Experimental study of surface plasmon resonance enhancement using bimetallic film technique

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Experimental Study of Surface Plasmon Resonance Enhancement Using Bimetallic Film Technique

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A thesis submitted to the Nanyang Technological University
in fulfilment of the requirement for the degree of
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SUMMARY

The rapid civilization process of mankind, swift advancement in technology and surging human population have prompted mankind to be consistently conscious of their health, environment and surroundings. Increasing awareness and concerns have spearheaded many research and development activities in the area of optical sensors. Surface plasmon resonance (SPR), since it was first applied for gas and biosensing almost two decades ago, has made great strides in instrumentation development and sensing applications. In this thesis, SPR enhancement with the two-layer silver-gold metallic film technique is systematically investigated. Predominantly, this configuration integrates the better evanescent field enhancement of silver, the improved SNR of the SPR curve achievable with silver, and the high chemical resistance and biocompatibility of gold.

First, a multi-layer model is employed to simulate the SPR curves, electric field intensity on the metal surface and penetration depth of the SP evanescent field during resonance, considering magnetic field continuity since SPs can only be stimulated with a p -polarized incident beam. Theoretical comparison between the popular single gold film SPR configuration and the bimetallic silver-gold structure with prism in-coupling scheme concludes that the latter yields better SNR, poorer sensitivity in terms of $d\theta/dn$, no distinctive differences for sensitivity in terms of $d\lambda/dn$ and better sensitivity in terms of sensing region. Experimental data with prism geometry reinforced the theoretical deduction that the bimetallic silver-gold configuration improves SNR of the SPR reflectivity curves.

The bimetallic configuration is extended to the waveguide geometry to examine the sensitivity differences ($d\lambda/dn$) achievable as compared to single film structures. The waveguide scheme is employed as it permits easy realization of wavelength interrogation, and its compact nature facilitates numerous lab-on-a-chip applications and potential commercial exploitation. Advanced lithography may reduce the overall cost as well. The first example of

a wavelength-modulated waveguide SPR sensor with a bimetallic silver-gold film for SPR sensitivity enhancement is reported. Wavelength interrogated sensing of glucose solution concludes that the bimetallic configuration achieves much better sensitivity ($d\lambda/dn$) than the single gold film waveguide SPR scheme.

Two new techniques for fabricating index-modulated embedded waveguides using commercial photosensitive materials are proposed and discussed in this thesis. The first, known as photothermal lithography, involves photo-induced polymerization of the UV-exposed regions under a patterned mask, and subsequently cross-linking of the unexposed regions via high thermal treatment, achieving an index contrast of $\sim 7.2 \times 10^{-3}$. The second technique, known as dual-UV exposure lithography, involves a UV expose – bake – UV expose procedure to marginally modulate the refractive index between the initially exposed and later exposed regions, resulting in a maximum index contrast of $\sim 8 \times 10^{-4}$.

Finally, the fabricated embedded waveguides are integrated with a fluorescence chamber for experimental observation of enhanced fluorescence emission due to bimetallic silver-gold layer induced SP wave extension. Rhodamine B solution, placed on top of metallic films with various silver and gold thickness combinations, are excited via SP evanescent fields and the intensity of the collected fluorescence presents a direct analysis of the SP fields penetration depth. The use of a bimetallic silver – gold layer allows us to obtain enhanced fluorescence emission of up to 4× improvement due to SP wave extension. Extending the bimetallic silver – gold SPR configuration for sensing, it was demonstrated that BSA aggregations occurring at increased distances from the metallic surface can be monitored as well. The better electric field enhancement of the two-layered metallic film configuration facilitates several applications. In SPR – fluorescence spectroscopy, less concentrated fluorophore-tagged analyte solution can be monitored. In sensing, biological bindings that occur further into the analyte can be probed and longer ligands can be immobilized on the sensing surface.

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CHAPTER 1

INTRODUCTION

1.1 Motivation

Since the dawn age, humans have always been interested to know more about the environment they interact with. The rapid civilization process of mankind, swift advancement in technology and surging human population have prompted mankind to be consistently conscious of their health, environment and surroundings. In the quest to build a Utopian environment for everyone to live in, the need to track and monitor environmental conditions is a fundamental necessity. Amidst the heightening global terrorist peril in recent years, which threaten mankind with the proliferation of nuclear, chemical and biological weapons, sensors capable of monitoring the quality of air, water and food is of paramount importance. Over the last decade, sensor technology has progressed into a new era where application into healthcare and bio-analysis is now taking the limelight. Sensitivity, accuracy, device miniaturization, low cost of production, ease of disposal and point-of-care diagnostics are the main focuses for most biosensor research and development projects. Generally, biosensors consist of a transducer and a biological recognition element which

interacts with a specific analyte. The fundamental principle of a biosensor is to detect this molecular recognition and translate it into another type of signal using a transducer. The recognition system provides the sensor with high degree of selectivity for the analyte to be measured. Most affinity biosensors can be categorized into one of three main classes, based on the transduction methods employed. These classes are namely the optical detection methods [1-6], electrochemical detection methods [7-9], and mass-sensitive detection methods [10, 11].

There are numerous advantages of employing optics for biosensing applications. Optical sensing schemes are sensitive since single photons can be detected, enabling sensing down to the single molecule level. Besides, as optical signals at different frequencies do not interfere with one another, multiple light signals can be employed concurrently. Different biological tissues, cells or proteins demonstrate unique response reactions when illuminated with light of different wavelength. Therefore, photoablation can be achieved by employing the wavelength with highest absorption, while generally non-destructive analysis will be possible by using a probing field with the least absorption. Last but not least, optical signals travel in an open path and hence, wires or other transmitting conduits are not necessary. This feature enables remote measurements to be made.

Thus far, optical sensors working on the principles of fluorescence [12-14], surface plasmon resonance (SPR) [15, 16], near-infrared absorption [17], reflectometric interference [18], Raman and surface enhanced Raman scattering [1, 19, 20] etc. have been reported, each of them having specific strengths for different applications. Among these techniques, fluorescence spectroscopy has shown to demonstrate single molecule detection (SMD) [21], with the aid of high sensitivity detectors such as cool photo-multiplier tube or cool CCD camera. However, the possibility of introducing toxicity and modifying the viability of labeled molecules and cells upon chemical binding with a fluorophore is a major concern

when employing this technique. Moreover, only one or several molecules can be observed at one time and the costly and bulky equipment involves confine the exploitation of fluorescence spectroscopy for SMD within clinical and research laboratories.

All optical detection schemes, including fluorescence spectroscopy and SPR, demonstrate unique advantages for different applications and should be exploited depending on requirements. Since its discovery, SPR has proven to be a strong contender in optical detection of biological and chemical analytes. Although the limit of detection for SPR sensors is generally higher than that of fluorescence's, the edge of SPR lies in the label-free sensing and imaging of specific analytes in real-time. In optical detection applications where real-time monitoring is desirable, label-free sensing is enviable, device simplicity is required, and SMD is an overkill, SPR will in no doubt be the most suitable candidate. Since the first application of the surface plasmon resonance phenomenon for gas sensing was demonstrated almost two decades ago [22-24], this method has made great strides in instrumentation development and sensing applications. SPR sensor technology is now commercially available, and a large number of researchers are currently working to further extend the merits of SPR sensors. Meanwhile, SPR has slowly crept its way into biosensing applications, becoming a useful tool for characterizing and quantifying biomolecular interactions. SPR makes it possible to monitor binding processes as a function of time by following the increase in refractive index that occurs when one of the interacting partners binds to its ligand immobilized on the sensing surface. Being a technique that does not require the reactants to be labeled is a major advantage, simplifying the data collection process. Very minute changes in binding affinity can be measured with good precision, which is a prerequisite for analyzing the functional effect and thermodynamic implications of limited structural changes in interacting molecules.

