sensitive enough to detect the differences of reflective index occurred by the different solution. Moreover, the study about the effect of the reflective index was able to be used to optimize and find the best angle for the experiment.

1.2. Objective of this work

To study and develop the chemical sensing instrument using the transmission-surface plasmon resonance technique and to develop the optical grating from optical storage discs which would be utilized in the instrument.

1.3. Scope of this work

This study involved the investigation of the transmission surface plasmon resonance spectroscopy using different kinds of grating made from CD-R disc, DVD disc and BD-R disc. The atomic force microscopy was used to study and compare the grating morphology which related to the data from T-SPR measurement. Moreover, the sensitivity of the instrument on the reflective index was also evaluated.

1.4. Theoretical background

1.4.1. Surface Plasmon Resonance

1.4.1.1. SPR

The surface plasmon (SP) is a quantum of an electron concentration wave that can exist at a dielectric-metal interface. This surface plasmon can be excited by an incident light wave under phase-matching conditions using a special technique including prism coupling and a grating coupling method. Therefore, an interesting phenomenon makes surface plasmon resonance (SPR) occurs when the frequency of the SP is coincident with the electromagnetic waves. The SPR effect in metal is important for many applications such as sensors, drug development and photonics devices. Surface plasmon resonance is one of the most widespread techniques to monitor and detect the target chemicals especially for biosensor. Normally, the SPR sensor based on grating relies on the extraordinary optical properties of noble metals such as gold (Au), silver (Ag) and Copper (Cu).

The SPR applications can be described by using Kretschmann configuration [6] (Figure 1.1). The interface of dielectric media is placed with a metal (normally gold or silver) film. Medium 1 with higher refractive index (n_1) is a prism or grating and the medium 2 with lower refractive index (n_2) can be the air or the solutions. Theoretically, only the p-polarized light is practical in the SPR sensing techniques.

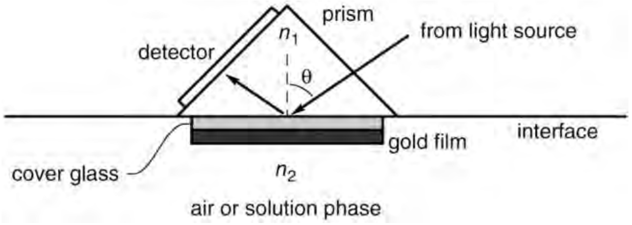


Figure 1.1 SPR setup [6]

The total internal reflection (TIR) can take place within medium 1 as long as the incident angle, θ, is greater than the critical angle, θ_c, where $\sin(\theta_c) = n_2/n_1$ when the light travels from the higher refractive index medium 1 to the lower refractive index medium 2. Evanescent waves are formed in the lower refractive index medium 2 under the condition of TIR. The amplitude of this type of standing waves decreases exponentially with the distance to the interface of the media 1 and 2. When a nonmagnetic gold film with suitable thickness is placed at the interface, the evanescent wave is enhanced, penetrating the gold film and existing in the medium 2. The magnitude of the parallel wave vector of the evanescent wave, $k_{evan,||}$, is expressed as

$$k_{evan} = \frac{2\pi}{\lambda} n_1 \sin(\theta) \qquad (1)$$

where λ is the wavelength of the incident light, n_1 is the refractive index of the higher refractive index medium 1, and θ is the incident angle.

Surface plasmons are quanta of plasma, a surface electromagnetic wave whose propagation is confined to the metal-dielectric interface. The magnitude of the wave vector of the surface plasmon (k_{SP}) is related to the dielectric constants of both the medium 2 and the gold film.

$$k_{SP} = \frac{2\pi}{\lambda} \sqrt{\frac{n_2^2 n_g^2}{n_2^2 + n_g^2}} \qquad (2)$$

where n_2 is the refractive index of medium 2 at the vicinity of the interface and n_g is the refractive index of the gold film.

The surface plasmon can be excited by the evanescent wave and this phenomenon is called surface plasmon resonance (SPR) leading to the sharp decrease of the intensity of the reflected light[7] . The decreases of the excited surface plasmon include energy conversion to phonons or photons. One requirement for the SPR is that k_{SP} equals to $k_{evan,||}$. Thus, using equation 1 and 2 gives

$$\theta_{SPR} = sin^{-1}\left(\frac{1}{n_1}\sqrt{\frac{n_2^2 n_g^2}{n_2^2+n_g^2}}\right) \qquad (3)$$

The angle required for the resonance, θ_{SPR}, is related to n_2 when n_1 and n_g are fixed. Adsorption and desorption on the gold surface as shown in figure 1.1 changes the refractive index of media 2 near the metal-dielectric interface and the resonance angle changes accordingly. Therefore, the monitoring of the θ_{SPR} change can be used to analyze the adsorption-desorption or association–dissociation activities that take place on the gold surface.

1.4.1.2. Polarized light

The polarized light waves are light waves in which the vibrations occur in a singular plane. Light waves that are produced by vibrating electric charges and vibrates in many planes are called unpolarized light. In order to change the unpolarized light into polarized light, the light has to go through the process called polarization. The two orthogonal linear polarization states that are the most important for reflection and transmission are s-polarization and p-polarization. S-polarized light has a rotational axis of substrate paralleled with the plane of incidence, while a rotational axis of p-polarized light is perpendicular to the plane of incidence. The common method of polarization is to use a polarizer which can block one of the two planes of vibration of electromagnetic waves. The desired polarization is transmitted through the reflective polarizers. A common type of this polarizer is a wire grid polarizer which consisting of many thin wires arranged parallel to each other. The light that is polarized perpendicular to these wires is transmitted, while the light that is polarized along these wires is reflected leading to the desired polarized light obtained in the experiment.

1.4.1.3. T-SPR

Unlike the conventional surface plasmon resonance, transmission surface plasmon resonance (T-SPR) is an unusual optical transmission that is more transparent at the certain wavelengths than expected by classical theory. This phenomenon occurs from the surface plasmon resonance excitations and the changing of the electrons on the surface of a metallic film causes the shifting in T-SPR spectra. Figure 1.2 showed the differences of the conventional technique and transmission technique. The strong electric field, which is very sensitive to the local dielectric properties of materials immediately adjacent to the metal, observed in the experiment is the result of the extraordinary transmission of light through the metal-coated grating. T-SPR which has high selectivity and easy setup procedure is highly useful technique in the present.

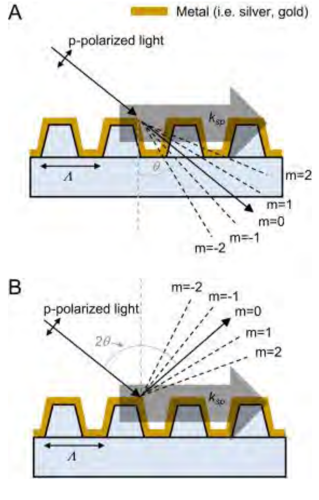


Figure 1.2 (A) TSPR and (B) conventional SPR configurations. In the diagrams, Λ is the diffraction grating pitch, θ is the incident light angle, and k_{sp} is the sensitive surface plasmon wavevector [6].

1.4.2. UV-visible spectroscopy

UV-visible spectroscopy is a fast, simple and inexpensive method to determine the concentration of an analyte in solution. It is used to measure the intensity of light which is absorbed by the sample in UV-visible range or 190-850 nanometers. Normally, the absorption property of the molecule in the sample will excite the electrons in the sample and change their energies into the higher levels when receive sufficient energy. The changing absorbance or transmission can be explained by Beer’s Law leading to the ability to identify or analyze the quantity or concentration of the sample. The UV-visible spectroscopy basically consists of the light source, sample holder and the detector to measure the intensity of absorbance or transmission.