The transduction principle involved in surface plasmon resonance sensors is based on the arrangement of a dielectric / metal / sample configuration such that when *p*-polarized light impinges on the metal surface from the dielectric medium, a surface wave is excited within the plasma formed by the conduction electrons of the metal under specific resonance conditions. By modulating the wavelength or incident angle of the incoming beam, the effect is observed as a minimum in the intensity of the light reflected off the metal surface. An SP evanescent field is generated due to the collective oscillations of electrons on the metal plasma, probing into the analyte medium within hundredths of nanometers from the metal surface. This electromagnetic field, from a mathematical perspective, has an imaginary Poynting vector and hence possesses no energy. Therefore, the penetration of the SP field into the adjacent medium is typically minimally invasive and non-destructive, paving a platform for clinical and biological applications. In addition, the plasmon resonance angle is naturally very sensitive to the dielectric constant of the sample medium immediately adjacent to the metal and therefore manifests itself for bioassay exploitation. Due to the intrinsic dependence of the index of refraction in the immediate proximity of the surface, surface plasmon resonance is popularly used as a sensor transducer to indicate when alterations at the surface occur. Refractive index changes in the order of $\sim 10^{-6}$ are easily detected with a conventional SPR sensor [25, 26], while even higher sensitivity ($\sim 10^{-8}$ RIU) can be achieved using phase interrogation [27] or interferometry. Common SPR applications include antibody–antigen recognition or DNA hybridization at the sensing surface.

Generally, there are two types of SPR immunoassays to measure the level of antibody–antigen binding, either competitive or non-competitive. In whichever assay format, ligands that are several tens of nanometer thick are immobilized on the metal surface in order to capture the desired analyte for sensing. Therefore, it is essential that the SP evanescent field, which penetrates into the biological sample, is strong enough to sense the ligand–analyte interactions. Since the evanescent field decays exponentially into the biological sample, it is

usually desirable that the size of the ligands immobilized on the metal surface is minimized so as to achieve the maximum sensitivity. In the quest of improvising SPR for more applications, it is enviable to enhance the surface electric field strength and extend the penetration depth of the SPs evanescent field. In sensing and imaging applications, it will be possible to increase sensitivity, better discriminate specific binding from nonspecific absorption [28], reach detection antibody in sandwich immunoassay, monitor/image bigger cells and immobilized longer ligands on the sensing surface if the evanescent wave possesses longer probing depths. Additionally, faster cells manipulation and sorting [29, 30] will be achievable with a stronger electromagnetic field.

Sarid [31] and several researchers [32, 33] have shown that it is possible to further extend the surface plasmon (SP) evanescent field through the excitation of long-range surface plasmons (LRSPs). Surface plasma waves existing on opposite sides of a thin metal surface couple together and eventually lead to sensitivity enhancement. Such technique requires an additional procedure to coat a Teflon or magnesium fluoride buffer layer, sandwiched between a prism and metal film. This added step is expensive and time consuming, and the surface quality of the buffer layer will directly affect the performance of the sensor. Additionally, one potential disadvantage of LRSPs for sensing in an imaging format is that the longer propagation length reduces the lateral resolution of the image.

For most SPR sensors, surface plasmons are excited on a single layer gold film to achieve stable test results as well as ride on the more matured ligand immobilization techniques on gold surfaces. In terms of practicality, gold is a good option as it is very resistant to oxidation and other atmospheric contaminants but sufficiently reactive to accommodate coating with a wide variety of binding molecules. Gold, though having excellent chemical stability and better bio-molecular adhesion, does not enhance the evanescent field as well as silver. Potentially, any enhancement to the SP evanescent field should yield a device capable of

monitoring bio-molecular interactions further into the analyte. In contrast, silver offers the greatest sensitivity due to the largest enhancement of the electric field and sharper SPR reflectivity dip (narrower FWHM). However, silver's high susceptibility to oxidation and bio-non-compatibility are its major limitations, especially for biosensing applications. Other metals are not as sensitive and practical: Indium (In) is too expensive; Sodium (Na) is violently reactive; Copper (Cu) and Aluminum (Al) yield broad SPR response.

In this thesis, the two-layer silver-gold configuration for SPs excitation is studied comprehensively. Potentially, this configuration integrates the better evanescent field enhancement of silver, the narrower FWHM of the SPR curve achievable with silver, and the high chemical resistance and biocompatibility of gold. Experimentally, this configuration can easily be realized as the films can be deposited consecutively in a single deposition process with the electron-beam evaporator without breaking vacuum.

1.2 Objectives

Surface plasmon, since its discovery, has been employed in multifarious applications. The main focus of this thesis is to study and explore the enhancement achievable with the two-layer silver-gold metallic film configuration for surface plasmon resonance applications, with specific emphasis on the improvement in signal-to-noise-ratio and sensitivities. Primarily, the prism and optical waveguide schemes are adopted for the coupling of photons into surface plasmons. Followings are the broadly classified objectives of the thesis:

a) Development of a theoretical model for bimetallic-film SPR

To model the SPR curve, electric field intensity on the metal surface and penetration depth of the SP evanescent field during resonance, considering a two-layer metallic film

SPR configuration. To discuss quantitatively, the difference in sensor's performance between the single and double metallic layer SPR configurations.

- b) Experimental comparison of the SNR achievable with the single and double metallic layer SPR configurations, using the prism in-coupling scheme

With the theoretical model, optimize the film thickness to achieve minimum resonance dip and hence maximum SP field enhancement. To verify experimentally the simulated SPR curves by employing the attenuated-total-reflection configuration to monitor analytes of various refractive indexes.

- c) Experimental comparison of the sensitivity ($d\lambda/dn$) achievable with the single and double metallic layer SPR configurations, using the waveguide in-coupling scheme

To fabricate surface relief waveguides with commercial photosensitive materials using contact-mask lithography. To employ the waveguide SPR scheme to monitor glucose solution of different concentrations with the single silver film, single gold film and two-layer silver-gold film configurations. To study and compare the sensitivity, in terms of the change in resonance wavelength for a given change in refractive index ($d\lambda/dn$), for the three metallic configurations.

- d) Fabrication of index-modulated embedded waveguides

To explore new techniques for the simple fabrication of planar embedded waveguides. To optimize the fabrication processes and characterize the fabricated waveguides.

- e) Experimental comparison of the sensitivity (probing depth) achievable with the single and double metallic layer SPR configurations, using waveguide SPR – fluorescence excitation

To design a fluorescence chamber and integrate it with the embedded waveguide to realize a waveguide SPR-fluorescence microchip. To exploit various metallic film configurations for the excitation of fluorescence dyes with SP evanescent fields. To examine the fluorescence emission spectrum from dyes on top of metal-coated waveguides for the various metallic configurations. Last but not least, to monitor in real-time, protein aggregations on top of metal-coated waveguides for the various metallic configurations.

1.3 Originality of Thesis

The major contributions of this thesis are on the study of surface plasmon resonance and the enhancement possible with a two-layer silver-gold metallic film for surface plasmons excitation. Predominantly, this configuration integrates the better evanescent field enhancement of silver, the improved SNR of the SPR curve achievable with silver, and the high chemical resistance and biocompatibility of gold. This thesis aims to investigate these enhancements and verify them experimentally.