Chapter 2

EXPERIMENTS

2.1. Chemicals, glassware and materials

2.1.1. Chemicals

1.	HNO_3 65%	AR grade	Merck
2.	Ethanol	AR grade	Merck
3.	Ethylene Glycol	AR grade	Merck
4.	4-Mercaptophenylboronic acid		Sigma Aldrich
5.	Deionized water		

2.1.2. Glassware

1.	Beaker	25ml	Pyrex
2.	Beaker	50ml	Pyrex
3.	Beaker	100ml	Pyrex
4.	Cylinder	10ml	Pyrex
5.	Cylinder	50ml	Pyrex
6.	Volumetric flask	100ml	Pyrex
7.	Dropper		
8.	Glass slide jar		
9.	Glass slides		

2.1.3. Materials

1.	CD-R		JVC
2.	DVD		JVC
3.	BD-R		That’s
4.	Syringe	10ml	
5.	Needle	0.8*25mm	NIPRO
6.	Gold target		

7. Forceps
8. Cell plate
9. Filter paper
10. Aluminum foil
11. Parafilm
12. Ultrasonic bath
13. Analytical balance

(Optical gratings) CD-R and DVD were purchased online from the website of JVC.BD-R was purchased from That's company. Other parts of the instrument were created by using a 3D printer. All the glassware was purchased from Pyrex. Ethanol, 65% Nitric acid, and Ethylene Glycol were purchased from Merck. All chemicals were analytical grade and used without any further purification. The glassware was cleaned with detergent and rinsed with DI water for several times before use.

2.2. Experimental

2.2.1. Grating Preparation

In order to get the optical grating from B-DR disc was drawn 4 pairs of parallel lines from one side to another, which had 2.5 cm width on the writable side. Each pair would have equal distance from other pairs and remain a hole of the BD-R disc in the middle of the pairs. The BD-R disc was carefully cut by following the predetermined lines. This results a total of 8 pieces of trapezoid-like BD-R. The top layer of the BD-R piece was peeled off and cut the sharp top of each BD-R and made another mark at the right bottom corner to remark the grating surface.

In the further steps, the chemical contamination on the grating was removed by chemical reactions. Firstly, all the cut BD-R pieces were placed in the glass slide jar and soaked in the 6.705M of HNO_3 solution for one minute and a half in order to cleanse the trace metals on the BD-R. Then, they were rinsed with DI water for several times until there is no residual of HNO_3 left on the BD-R pieces. The cut cleansed BD-R were soaked in diluted dishing soap solution with sonication for 15 minutes. The cut cleansed BD-R were later washed with DI water for several times. The process of washing the BD-R with soap solution would be repeated for 2 more times. After that, the cut cleansed BD-R was dried using a blower and kept them in a clean cell plate sealed with parafilm to prevent dusts and moisture from the air. Same preparation process was also performed for DVD and CD-R to obtain bare grating substrate. The overall process grating preparation process was summarized in Figure 2.1.

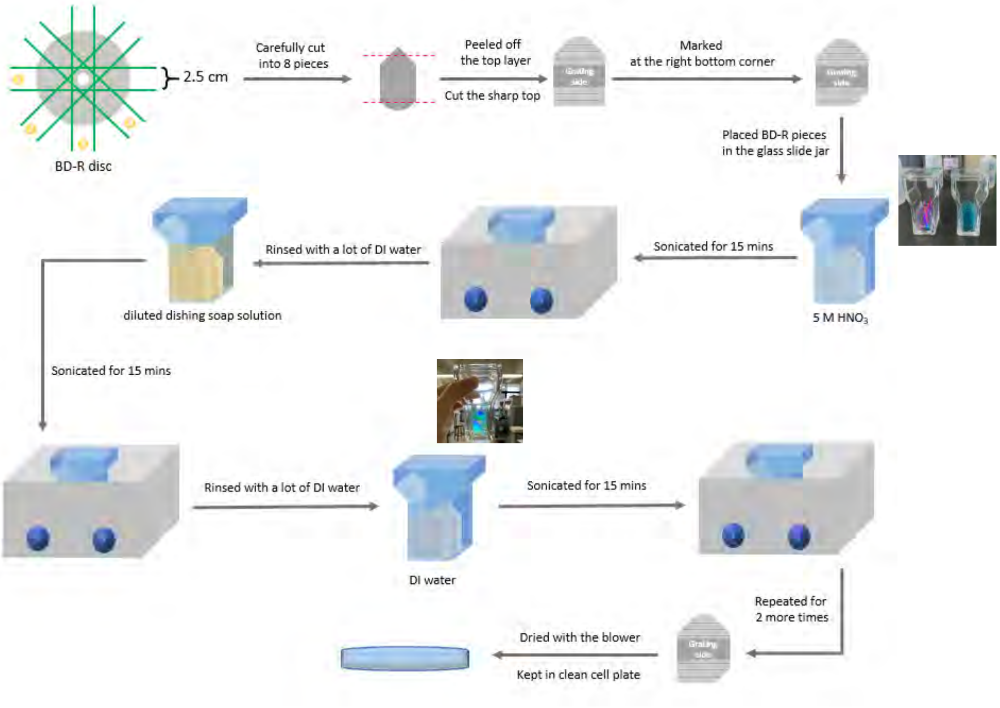


Figure 2.1 Preparation process of grating substrate from BD-R, CD-R and DVD disc

2.2.2. Gold sputtering

The cleansed BDR was coated gold using sputtering instruments (Leica EM ACE600) and the target gold received from Electron Microscopy Sciences. Six pieces of cleansed BD-R were glued to the LARS (low angle rotary shadowing) stage with thin glue tape. The LARS stage then was installed back into the sputtering machine. In the sputtering process, the thickness of deposited gold was set to 40 nm. Finally, kept all of the coated grating in the vacuum bag to prevent dusts and external moisture in the air. The overall process was summarized in Figure 2.2.

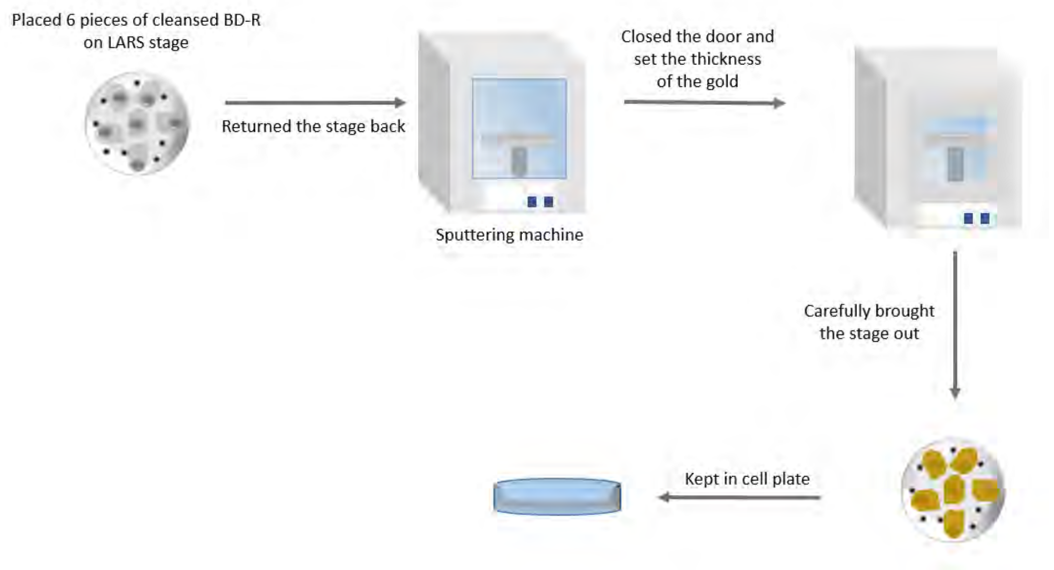


Figure 2.2 Gold sputtering method to fabricate the gold-coated grating substrate

According to the structure of grating consisting of uniformly repeated grooves, it has 3 main parameters including the groove depth (*h*), groove width (*d*) and groove period (*l*) as shown in Figure 2.3. The surface plasmon resonance phenomena could be affected by these grating parameters. The parameters will be continuously referred throughout the study. The surface morphology with all the grating parameters of gold deposited grating was investigated by Atomic Force Microscope (AFM).

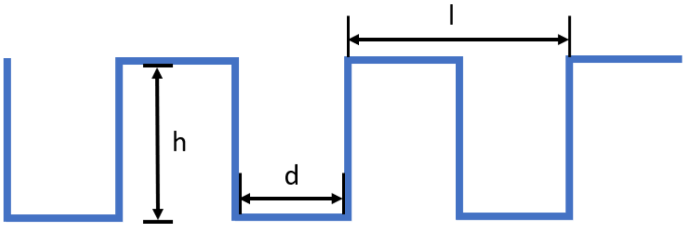


Figure 2.3 Cross section of a grating substrate including the parameters of the groove depth (*h*), groove width (*d*) and groove period (*l*)

2.2.3. Transmission SPR meansuremants

2.2.3.1. Instrument set up

The in-house instrument was constructed with three main parts including part A, B and C as shown in Figure 2.4. Part A demonstrated the optic setting including polarizer that allow light wave of specific polarization pass through while blocking light waves of other polarizations, iris diaphragm was used to control the intensity of the light and the size of passage light passing through aperture, collimator was used to align the light to be in the specific direction and make the light parallel, sample holder with the adjustable angle, collimator, and detector. In this study, the specific polarization passed through the iris polarizer and measured by the detector was p-polarized light which had the electric field polarized parallel to the plane of incident and perpendicular to the plane of grating while s-polarized which had the electric field polarized perpendicular to the plane of incident and parallel to the plane of grating was blocked as it could disturb the observation of SPR signal. Starting with turned on the computer and light source. In part B, there are the light source lamp, which helium light bulb was used as a light source. The light would go through optical fiber and optical setup Part A until it finally reached the detector. Finally, part C was the computer which was installed with SpectraSuite program. This program was used to collect all the data from the experiment in the form of spectrum. The instrumental part was listed in Table 2.1 and the alignment of the instrument used in the study was shown in Figure 2.4.

Table 2.1 List of instrumental parts

Part A	**Part B**	**Part C**
1. Light source 2. Polarizer (wire grid) 3. Iris diaphragm 4. Collimator 5. Sample holder 6. Collimator 7. Detector (Ocean optics; USB2000)	1. Light source (lamp) (Halogen light bulb) 2. Optical fiber	Computer with SpectraSuite program

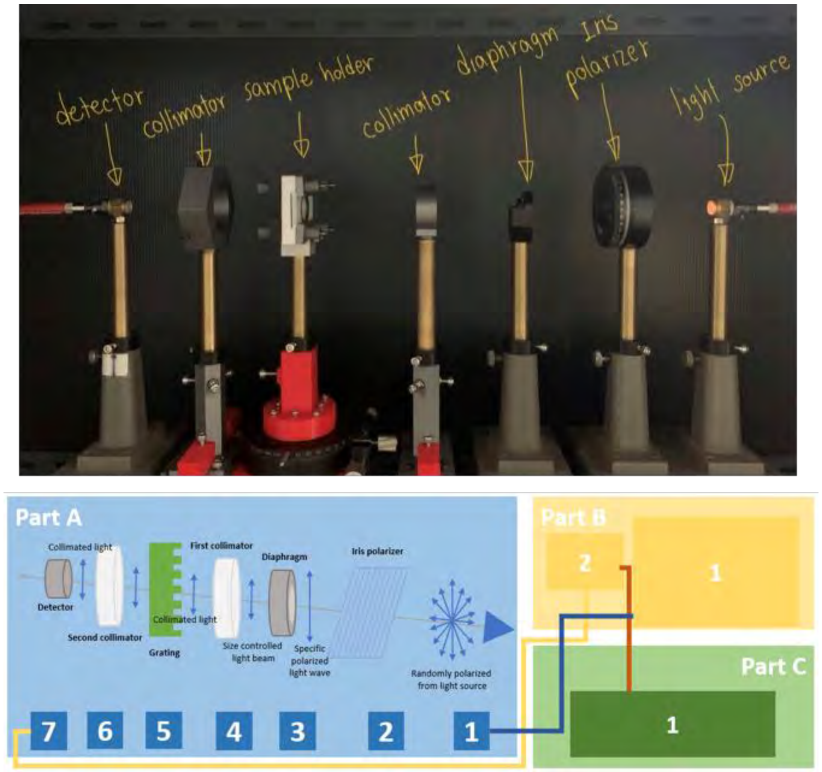


Figure 2.4 Image of optical set up for Transmission-SPR measurement including the schematic details of the optical alignment combined with light source, detector and computer with SpectraSuite to monitor SPR phenomenon

Before starting the measurement, the instrument had to be carefully undergone an optical alignment. This alignment is required in order to minimize the reflection of the light while it travelled through each part(lens) of the instrument at the incident angle of 0°. The alignment in this study started from checking the reflective beam from the grating which should be in the center of the first collimator. The position of the beam at the detector was later checked whether it was in the center of the detector or not. Other parts of the instrument remained in the same position and were not adjusted. To align the optical parts, the photon intensity was monitored. The highest intensity would be obtained when the optical parts were already aligned at incident angle of 0°.

2.2.3.2. T-SPR measurement

2.2.3.2.1. Sample holder preparation

The sample holder was in-house designed and printed with 3D-printer (Prusa i3 mk3). The cell body is capable for measuring T-SPR of a sample in liquid form. In order to insert liquid sample in the sample holder, the sample holder had to be carefully prepared. Four threads were screwed on every corner of the holder base, which was used as the cell body. The rubber ring was then placed on the rim of middle hole of the PTFE square spacer. Then, the glass slide was placed over the rubber ring and locked with 2 pieces of rectangle plastic lock on the side of cell body over the rim of the glass slide. Everything was later locked by screwing 4 pieces of plastic nuts on every thread. After finished on this side, the cell was flipped over to the other side. The rubber ring was placed on the rim of middle hole on that side. Then, the gold coated grating was carefully placed on the rubber ring by turned its gold coated side inside the cell body while its mark should be on the right bottom of the white part and the injection hole was upward. Followed by the same process as the other side, 4 pieces of plastic nuts was screwed on every thread over two plastic locks. The sample media, which could be any kind of liquid, for example, water and ethylene glycol, was injected into the cell which was later returned to the sample holder stage and was ready to be used in the experiment.