First, a multi-layer model was employed to simulate the SPR curves, electric field intensity on the metal surface and penetration depth of the SP evanescent field during resonance, considering magnetic field continuity since SPs can only be stimulated with a p -polarized incident beam. With this theoretical model, the film thicknesses for different metallic configurations (single silver film, single gold film and bimetallic silver-gold) are optimized to achieve maximum SP field enhancement. Thereupon, the differences in the FWHM of the reflectivity curves, changes in resonance wavelength for a given change in refractive index ($d\lambda/dn$) and coverage of the sensing region for the various metallic film configurations are meticulously discussed.

To verify the reflectivity curves experimentally, the Kretschmann prism in-coupling scheme is employed to monitor various analytes using different metallic configurations. The experimental results yield the FWHM of the reflectivity curves and the change in resonance angle for a given change in refractive index ($d\theta/dn$) of the respective configurations, enabling a comprehensive study of these two parameters. In addition, the waveguide in-coupling scheme is exploited for SPR sensing of glucose solutions with different concentrations. Surface relief waveguides are first fabricated in-house using contact mask lithography, before various metallic films are deposited on the waveguides' surface. The first example of a wavelength-modulated waveguide SPR sensor with a bimetallic silver-gold film for SPR sensitivity enhancement is reported. In the aforementioned studies, only the SPR reflectivity/transmission curves are investigated. An alternative technique is necessary for the examination of the SP field penetration depth.

Integrating SPR with fluorescence spectroscopy, it will be possible to evaluate the SP field induced on different metallic films. This SP evanescent field can excite fluorescence dyes in the vicinity of the metal surface, and the emission intensity enables the comparison of the SP field. Conceptually, SPR-excited-fluorescence can be realized using either the prism or waveguide in-coupling scheme. The investigation of waveguide SPR for fluorescence is of significant research value, especially in recent years where lab-on-a-chip, decentralized-diagnostics-home-healthcare and point-of-care diagnosis initiatives are widespread. Advanced lithography enables the fabrication of such elements at reduced cost as well. Hence, it will be a prudent choice to exploit waveguide SPR for on-chip fluorescence studies. To realize this, index-modulated embedded waveguides are necessary for the firm integration between the fluorescence chamber and planar waveguides.

Two new techniques for fabricating step-index embedded waveguides using commercial photosensitive materials are proposed and discussed in this thesis. Briefly, both methods

achieved refractive modulation through thermal densification of the photosensitive material, prior to polymerization. The first, known as photothermal lithography, involves photo-induced polymerization of the UV-exposed regions under a patterned mask, and subsequently cross-linking of the unexposed regions via high thermal treatment. The second technique, known as dual-UV exposure lithography, involves a UV expose – bake – UV expose procedure to marginally modulate the refractive index between the initially exposed and later exposed regions.

Prior to the experimental study of waveguide SPR-fluorescence, a rectangular fluorescence chamber is engraved onto a double-sided adhesive Mylar (100 μm thick) using a direct-write CO_2 -laser cutting system and pasted onto the planar waveguides. Fluorescence (Rhodamine B) solution is injected into the chamber and a thin Mylar film (30 μm thick) is placed on top of the double-sided adhesive Mylar to seal up the chamber. The emission spectrum of the fluorescence particles excited with the SP evanescent field induced on various metallic films are measured and analyzed. Furthermore, the proposed SPR configuration has also been employed for real-time monitoring BSA aggregations on the metallic surface, based on transmission measurement. An in-depth account of the two-layer metallic film induced SP field enhancement is given in this thesis.

1.4 Organization of Thesis

This thesis is organized into eight chapters and the flow is structured as below.

Chapter 1 presents the introduction, encompassing the motivation for this work, objectives, major contributions and the organization of this thesis.

Chapter 2 contains a detailed description of the background on the topics and techniques used in the exploration of this thesis. In particular, the evolution and impact of surface plasmons, fundamentals of the SPR phenomenon, modulation techniques, and applications of SPR are discussed.

Chapter 3 outlines the mathematical modeling of a multi-metallic-layer SPR configuration, with specific focus on the SPR curves, electric field intensity on the metal surface and penetration depth of the SP evanescent field during resonance.

Chapter 4 depicts the experimental verification of the SPR reflectivity curves for various metallic configurations using the Kretschmann prism in-coupling scheme. An in-depth discussion on the enhancement possible with the two-layer silver-gold configuration is presented.

Chapter 5 reports a wavelength-modulated waveguide SPR sensor with a bimetallic silver-gold film for SPR sensitivity enhancement.

Chapter 6 presents two new techniques for fabricating index-modulated embedded waveguides using commercial photosensitive materials. The quality of the waveguides fabricated with these two processes is evaluated.

Chapter 7 describes an extension of SPR for fluorescence and continuous protein monitoring applications. The measured fluorescence intensities and waveguide transmittance allow us to proceed with a comparative study on the SP field excited on various metallic structures.

Chapter 8 concludes the report, as well as presents a proposal for the future direction of this research project.

CHAPTER 2

SURFACE PLASMONS & ITS METHODOLOGY

2.1 A glimpse in history: How it all started

The advancement of mankind's understanding of optics has taken numerous milestones spanning across several centuries from 1600s to 1800s, notably significant contributions in chronological order from Isaac Newton, Christiaan Huygens, Thomas Young, Augustin Jean Fresnel, Joseph Fraunhofer, Michael Faraday and James Clerk Maxwell. Surface Plasmon (SP), from an analytical perspective, is a marriage between optics and electronics. Parallel progressions of optics and electronics have brought about the evolution of surface plasmons less than a century ago. It all began in 1925 when Langmuir noticed that a stream of electrons projected onto ionized mercury vapour at a given velocity will be scattered in such a way that some of the electrons will acquire a considerable increment in velocity and a comparable number of electrons would have been retarded by the same amount [34]. Subsequently, he moved on to develop the theory of electronic and ionic oscillations in an ionized gas together with Tonks in 1929 [35]. Two decades later, Bohm and Gross proposed the theory of electrons oscillations of an unbounded plasma, discussing the origin of medium-liked behavior [36] and the excitation and damping of electronic oscillations [37].

The breakthrough of plasmons began in 1952, where Pines and Bohm attributed energy losses of fast electrons passing through foils to excitation of plasma oscillations in a sea of conduction electrons [38]. Consequently, Ritchie demonstrated in 1957 [39] that electron charges on a metal boundary can perform coherent fluctuations, bringing about wide recognition of surface plasmons (SPs) in the field of surface science. A major leap in SPs application was in 1959 where Turbadar first observed and predicted from thin film theory the complete absorption of an incident beam by a thin aluminum film [40]. In 1968, Otto proposed a new configuration for the optical excitation of SPs, employing evanescent waves produced by attenuated-total-reflection to stimulate SPs on bulk metal, across a very thin air gap [41]. Concurrently in the same year, Kretschmann and Raether furthered Turbadar's work and proposed a practical and commonly used method to excite plasmons on a thin metal film, where they bridged the momentum mismatch between light and SPs using an angle modulated incident beam through a prism [42]. The potential of SPR for characterization of thin films and monitoring process at metal surfaces slowly gained more recognition in the late seventies. In 1982/83, the use of SPR for gas detection and biosensing was first demonstrated by Nylander and Liedberg [22, 23] and this triggered a series of researches into this area.

As a result of these initial observations, Pharmacia of Sweden became interested in using SPR as an instrument for the study of interactions between biomolecules. Together with the Linköping Institute of Technology and the Swedish National Defense Research Institute (FOA), they created Pharmacia Biosensor AB in 1984, the company which later became Biacore AB in 1996. After years of very successful growth, Biacore was acquired by GE Healthcare recently in 2006. Other than Biacore (<http://www.biacore.com>), there are at least seven other companies that manufacture a variety of biosensor hardware, and they are Affinity Sensors (www.affinity-sensors.com), Windsor Scientific Limited (www.windsorscientific.co.uk), Ssens (www.ssens.nl), IBIS Technologies (www.ibis-spr.nl),

Hofmann Sensorsysteme (www.plasmonic.de), GWC instruments
(www.gwctechnologies.com) and Sensata Technologies (www.sensata.com) [43], to mention a few. SPR sensors are creeping into clinical assays for detection of biologically active compounds because of their simpler and quicker detection in comparison with labeling assay methods.

2.2 **Impact of surface plasmons (SPs)**

Thus far, surface plasmon has been exploited extensively in numerous areas. The use of SPs for sensing has erupted since Nylander and Liedberg demonstrated the use of SPR for gas detection and biosensing. One of the many advantages of employing SPR in biosensing lies in its capability of monitoring binding interactions continuously, without the need for any labelling of the biomolecules. Any binding events can be observed in real-time providing potentially rapid response. Beside, generic SPR platforms can be tailored for detection of any analyte, as long as a biomolecular recognition element recognizing the analyte is available. The analyte does not have to exhibit any special properties such as fluorescence or characteristic absorption and scattering bands. SPR has shown great promise in the real-time determination of the concentration, kinetic constant and binding specificity of individual biomolecular interactions. Antibody-antigen interactions, peptide-protein interactions and DNA hybridization conditions can all be analyzed [3, 44-47]. Furthermore, the potential of SPs for microscopy and high contrast imaging applications was first proposed by Rothenhäusler and Knoll in 1988 [48]. As the penetration depth of SPs evanescent field extends from sub-nanometer to hundreds of nanometers, surface plasmon resonance imaging (SPRi) is capable of mapping 3-dimensional topographies of cells with nanometres vertical resolution [49]. Additionally, SPRi has popularly been used in microarray based immunoassays for the high-throughput detection of biological binding interactions [50, 51], as well as in the measurements of ultra-thin organic films [52]. Lately, the SPs evanescent

field has found further applications in fluorescence spectroscopy [53-55]. The versatility of SPs for probing real refractive index changes and exciting fluorophores makes it an attractive technique for both sensing and fluorescence applications.

When gold are reduced from bulk form into colloids of tens of nanometers, they exhibit totally new optical characteristics. Noble metal nano-particles, usually silver or gold, strongly interact with visible light triggering a resonance in the collective oscillations of the conduction electrons within the particles, in response to incident electromagnetic field. As a result, local electromagnetic fields near the particle surface are orders of magnitude higher than that of incident fields, and the incident electromagnetic wave is heavily scattered by the particles at the resonant peak wavelength. The recent progress in nanoshells, a new class of nanoparticles with tunable plasmon resonance, allows materials to be specifically designed to match the wavelength required for a particular application, for instance to fall within near-infrared (NIR) regions where light penetration through tissue is optimal [56]. Basically, the structure of a nanoshell comprises a dielectric core coated with a thin layer of noble metal. By varying the composition and dimensions of the layers of the nanoparticles, nanoshells can be tailored to resonant plasmonically from the visible to infrared regions of the spectrum. Upon excitation by the resonance wavelength, nanoshells generate localized heating due to localized excitation of SPs, potentially enabling nanoshell-mediated thermal ablation therapies for applications such as cancer treatment. Thermal ablation therapies can provide a minimally invasive alternative to surgical excision of tumors and are particularly attractive for situations where surgery is not possible [57]. Nanoshell has already been successfully implemented in photo-thermal ablation of tumor in mice [58], imaging and *in vitro* therapy on breast cancer cells [59], and probably in clinical therapies and numerous biomedical applications in the near future.

In the current knowledge-based economy where digital information needs to be transmitted from one point to another at high bit rates, the performance of electronic circuits is now becoming rather limited. Photonics offers an effective solution to this problem through implementing high capability optical communication systems based on optical fibres and photonic circuits. Unfortunately, the micrometer-scale bulky photonics components have limited the integration of these components into electronic chips, which are now advancing into the size of nanometers. Surface plasmon-based circuits, which merge electronics and photonics at the nanometer-scale, has recently been proposed as a solution to this size-compatibility problem [60]. SPs can be exploited for plasmonic waveguides [61], switches or modulators, all in the size range of nanometers.

In recent years, SP has also been proposed to be employed in photolithography for the fabrication of nanometers-sized structures. Basically, there are two methods of achieving nano-fabrication. The first make use of interference between two SP fields, excited with the attenuated-total-reflection coupling method, for large area fabrication of structures. Finite-difference-time-domain simulations indicate that features as small as 60 nm can be fabricated [62]. The second technique relies on the enhanced optical transmission of light beaming through subwavelength apertures [63, 64]. Light usually diffracts in all directions when it emerges from a subwavelength aperture, which puts a lower limit on the size of features that can be used in photonics. However, the diffraction limit can be overcome by creating a periodic texture on the exit side of a single aperture in a metal film. Extraordinary transmission of light with small angular divergence occurs due to coupling of the light with the SPs of the two-dimensional array of sub-wavelength holes. With a 365 nm light source, dot array patterns of diameter 90 nm and period 170 nm, well beyond the diffraction limit of far-field optical lithography, has been fabricated [65]. Additionally, the enhanced transmission of light through nano-hole array can be applied for sensing as well, with the potential of monitoring refractive index changes in the order of 10^{-6} [66]. More recently,

surface plasmon excited with periodic subwavelength structures has taken another huge leap forward due to the breakthrough research on designer SPs, which promises the ability to engineer SPs at almost any frequency [67, 68]. This enables the excitation of SPs even in the terahertz range, a regime where SPs localization was never possible before!

2.3 Methodology of surface plasmon resonance (SPR)

2.3.1 Plasma theory

The free electrons of a metal may be treated as an electron liquid of high density. Therefore, it will be possible for longitudinal density fluctuations (plasma oscillations) to propagate through the volume of the metal with a plasma frequency, given by the equation [69, 70]:

$$\omega_p = \sqrt{\frac{Nq^2}{m\epsilon_0}} \dots\dots\dots (2-1)$$

where N is the equilibrium electron density, q is the electron charge, m is the rest mass of an electron and ϵ_0 is the permittivity of free space. In turn, the plasma dielectric constant of the electron gas in metal as a function of frequency, ω , in the non-collision case can be approximated from the Drude model [71] to arrive at the dielectric function model defined by [72]:

$$\epsilon_m(\omega) = 1 - \frac{\omega_p^2}{\omega^2} \dots\dots\dots (2-2)$$

Below the plasma frequency, the electrons in the metal are able to screen the electric field of an incoming beam and reflect a large portion of light. As the refractive of metal is complex, the transmitted portion of the incident wave decays exponentially within it. On the contrary,

above the plasma frequency, the electrons in the metal cannot respond fast enough to screen an incident electromagnetic wave. The refractive index becomes real, and the incident beam can propagate through the metal. In most metals, the plasma frequency usually lies in the ultraviolet region, making them shiny and reflective in the visible range. Some metals, for instance copper, have a plasma frequency in the visible range, yielding its distinct colour.