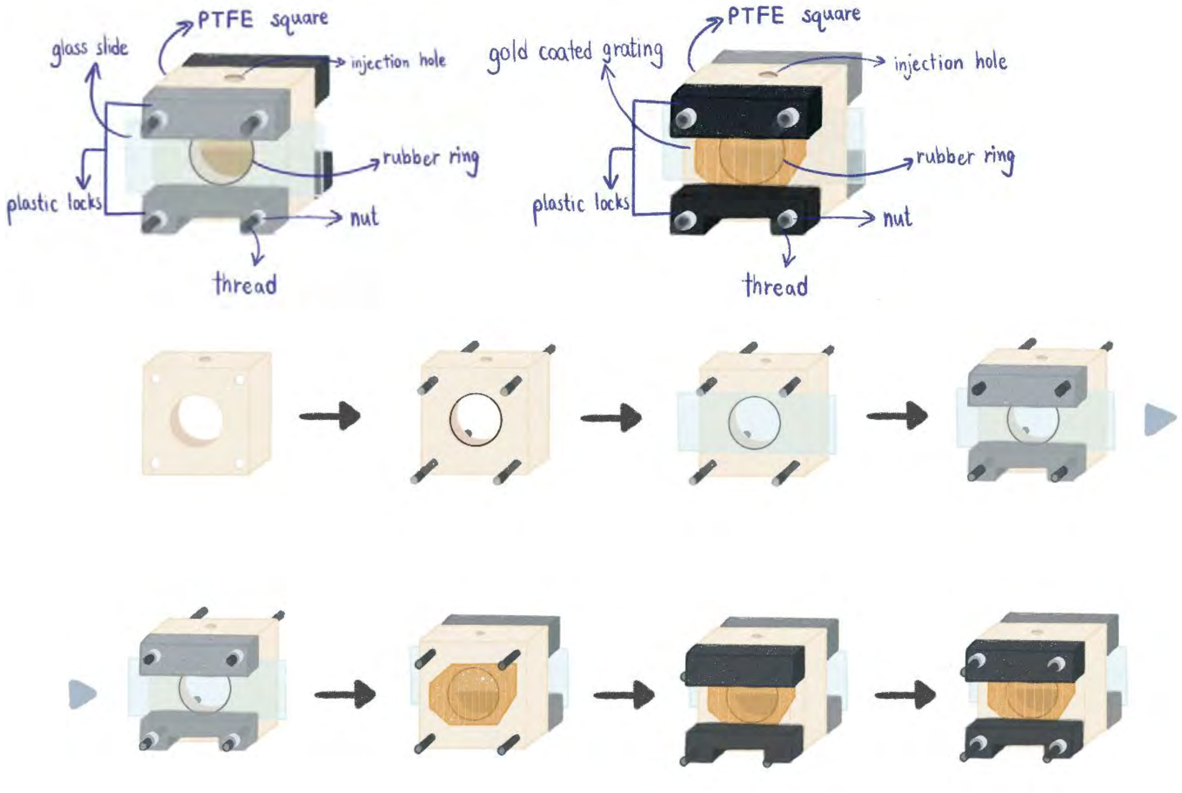


Figure 2.5 Image of sample holder components

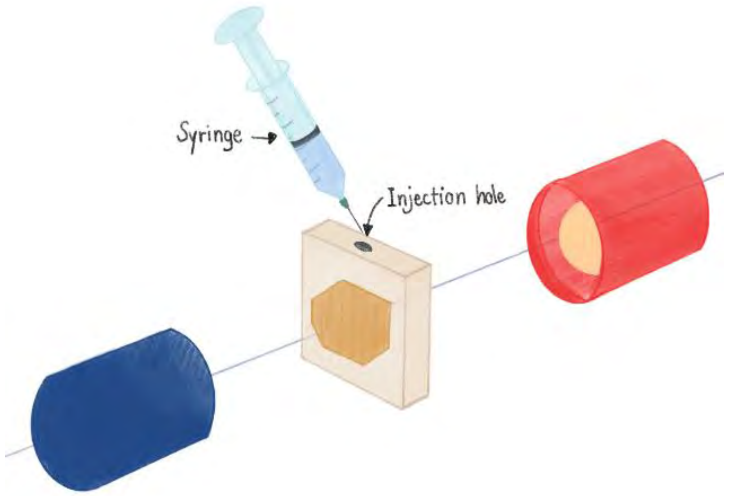


Figure 2.6 Injection of liquid sample in the prepared sample holder

2.2.3.2.2. Adjustment of incident angle

The stage was constructed with the angle adjustable plate. This can be freely rotated in both clockwise and anti-clockwise directions. In this experiment, the plate was rotated with only anti-clockwise direction to control the light diffusion on the grating.

The incident angles were calculated from the angle of incident light and perpendicular line of the grating as shown in Figure 2.7. In this experiment, the detected angle started from 0° and increased every 4° until the spectrum was not detectable (which was around 40°-52° depending on the grating and the medium) and the spectrum range were selected from 450 to 850 nm which were the wavelength of visible light.

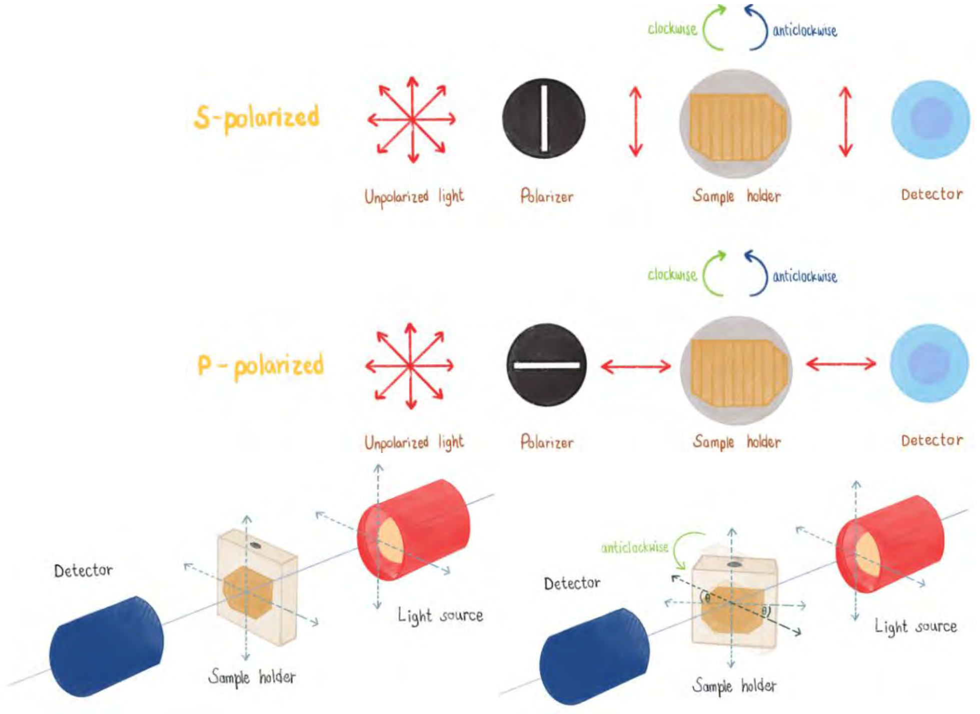


Figure 2.7 Detection of T-SPR with P- and S- polarized and the scheme of incident angle adjustment

2.2.4. Reflective index sensitivity by T-SPR

Apart from the grating parameters, a significant factor which could affect the nature of the SPR spectrum were investigated. Generally, the reflective index of the solutions was strongly influenced by concentration and type of the solution. Variation of the reflection index from the solution could strongly affect the nature of the T-SPR of the grating. Therefore, this is a high possibility to quantify concentration of the target analyte by measuring characteristic changes of T-SPR band. This is a strong impact for biological and chemical applications. To demonstrate the possibility to use our in-house T-SPR instrument with our modified grating as chemical sensors, the system of mixture between ethylene glycol and water was used as a reference system. In fact, the mixture of ethylene glycol and water is a well-known solution to monitor the changes of reflective index. The reflective index will increase from 1.33300 to 1.38313 when amount of ethylene glycol is varied from 0% to 50% w/w, respectively.