2.3.2 Fundamentals of surface plasmons

Consider a neutral plasma (example, free-electron-like metals), consisting of a gas of positively charged ions and negatively charged electrons. If one displaces all of the electrons by a tiny amount with respect to the ions, the Coulomb force pulls back, acting as a restoring force. Hence, on the plasma surface, collective resonating oscillation of free electrons may exist, producing a charge density wave propagating along the plasma surface. These charge fluctuations, called the surface plasmon, are accompanied by a mixed transversal and longitudinal electromagnetic field which disappears at $|z| \rightarrow \infty$, and has its maximum on the metal surface, typical for surface waves. This explains their sensitivity to surface properties [73].

Figure 2-1: (a) Charge oscillations on metal surfaces and the electromagnetic field components of the SPs, and; (b) Exponential decay of the SP field in metal and dielectric (redrawn from [61])

The electric field of the surface plasmon propagating on a metal surface ($z = 0$) in the x -direction can be expressed as:

$$E_{SP}(x, z) = E_0 \exp[i(k_{SP}x - k_z |z|)] \quad \dots\dots\dots (2-3)$$

This field corresponds to a surface mode propagating along the metal surface with wavevector k_{SP} and decaying from the surface exponentially with decay function $\exp[i(k_z |z|)]$. The surface plasmon dispersion relation on a smooth semi-infinite metal surface is given by:

$$k_{SP} = \frac{\omega}{c} \left(\frac{\epsilon_2 \epsilon_3}{\epsilon_2 + \epsilon_3} \right)^{1/2} = k'_{SP} + i k''_{SP} \quad \dots\dots\dots (2-4)$$

where ω/c is the wavevector of light in vacuum, ϵ_3 is the dielectric constant of the adjacent medium and $\epsilon_2 = \epsilon'_2 + i\epsilon''_2$ is the dielectric constant of the metal. If we assume that both ω and ϵ_3 are real, and $\epsilon''_2 < |\epsilon'_2|$, we obtain a complex $k_{SP} = k'_{SP} + i k''_{SP}$ with:

$$k'_{SP} = \frac{\omega}{c} \left(\frac{\epsilon'_2 \epsilon_3}{\epsilon'_2 + \epsilon_3} \right)^{1/2} \dots\dots\dots (2-5)$$

$$k''_{SP} = \frac{\omega}{c} \left(\frac{\epsilon'_2 \epsilon_3}{\epsilon'_2 + \epsilon_3} \right)^{3/2} \frac{\epsilon''_2}{2(\epsilon'_2)^2} \dots\dots\dots (2-6)$$

The real part of k_{SP} , k'_{SP} , represents the propagation constant while the imaginary part, k''_{SP} , represents the damping (internal absorption) constant of the surface plasmon oscillations. In order to obtain a real k'_{SP} , it is required for $\epsilon'_2 < 0$ and $|\epsilon'_2| > \epsilon_3$, which can only be fulfilled in a metal as well as a doped semiconductor near the eigen frequency. From Equation 2-5, it follows that k'_{SP} approaches an infinitely large value when $\epsilon'_2 = -\epsilon_3$. Correspondingly, this also implies that the wavelength approaches the short wavelength limit and at this limit, the surface plasmons become a localized fluctuation, also known as localized surface plasmon resonance. Substituting into Equation (2-2), the surface plasmon cutoff frequency, ω_{SP} , is given by:

$$\omega_{SP} = \frac{\omega_p}{\sqrt{\epsilon_3 + 1}} \dots\dots\dots (2-7)$$

The surface plasmon cutoff frequency can be viewed as the minimum wavelength to excite SPs and is approximately 500 nm for gold, 400 nm for silver, 550 nm for copper and 200 nm for aluminum, with air as the analyte [74]. The intensity of SPs propagating along a smooth surface decreases as $\exp(-2 k_{SP}''x)$. The length L_i after which the intensity decreases to $1/\exp(1)$ is given by [73]:

$$L_{SP} = 1/(2k_{SP}'') = \frac{c}{\omega} \left(\frac{\epsilon'_2 + \epsilon_3}{\epsilon'_2 \epsilon_3} \right)^{3/2} \frac{(\epsilon'_2)^2}{\epsilon_2''} \dots\dots\dots (2-8)$$

The dispersion relation of surface plasmons on smooth surfaces (line 2) is shown in Figure 2-2, together with the photon dispersions in vacuum (line 1) and in a dielectric medium (line 3) of dielectric constant ϵ_l [75, 76].

Figure 2-2: Dispersion relations of nonradiative surface plasmons on a smooth semi-finite metal surface, light in vacuum and light in a dielectric medium

It is well known that the momentum of optical light, p , is related to its wavevector, k , linearly via the relationship: $p = \hbar k$, where $\hbar$ represents the Dirac constant (reduced Planck constant). As seen from the figure, at frequency ω_l , light in vacuum has a momentum ($\hbar \times \Delta k_{x1}$) lower than that of surface plasmons and hence, will not be able to excite them due to insufficient momentum. On the other hand, light in an optically denser medium possesses a momentum ($\hbar \times \Delta k_{x2}$) higher than that of surface plasmons and hence, are not able to resonate with SPs as well due to the momentum mismatch. The resonating condition for SPs excitation occurs only when the momentum (or wavevector) of the incident beam matches exactly with that of SPs. Two possible scenarios to bridge the momentum disparity at frequency ω_l , i.e. either (1) increase the wavevector of light in vacuum by exactly Δk_{x1} or (2) decrease the wavevector of light in an optically denser medium by exactly Δk_{x2} , will be discuss in detail in subsections 2.3.5.4 and 2.3.5.2 respectively. Several modulation schemes to realize the strict SPs

excitation condition will be discussed in the later portion of this chapter as well. An interesting point to note is that at large k_x , the group velocity and the phase velocity of SPs approach zero. Therefore, the SPs resemble a localized fluctuation of the electron plasma.

The factor k_z , which determines the SP field decay from the surface, is given as:

$$k_{z3} = \sqrt{\epsilon_3 \left(\frac{\omega}{c} \right)^2 - k_{SP}^2} \quad \text{in dielectric} \quad \dots\dots\dots (2-9)$$

$$k_{z2} = \sqrt{\epsilon_2 \left(\frac{\omega}{c} \right)^2 - k_{SP}^2} \quad \text{in metal} \dots\dots\dots (2-10)$$

The value of the depth (skin depth) at which the field falls to $1/\exp(1)$ is defined as the reciprocal of the magnitude of k_z . As the relative permittivity of a metal is usually greater than the dielectric constant of a dielectric, the SP field penetrates deeper into the adjacent dielectric as compared to the metal.

2.3.3 Internal and radiation damping

Surface plasmons can undergo two damping processes on a smooth metal surface, namely the internal damping and radiation damping.

Internal damping, as described by Equations 2-4 and 2-6, occurs when the electromagnetic field of the SPs excites electron-hole pairs at the Fermi-level. The following de-excitation produces phonons and thus heating. It has been shown that for rough metal surfaces, the energy reduction in the reflected light can be measured from the heat energy in the metal film. Therefore, the power lost from light decaying into SPs can be detected. However, the signal is below the limit of measurements at smooth surfaces.

Radiation arises when SPs propagate on a smooth metal film of finite thickness, which is coated on a dielectric material. The SPs, an evanescent wave, transform into a plane wave in the dielectric medium and this is known as radiation damping, characteristic for an asymmetric two-interface medium. The presence of SPs radiation damping on a thin metal film alters the SP dispersion relation given by Equation 2-2, which is valid only for SPs on a semi-infinite metal. The extent of radiation damping varies depending on the thickness of the metal film. As the film thickness increases, radiation damping approaches zero and the SP dispersion relation transforms back into Equation 2-2.

2.3.4 Modulation techniques for SPs excitation

In order to achieve matching conditions between the incident light and surface plasmons, we can modulate several parameters of the optical SPR system. Revisiting Figure 2.2 which depicts the momentum mismatch between SPs and light in vacuum or a denser medium, we analyze the possible modulation methods to realize momentum matching. Primarily, this involves shifting the dispersion relations of either the incident beam or SPs by changing some experimental parameters.

2.3.4.1 Angle modulation

Although laser technology has advanced by leaps and bounds since its discovery, they are available only in specific wavelengths due to the discrete bandgap of the gain medium used for lasing. Therefore, excitation of SPs at a particular frequency ω_1 is an important consideration. As described in Figure 2-3, the momentum of light in vacuum/air is insufficient to fulfill the resonance condition of SPs. On the contrary, light in a denser medium possesses momentum far greater than that of SPs. Principally, resonance will be possible if the momentum of light in the optically denser medium is reduced by Δk_{x2} . This

reduction of momentum can easily be achieved if the input beam is incident onto the metallic film at an angle. Therefore, the component of light, resolved in the direction of SPs possesses a wavevector k_x given by $k \sin \theta$. This approach of altering the momentum of the incident beam is termed as angle modulation, which will be discussed in more details in subsection 2.3.5.2.

Figure 2-3: Reducing the momentum of light in the denser medium to achieve SPs resonance on a smooth semi-finite metal surface

2.3.4.2 Intensity modulation

Another alternative to achieve SPs resonance will be to shift the SPs dispersion relation downwards, as shown in Figure 2-4. From the SPs dispersion relation equation, it is obvious that the SPs excitation condition is dependent on the dielectric constant, ϵ_3 , of the adjacent sample medium. Modulation of the analyte sample's dielectric constant, from ϵ_3 to ϵ_3' , can be achieved simply by changing the concentration of the sample solution. This method of altering the momentum required for SPs excitation is termed as intensity modulation. However, this is not a feasible option as it requires samples of known refractive indexes and SPR is usually used for monitoring analytes of unknown concentrations.

Figure 2-4: Downshifting the SPs dispersion condition to achieve SPs resonance on a smooth semi-finite metal surface

2.3.4.3 Wavelength modulation

A third approach to realize momentum matching will be to use an array of wavelengths concurrently, for instance a broadband light source. One of the many wavelengths will be able to coincide with the strict SPs resonance condition. This approach of using incident beams with an array of momentums is termed as wavelength modulation (an in-depth discussion is presented in sub-section 2.3.5.3). However, one important consideration to take note is that SPs can only be excited using a *p*-polarized beam. Hence, collimating and polarizing light from a broadband source will be a challenge in this approach.

Figure 2-5: Employing a broadband light source with a broad spectrum to achieve SPs resonance on a smooth semi-finite metal surface

2.3.4.4 Other modulation techniques

The three modulations techniques mentioned earlier involve shifting the dispersion relations to realize the resonance condition for SPs excitation. These are generic modulation techniques which apply in all scenarios, including SPs excitation on bulk metal with the Otto setup for instance. Over the years, new modulations methods have surfaced to advance the capability of SPR sensors, including but limited to techniques such as phase [77, 78] and polarization [79, 80] modulations. However, these techniques can only be employed for metallic films of finite thicknesses, where SPs excitation is possible merely with the in-coupling schemes that will be discussed in the next section. As these techniques are not widespread at the moment, and neither referred to in the later portion of this thesis, in-depth methodologies will not be discussed in the section.

2.3.5 Schemes for SPs excitation

As seen from the SP dispersion relations, the SP wavevector is often larger than the photon wavevector. Therefore, light illuminating a smooth surface cannot be directly coupled to surface plasmons. In order to bridge the wavevector mismatch between SPs and the incident beam with the modulation techniques discussed earlier, several possible schemes can be employed. In this section, we discussed the Otto and Kretschmann schemes, as well as two other widely used configurations, the optical waveguide based-SPR system and grating coupler based-SPR system [25].

2.3.5.1 Otto configuration

Primarily, optical excitation of SPs on thick metal films can be realized with the Otto configuration geometry, who first proposed this scheme in 1968. Here, a prism is placed close to the metal surface, and an incident beam illuminates the prism hypotenuse at an angle larger than critical angle. The wavevector of light is increased in the optically dense medium and the occurrence of total-internal-reflection permits photons to tunnel through the thin air gap (as evanescent waves) between the prism and the metal surface. If the wavevector of the photons matches the condition for SPs excitation on the bulk metal (Equation 2-4), resonance energy transfer occurs and the intensity of the reflected light, R , plunges sharply at the specific angle of incidence.

Figure 2-6: Otto geometry for resonance excitation of SPs on bulk metal

Otto deserves great credit for being one of the pioneers to successfully propose a configuration for SPs excitation. However, this geometry is not largely employed nowadays for many applications due to the difficulty in maintaining a constant air gap to achieve repeatable results.

2.3.5.2 Kretschmann prism coupler

Figure 2-7: Kretschmann prism coupler based-SPR configuration

The Kretschmann prism coupling geometry is somewhat similar to Otto geometry, except that a thin metal film is now deposited on the prism surface instead of a thin air gap separating the two mediums. As the design is much easier to realize and results are highly repeatable, the Kretschmann geometry is one of the most popularly employed scheme for SPR. Based on the attenuated-total-reflection principle, light illuminates a metal surface through a dielectric prism at an angle larger than the critical angle of the prism-sample interface. Therefore, the wavevector of light is increased in the optically dense medium. At a certain angle of incidence where the in-plane photon wavevector in the prism matches the SP wavevector on the sample-metal interface, resonant light tunnels through the metal film and excites the surface plasmons. The in-plane photon wavevector in the prism is given by:

$$k_x = \frac{\omega}{c} \sqrt{\epsilon_1} \sin \theta \quad \dots\dots\dots (2-11)$$

In addition to angle modulation, wavelength interrogation for SPR will be possible as well. At a fixed incidence angle, θ , a change in wavelength alters the in-plane photon wavevector, k_x , as well. Under these resonant conditions, a sharp minimum is observed in the reflected intensity, R , from the prism as light can be coupled to SPs on the metal-dielectric sample interface with almost 100 % efficiency. A point to note is that SPs cannot be excited on the prism-metal interface as the wavevector of the SPs at this interface is greater than the photon wavevector in the prism at all angles of incidence. The SP dispersion relation given earlier in Equation 2-2 applies only for a semi-infinite metal film and slight modification is needed to be valid for a finite metal film of thickness d . The modified resonance wave vector in this context is defined as:

$$k_{SP} = k'_{SP} + ik''_{SP} + \Delta k_x = k'_{SP} + \text{Re}[\Delta k_x] + ik''_{SP} + \text{Im}[\Delta k_x] \quad \dots\dots\dots (2-12)$$

where $\text{Re}[\Delta k_x]$ causes a displacement of the resonance position (k'_{sp}); and $\text{Im}[\Delta k_x]$ gives a radiation damping term in addition to the internal damping (ik''_{sp}). The quantitative description of the minimum reflectivity can be given by Fresnel's equation for the three-layer system 1/2/3: 1 – prism; 2 – metal film of thickness d ; 3 – dielectric sample, e.g., air, water, biological samples, as shown in Figure 2-8.