To monitor characteristic changes of T-SPR peak, the ethylene glycol solutions were prepared in 5 different concentrations involving 10%, 20%, 30%, 40% and 50% w/w in the 100 ml volumetric flasks. These solutions were used as a sample. All the data was collected and interpreted the spectrum by using origin program. The changes of SPR spectrum were observed by observing the shift of (P-S)/S or the intensity from 0% (DI water) to 50% w/w of ethylene glycol at the same incident angle. The maximum intensity of the peaks at the same angle was collected and plotted in the excel to collect the R-squared and slope at every angle in order to find the best angle for detection by using T-SPR.

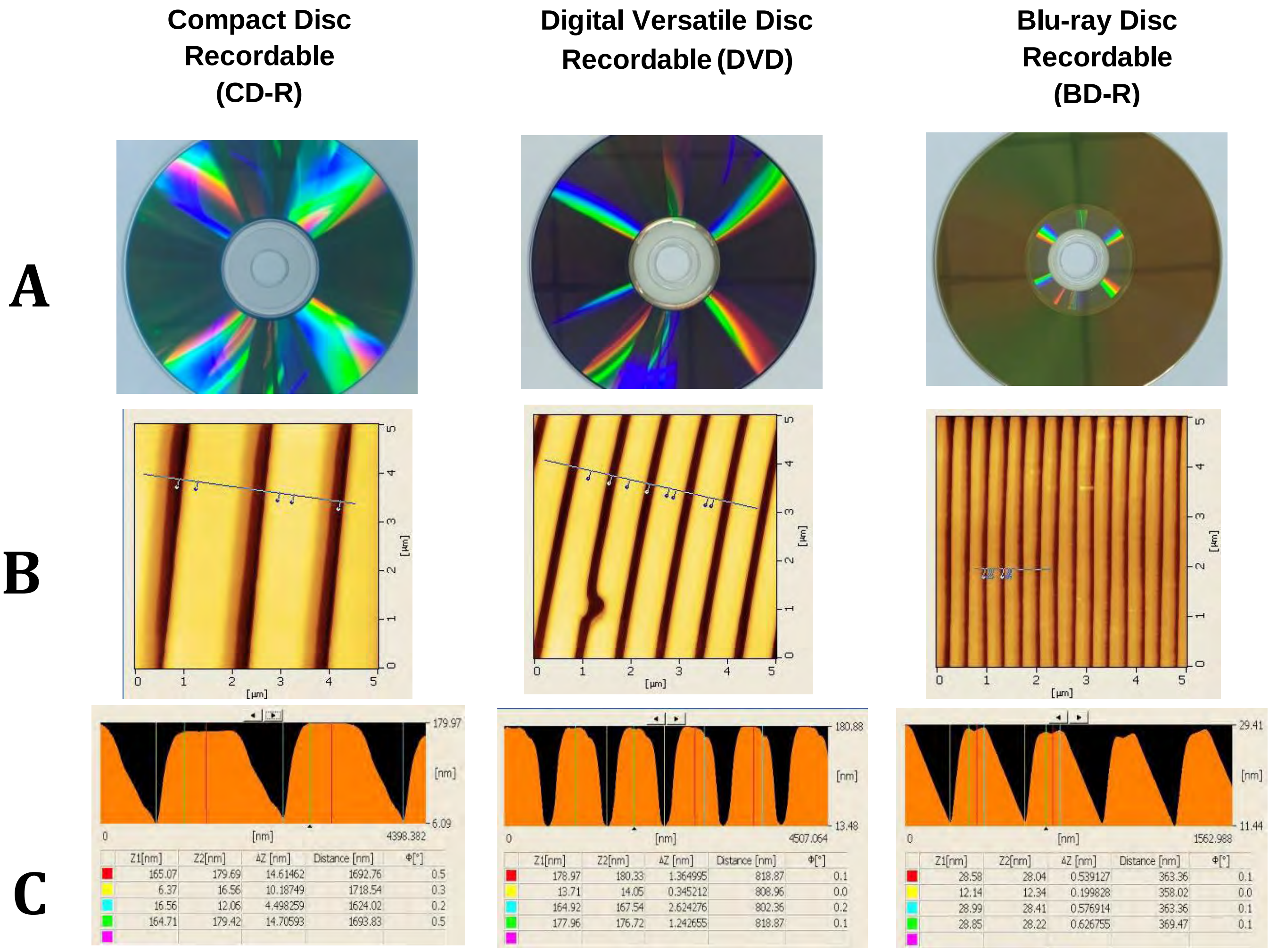
Chapter 3

RESULTS AND DISCUSSION

3.1. Grating morphology analysis

From digital images of discs (CD-R, DVD and BD-R), the physical appearances of each grating were different (shimmering color). This might relate to the reflection of the light on their surfaces affected from the grating structure of each optical storage disc. The grating structure of each storages disc is directly related to their record capacity. To get insight of the grating structure, AFM with NanoNavi built-in program was used to visualize their surface morphologies as shown in Figure 3.1 B. The rectangle-like shapes of grating were observed from the three types of discs. The grating structures with rectangle shape are similar even though their groove depth (*h*), groove width (*d*) and groove period (*l*) were obviously different. The NanoNavi program was able to measure the grating parameters. However, the grating structure on disc is in poor uniformity. Therefore, an alternative program, which is the imageJ program, was used to reassure the measurement data from the NanoNavi program. It was found that there was no significant different between both programs. For CD-R disc, the grating parameters including *h*, *l* and *d* of CD-R was observed at 172.22 nm, 1693.3 nm and 1079.5 nm, respectively. In case of DVD, the parameters of *h*, *l* and *d* were 174.4 nm, 813.41 nm and 479.38 nm, respectively. For BD-R disc, the grating parameters of *h*, *l* and *d* were 28.52 nm, 365.4 nm and 280.08 nm, respectively. From grating parameters of the grating substrates, it was found that there is no significant difference of the groove depth between the grating made from CD-R and DVD. The BD-R shows the thinnest grating with the lowest groove depth. The groove period and groove width are directly related to the storage capacity. The smaller groove period, the larger storage capacity. In our case, the BD-R contain the lowest groove period resulting is the highest storage capacity. The important factor which strongly affect further SPR investigation is groove period.

Generally, the frequency of SPR should be well matched with the groove period of the substrate in order to generate T-SPR phenomena. Only groove period of DVD and BD-R is in visible region, while the groove period of CD-R is out of visible range.



	Z1[nm]	Z2[nm]	ΔZ [nm]	Distance [nm]	Φ[°]
🟥	165.07	179.69	14.61462	1692.76	0.5
🟨	6.37	16.56	10.18749	1718.54	0.3
🟦	16.56	12.06	4.498259	1624.02	0.2
🟩	164.71	179.42	14.70593	1693.83	0.5
🟪					

	Z1[nm]	Z2[nm]	ΔZ [nm]	Distance [nm]	Φ[°]
🟥	178.97	180.33	1.364995	818.87	0.1
🟨	13.71	14.05	0.345212	808.96	0.0
🟦	164.92	167.54	2.624276	802.36	0.2
🟩	177.96	176.72	1.242655	818.87	0.1
🟪					

	Z1[nm]	Z2[nm]	ΔZ [nm]	Distance [nm]	Φ[°]
🟥	28.58	28.04	0.539127	363.36	0.1
🟨	12.14	12.34	0.199828	358.02	0.0
🟦	28.99	28.41	0.576914	363.36	0.1
🟩	28.85	28.22	0.626755	369.47	0.1
🟪					

Average groove depth (*h*) : 172.22 nm
Average groove period (*l*): 1,693.30 nm
Average groove width (d) : 1079.48 nm

Average groove depth (*h*) : 174.41 nm
Average groove period (*l*): 813.57 nm
Average groove width (d) : 479.38 nm

Average groove depth (*h*) : 28.52 nm
Average groove period (*l*): 365.40 nm
Average groove width (d) : 280.08 nm

Figure 3.1 (A) Digital images of three different type of disc including CD-R, DVD and BD-R. (B) The surface morphology of grating substrate monitored by AFM. (C) Grating properties including Groove depth (*h*), Groove period (*l*) and Groove width (*d*) of CD-R, DVD and BD-R investigated by AFM.

3.2. T-SPR

To prove our set up of T-SPR measurement, the glass slide coated with gold at thickness of 40 nm was used to monitor the phenomenon of T-SPR. The surface of glass slide was completely flat after coated with gold, therefore, it should not provide any behavior of T-SPR. Generally, the T-SPR spectrum could be obtained from the substrate contain high reflective index compared to the media which involve some precious metals (e.g. gold, silver, platinum) and the surface structure which could diffract the light especially in different incident angle (e.g. groove, grating). In this case, the characteristic T-SPR band should not be occurred during the measurement as the surface of the substrate is completely flat. From Figure 3.2, it ensured that there are no characteristic SPR band on the spectrum when the incident angle was adjusted from 0° to 32°. In the case, it could be confirmed that our T-SPR instrumental set up (optics and flat substrate) do not provide any artifact SPR peaks in the spectrum. If the characteristic SPR band appear in the further analysis, it should reflect the existed interaction of light, sample and grating substrate.

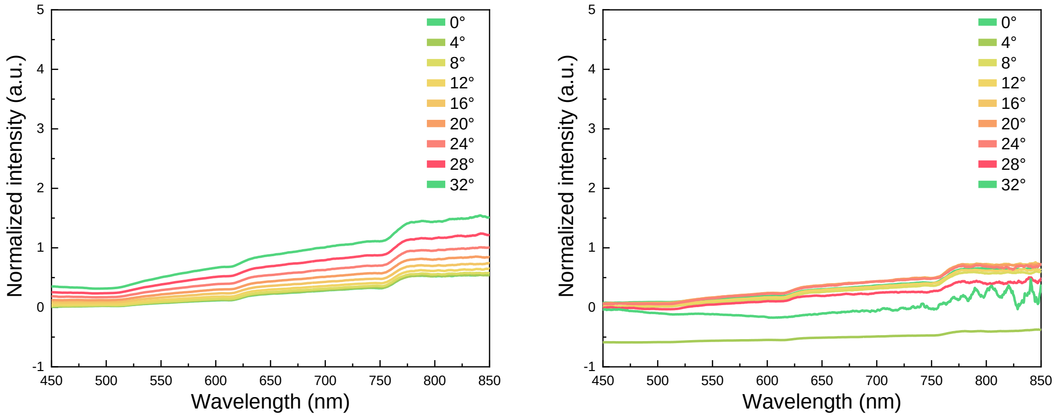
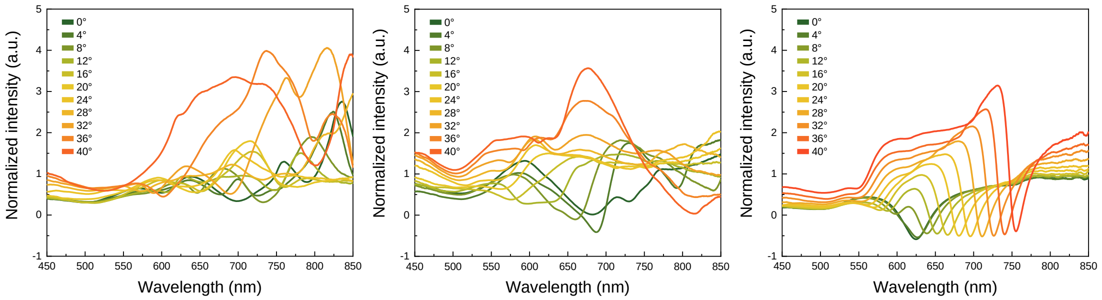


Figure 3.2 Transmission-SPR spectra of the gold-coated glass slide in the two different medias which were (A) air and (B) water. The T-SPR spectra were acquired at different incident angle varied from 0° to 32°.

The relationship between the grating parameter and the obtained T-SPR peaks was investigated in two different media which are air (Figure 3.2A) and water (Figure 3.2B). The grating parameters of the three different types of disc were already mentioned in Figure 2.3. Due to the differences of the grating parameters, T-SPR spectra were expected to be different as well. For CD-R and DVD, unpredictable T-SPR spectrum was obtained. There is no relationship between incident angle and the SPR band in the spectrum. However, the T-SPR patterns was obtained when BD-R was used as a grating substrate. The SPR band was clearly redshifted, when the incident angle increased. These patterns were obtained when either air or water was used as medium. The observed phenomena might be associated with the interaction of light frequency and gratin parameters. In our case, only BD-R could provide an appropriate groove period which well match with the light in visible region, while the groove period of CD-R and DVD is out of visible range (larger than visible light wavelengths).

A. Air



B. Water

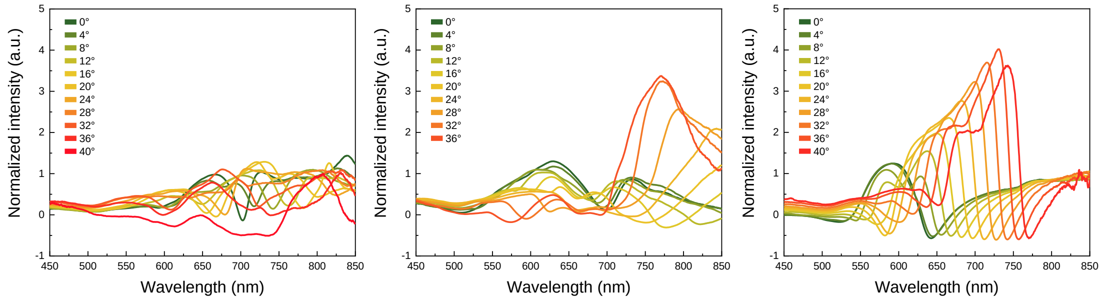


Figure 3.3 The T-SPR spectra using grating from CD, DVD and BD-R as the substrate. T-SPR spectrum was acquired at different incident angle (0° – 40°) in (A) air and (B) water.