Figure 2-8: Multiple reflections consideration of a three-layer system to simulate the SPR phenomenon

With E_0 as the incoming and E_r as the reflected electric field, the reflected intensity, R , for p -polarized light is given by [73]:

$$R = |r_{123}^p|^2 = \left| \frac{E_r^p}{E_0^p} \right|^2 = \left| \frac{r_{12}^p + r_{23}^p \exp(i2k_{z2}d)}{1 + r_{12}^p r_{23}^p \exp(i2k_{z2}d)} \right|^2 \quad \dots\dots\dots(2-13)$$

$$\text{where } r_{ik}^p = \left(\frac{k_{zi}}{\epsilon_i} - \frac{k_{zk}}{\epsilon_k} \right) / \left(\frac{k_{zi}}{\epsilon_i} + \frac{k_{zk}}{\epsilon_k} \right) \quad \dots\dots\dots(2-14)$$

The physics behind the reflectivity minimum is that the light wave, having passed the prism, is partially reflected at the prism-metal interface. Partially, it traverses the silver film of

thickness d , as an exponentially decaying wave. At the metal-dielectric sample interface, the exponentially decaying wave induces excitations which radiate light back into the silver film. If the metal thickness increases, the back-scattered film disappears due to increased absorption by the metal. Therefore, the value of R approaches a constant value for all angles of incidence. For decreasing metal thickness d , the back-scattered field increases. As the back-coupled light beams ($t_{12}r_{23}t_{21}$, $t_{12}r_{23}r_{21}r_{23}t_{21}$ etc.) are in anti-phase with the first reflection of the incoming wave (r_{12}), they interfere destructively at the prism-metal boundary and results in the minimum reflected intensity phenomenon. In the case of $R = 0$, no photons leave the prism. Energy conservation requires that the sum of the relative reflection, absorption and transmission, $R + A + T = 1$. Since $T = 0$ at angles larger than critical angle, and that $R = 0$ at the resonant condition to generate surface plasmons, it implies that all the energy is absorbed by the metal film. This leads to the generation of surface plasmons in the metal-dielectric sample interface and heat generation due to internal damping of surface plasmons.

2.3.5.3 Waveguide coupler

Essentially, light propagation through a waveguide relies on the principle of total-internal-reflection. Therefore, the SP excitation process in an optical waveguide based SPR system is in principle similar to that in the Kretschmann prism coupling geometry. A light wave is guided by the waveguide and as it enters the region with a thin metal overlay, it evanescently penetrates through the metal layer. If the resonance requirement of SPs coincides with the wavevectors of the guided modes, SPs will be excited on the metal-dielectric interface.

Figure 2-9: Optical waveguide based-SPR configuration

Comparing with the Kretschmann geometry, SPs excitation via a waveguide is usually modulated with the wavelength of the guided wave rather than the angle of incidence. This is because only modes that fulfill a self-consistency condition are supported in a waveguide. From the ray optics point of view, these modes strike the waveguide's boundaries at discrete angles. Therefore, it is an uphill task to excite SPs by angle modulation. Instead, observing the SPR phenomenon could be achieved by interrogating the wavelength of the guided optical wave. The matching condition between the guided mode of an integrated optical waveguide and surface plasmons may be fulfilled only within a narrow spectral band. Thus, when a broadband light is launched into the waveguide, the transmission spectrum exhibits a narrow dip at a particular wavelength due to SPR [81, 82].

The self-consistency condition, which supports mode propagation in waveguides, is such that the wave reproduces itself after every round trip. This condition can be satisfied if [83]:

$$\frac{2\sqrt{\epsilon_1}\pi}{\lambda} 2t \sin \theta_i - \gamma_1 - \gamma_2 = 2m\pi, m = 0, 1, 2, \dots \quad \dots\dots\dots(2-15)$$

where ϵ_1 is the dielectric constant of the waveguide, λ is the wavelength of light in vacuum, t is the thickness of the waveguide, θ_i is the angle of incidence, γ_1 is the phase shift introduced by the internal reflection at the waveguide-substrate interface and γ_2 is the phase change of the incident light upon reflection at the waveguide-air interface. The reflection phase shift, γ ,

is a function of the incident angle, θ , and it also depends on the polarization of the incident wave. In our discussion, we focused only on the scenario where the magnetic component of the incident light is perpendicular to the plane of incidence as surface plasmons can only be excited by p -polarized light. For TM polarization, γ can be obtained numerically by solving:

$$\tan \frac{\gamma}{2} = \frac{(\sin^2 \theta_i - \sin^2 \theta_c)^{1/2}}{\cos \theta_i \sin^2 \theta_c} \dots\dots\dots (2-16)$$

After determining the discrete incident angles of the supported modes, the photon wavevector in the direction of light propagation will essentially be similar to the prism's case, i.e. Equation 2-11, as shown in Figure 2-10.

Figure 2-10: Ray optics consideration of the optical waveguide SPR configuration

2.3.5.4 Grating coupler

If a metal surface is periodically distorted, an incident optical wave will be diffracted from the surface into a series of beams with various wavevectors. Therefore, components of the diffracted light whose wavevector coincides with the SPs wavevector will be coupled into surface plasmons.

Figure 2-11: Grating coupler based-SPR configuration

The general expression of the diffracted in-plane wavevector, taking into account that the incident beam may impinge onto the gratings at any angle, θ , from a medium (air/vacuum/glass etc) of dielectric function ϵ_1 , is given by [84]:

$$k_x = \frac{\omega}{c} \sqrt{\epsilon_1} \sin \theta \pm m \left(\frac{2\pi}{a} \right) = \frac{\omega}{c} \sqrt{\epsilon_1} \sin \theta \pm \Delta k_x \quad \dots\dots\dots(2-17)$$

where m is an integer and a is the grating constant. The grating coupler configuration can provide efficient coupling to both air-metal and dielectric sample-metal SP modes if the film thickness and grating profile depth is optimized. Resonance is observed as a minimum in the intensity of the reflected light. It is interesting to note that the depth of this minimum depends on the height, h , of the grating. There is a critical amplitude, h_c , at which the reflected intensity becomes zero, which indicates the strongest coupling of photons with SPs.

Additionally, SPs can be excited on a rough surface as well. Diffraction of light on surface features permits coupling to SP modes on both air-metal and dielectric sample-metal interfaces. This is possible since in the near field region, the diffracted components typically encompass all the wavevectors. However, the disadvantage with random roughness is the

coarse SP excitation conditions, which result in the low efficiency of light-to-SP coupling. Contrary to the excitation of SPs by photons, the reverse can take place too. SPs propagating along a grating or rough surface undergoes reduction in their wavevector such that the SP is transformed back into light.