To reveal the correlation of incident angle on T-SPR band, λ_{max} was collected and plotted against the incident angle as shown in Figure 3.4. From both mediums (air and water), it showed the similar trends. There is no linear relationship between λ_{max} and incident angle from CD-R and DVD, while the linear relationship with $R^2 = 0.9989$ and 0.9978 were obtained using BD-R as grating substrate. The linear relationship of λ_{max} on different incident angle demonstrate that the absorption band was originated from plasmon on the grating surface neither from the scattering effect nor substrate adsorption. It was proven that the grating groove is an important factor which should be well match with the wavelength of the incident light. After this experiment, the grating substrate from only BD-R will be used in the further experiments.

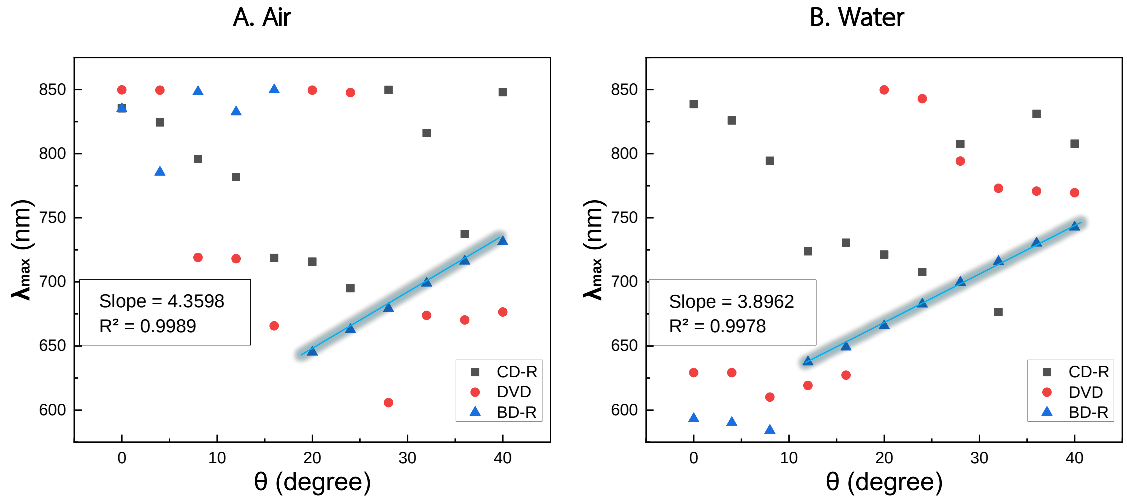


Figure 3.4 The λ_{max} of characteristic T-SPR band obtained at different incident angle using CD-R, DVD and BD-R grating substrate in (A) air and (B) water medium

In this study, the changes of reflective index affected to the SPR peak was investigated. At each incident angle, the experiment was conducted by monitoring the SPR peaks at different concentration of ethylene glycol. In the case, it is important to determine the best incident angle which provides either the clearest shifts or the highest sensitivity due to the reflective index on the interface of grating. The correlation of incident angles on SPR band was monitored by a graph of collected intensity at λ_{max} and the incident angles. R^2 value and the slope of regression model was used as performance indicator to demonstrate the sensitivity of the grating substrate on the changes of reflective index as shown in Figure 3.5A. The higher R^2 value and slope, the more sensitivity of the grating substrate. The best angle which provided the most sensitive SPR response was 28°. Figure 3.5B shows the SPR spectra at different %ethylene glycol. It shows that the intensity at 700 nm is clearly changed due to the concentration of ethylene glycol. The good correlation of intensity and %ethylene glycol was observed as shown in Figure 3.5C (R^2 value = 0.8962). This suggests that our grating substrate from BD-R is sensitive to detect the target chemical interacted with the grating surface with good sensitivity.

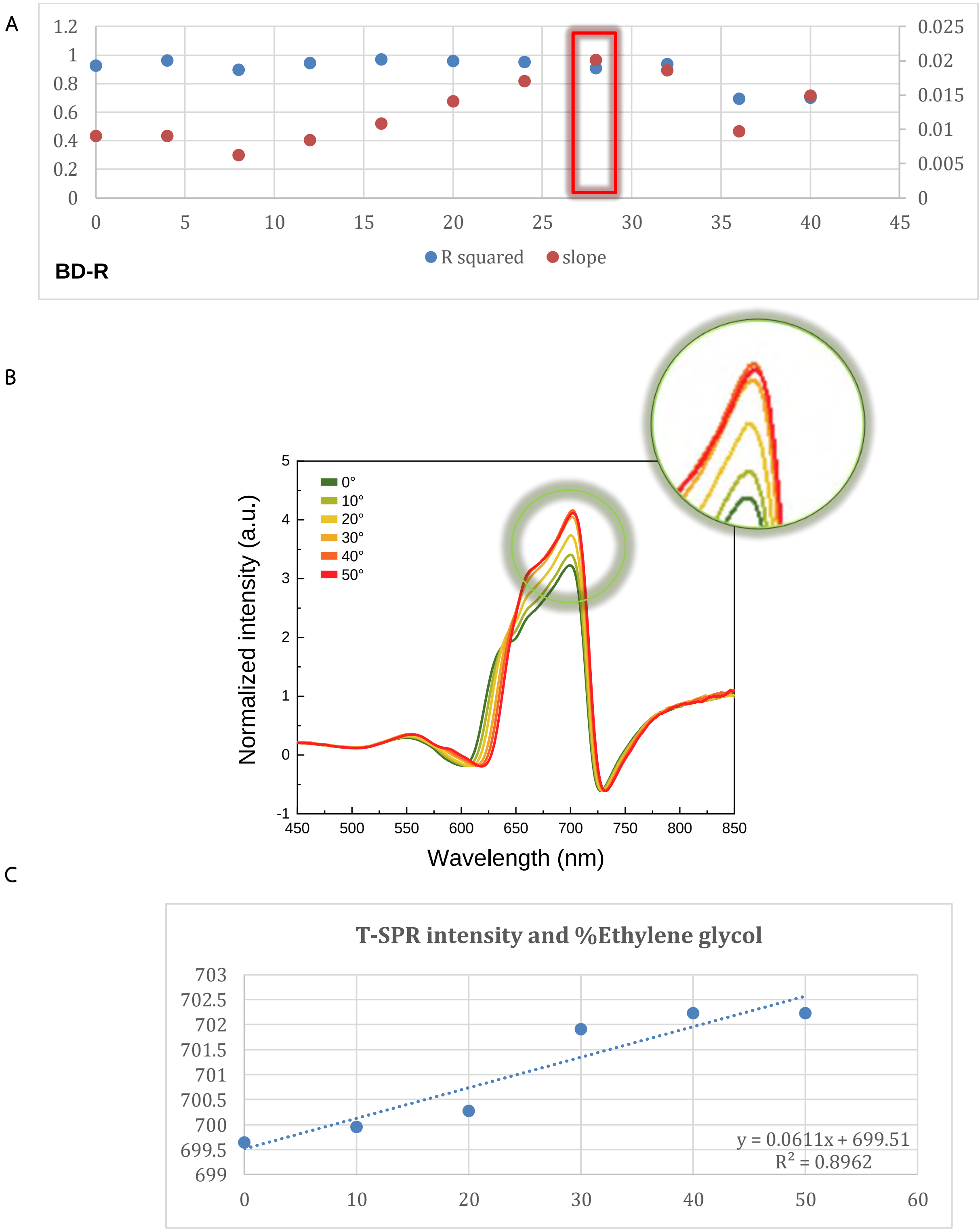


Figure 3.5 (A) the R^2 value and slope on the determination of %ethylene glycol at different incident angle, (B) the T-SPR spectra at 28° provided good determination with high R^2 (0.8962) and slope (0.0611) and (C) the linear relation of T-SPR intensity and %ethylene glycol

2.1 Developing

It is well-known that thiol compounds could react to gold surface to generate Au-S bond. This chemical reaction is important in order to modify the gold surface for several applications because the reaction is spontaneous and generate the strong chemical bond of modification agent (thiol compound) and gold surface. This chemical bond of Au-S is very strong and stable which could not be removed by simple cleaning processes. However, to monitor the coverage of thiol interacted on gold surface is difficult. The incubating with long period of time will be used to ensure that the thiol compounds are fully interacted and coverage the gold surface. The electrochemistry was conventionally used to investigate this phenomenon, but it contained a weak point that electric could react to thiol compound instead of gold. In this study, we would like to offer a new alternative way to monitor this thiol-gold reaction by T-SPR measurement as T-SPR spectrum was sensitive to the reflective index of a grating substrate. The reflective index of grating surface might be changed when chemical bond of Au-S is existed. The 4-Mercaptophenylboronic acid 90% (MPBA) was used as modification thiol agent. The T-SPR spectrum was monitored at the interval of 24 hours and the process was repeated for 3 times (1st - 3rd soaked). Figure 3.6 B show the SPR peaks when the grating substrate was incubated with the MPBA solution. It reveals the intensity shift when the incubation time was increased. The results are promising that the T-SPR measurement could be further developed to monitor chemical rate reaction of thiol-gold surface.

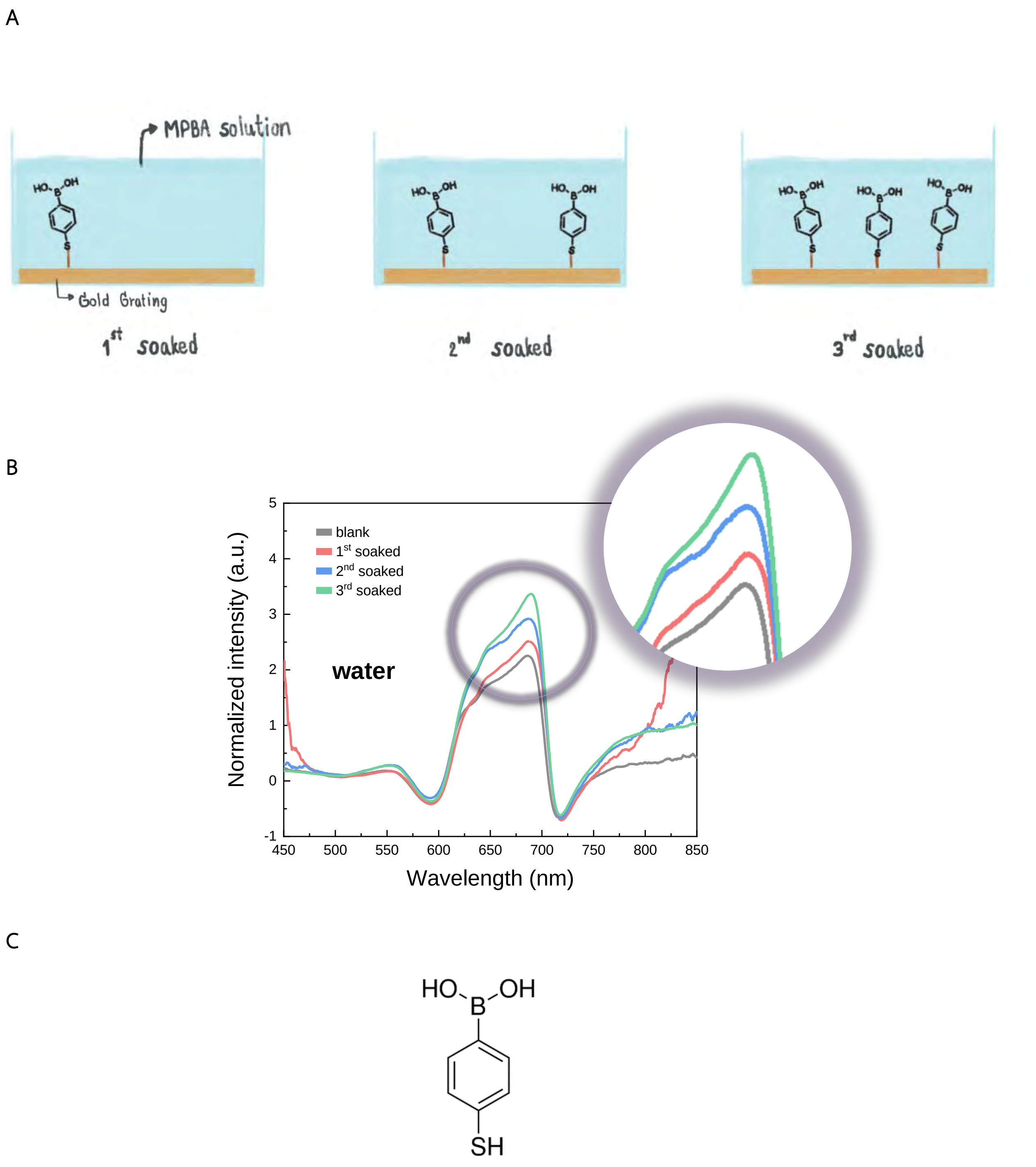


Figure 3.6 (A) Experimental set up to monitor reaction of thiol-gold using T-SPR measurement (B) T-SPR spectra of a BD-R grating substrate interacted with thiol (MPBA) (C) Structure of 4-Mercaptophenylboronic acid 90% (MPBA)

Chapter 4

CONCLUSIONS

This study has demonstrated the potential application of transmission surface plasmon resonance spectroscopy to be used as chemical sensor by investigating the shifts in SPR spectra. Grating from CD-R, DVD and BD-R coated with gold with thickness of 40 nm was used as a substrate to acquire T-SPR spectra. The surface morphologies of gratings were monitored by atomic force microscopy (AFM). The grating parameters including groove period (l), groove height (h) and groove width (d) were estimated using the captured AFM images with ImageJ program. In the case, the grating period is a major factor which affects to the T-SPR signal. The groove period of CD-R, DVD and BD-R were 1693.30 nm, 813.57 nm and 365.40 nm, respectively. It should be noted that only groove period from BD-R is well matched with the visible light wavelengths. The SPR spectra from CD-R, DVD and BD-R using air and water as medium was acquired with different incident angle (θ = 0° – 40°). Only the intensity and λ_{max} of the SPR spectra from BD-R grating showed the good linearity with incident angle with R^2 = 0.9978 – 0.9989, while the random correlation was observed for CD-R and DVD gratings. It was possible that the groove width, groove height and groove period of BD-R were appropriately matched with the wavelength from incident light rather than CD-R and DVD. Therefore, this experiment could reveal the influences of grating parameter on the obtained SPR spectra.

The effect of reflective index of sample on the T-SPR spectra was investigated by using the mixture of ethylene glycol (10 – 50 %w/w) as a testing system. In this study, it showed that T-SPR spectra acquired by using the BD-R grating substrate is sensitive to the reflective index of the medium. It provided the linear range R^2 of 0.8962 when the incident angle of 28° was used. The transmission surface plasmon resonance spectroscopy was a potential technique to monitor and detect the changes of the reflective index on the grating substrate. The reflective index of the substrate is very sensitive to the interaction of the target molecule and the grating surface. Therefore, T-SPR is a promising method to be used as chemical sensors and used to reveal the interaction of surface with various type of chemicals.

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