2.3.6 Field enhancement due to surface plasmons

The excitation of surface plasmons on the metal surface produces an exponentially decreasing SP evanescent field in the analyte medium. This SP evanescent field has an enhancement many times of the incident electromagnetic field. The maximum value of this enhancement is given by the ratio of the maximum field intensity on the metal-dielectric sample interface, $|H(3/2)|^2$, to the incoming field intensity in the medium (prism/waveguide), $|H(1/2)|^2$ for p -polarized light [85].

$$\left(\frac{|H(3/2)|^2}{|H(1/2)|^2} \right)_{\max} = \frac{1}{\epsilon_3} \frac{2|\epsilon_2'|^2}{\epsilon_2''} \frac{a}{1+|\epsilon_2'|} \dots\dots\dots (2-18)$$

$$\text{where } a^2 = |\epsilon_2'|(\epsilon_1 - 1) - \epsilon_1 \dots\dots\dots (2-19)$$

Equation 2-18 conveniently gives the maximum magnetic field intensity enhancement factor as the magnetic field is continuous across all boundaries for p -polarized light. To avoid confusion since the electric field intensity is not continuous across interfaces for p -polarized light, the $N/(N-1)$ interface is referred to as the N side of this interface and vice-versa. In general, the enhancement of the electric field intensity can be obtained from:

$$\frac{|E(3/2)|^2}{|E(1/2)|^2} = \frac{\epsilon_1}{\epsilon_3} \left(\frac{|H(3/2)|^2}{|H(1/2)|^2} \right) \dots\dots\dots (2-20)$$

Assuming that $\epsilon_3 = 1$ (air), $\epsilon_f = 2.2$ (quartz) and at a wavelength of 600 nm, the maximum enhancement factors achievable for silver, gold, aluminum and copper are approximately 200, 30, 40 and 7 respectively. The choice of the metal to be used determines to a large extent, the sensitivity of the biosensor. As can be seen, the maximum enhancement of the electric field by thin silver film is much greater than the other metals. Therefore, in order to achieve a biosensor with the greatest sensitivity in terms of sensing range, silver will be the best option.

2.5 Conclusion

This chapter presents a detailed description on the pioneering works on SPs and its impact in spearheading numerous applications in green pastures. Additionally, the fundamentals and methodology of the SPR phenomenon, modulation techniques and popular configurations are also discussed comprehensively. This forms the groundwork for further research work which will be presented in the subsequent sections in this thesis.

CHAPTER 3

BIMETALLIC FILM SPR CONFIGURATION – THEORETICAL MODELING & SIMULATION

3.1 Overview of Chapter 3

The prospect of enhancing the performance of SPR sensors certainly extends the use of SPR for numerous applications potentially. Several research ideas for SPR enhancement have been proposed and employed by scientists and researchers globally. In the initial portion of this chapter, a brief overview of SPR enhancement techniques is discussed. Subsequently, the two-layer silver-gold film SPR configuration is modeled with the multi-layer beam interference approach.

3.2 A synopsis of SPR enhancement techniques

In order to monitor surface interactions in a sensitive and stable manner with SPR biosensors, there are several critical areas of interest. It is desirable that the SPR sensor is highly sensitive to binding interactions within the sensing region, the reflectivity dip has a

narrow full-width-half-maximum (FWHM), and the metal film is highly stable even under extreme environmental conditions, to mention a few. Due to different optical and chemical properties of individual metal films, the realization of all conditions is not possible concurrently with a conventional single metallic film SPR sensor.

Sarid [31], Nenninger [32] and Wark [33] *et al.* have shown that it is possible to further extend the SP evanescent field through the excitation of long-range surface plasmons (LRSPs). Surface plasma waves existing on opposite sides of a thin metal surface couple together, leading to sensitivity enhancement. To realize this technique, an additional procedure to coat a Teflon or magnesium fluoride buffer layer, sandwiched between a prism and metal film, is essential. This added step increases experimental cost, is time consuming, and the surface quality of the buffer layer will eventually affect the performance of the sensor. Moreover, one major disadvantage of LRSPs for imaging applications is that the longer propagation length reduces the lateral resolution of the image. Thus far, LRSPs have been demonstrated with the Kretschmann prism coupling geometry and there has been no related works on LRSPs excitation via other configurations.

3.3 Introducing the two-layered metallic film for SPR and the achievable advantages

In order to have a clearer understanding of SPs propagation on metal surfaces, the knowledge of metal properties under electromagnetic interference is of paramount importance. To be useful for SPR, a metal must have conduction band electrons capable of resonating with light at a suitable wavelength. The visible and near-infrared parts of the spectrum are particularly convenient because optical components and high performance detectors appropriate for this region are readily available. A variety of metallic elements satisfy this condition. They include silver, gold, copper, aluminum, sodium, and indium.

There are two critical limitations on the selection of a metal for sensor construction. The surface exposed to light must be pure metal. Oxides, sulfides and other films formed by atmospheric exposure interfere with SPR. The metal must also be compatible with the chemistries needed to perform assays. Specifically, the chemical attachment of antibodies or other binding molecules to the metal surface must not impair the resonance.

From the list of possible metals, silver (Ag) offers the greatest sensitivity due to the largest enhancement of the electric field, and highest SNR due to the sharper SPR reflectivity dip. However, silver begins to oxidize almost immediately upon exposure to atmosphere and demonstrates non-compatibility with most biological substances, rendering it unsuitable for biosensing applications. Other metals are not as sensitive and practical: Indium (In) is too expensive; Sodium (Na) is violently reactive; Copper (Cu) and Aluminum (Al) yield broad SPR response.

For most SPR sensors, a single gold film layer is employed for sensing so as to achieve stable test results as well as ride on the more matured ligand immobilization techniques on gold surfaces. In terms of practicality, gold (Au) is a good option as it is chemically resistant to oxidation but sufficiently reactive to accommodate coating with a wide variety of binding molecules. Although gold is widely known to possess excellent chemical stability and better bio-molecular adhesion, it does not enhance the SP evanescent field as well as silver during resonance. Potentially, any enhancement to the SP evanescent field should yield a device capable of monitoring bio-molecular interactions further into the analyte.

Further extension of the SP evanescent wave might not necessary be an edge for all sensing and imaging techniques, for example when selective excitation of fluorophores very near the sensing surface is required [86, 87]. Nevertheless, evanescent field extension does provide considerable advantages in several applications. In sensing and imaging applications, it will be possible to increase sensitivity, better discriminate specific binding from nonspecific

absorption [28], reach detection antibody in sandwich immunoassay, monitor/image bigger cells and immobilized longer ligands on the sensing surface if the evanescent wave possesses longer probing depths. Additionally, faster particles / cells manipulation and sorting [88-90] will be achievable with a stronger electromagnetic field.

The dispersion relations of SPs propagating on thin metal films coated on bulk metal substrates had extensively been discussed by several researchers since four decades ago [91, 92]. The idea of a thin underlying silver with a thin overlying gold film for SPR sensing with the attenuated-total-reflection configuration was first suggested in 1981 [93]. However, no further research has been performed thereupon till recently. In 2002, Wu and Ho discussed the SPR reflectivity curve simulation for the silver-gold configuration [94]. Simultaneously in the same year, Zynio presented some experimental SPR reflectivity curves [95]. In 2005, Gupta and Sharma suggested extending the bimetallic film configuration for optical fibre SPR sensing, presenting in-depth simulations and theoretical analyses on the achievable signal-to-noise-ratio and sensitivity [96, 97]. However, in the aforementioned articles, only the mathematical modeling for the bimetallic configuration is presented (except Zynio who presented some experimental results), and discussions focused mainly on the SPR reflectivity curves only.

In the following sections, the two-layer silver-gold configuration for SPs excitation is modeled with more considerations. Potentially, this configuration integrates the better evanescent field enhancement of silver, the narrower FWHM of the SPR curve achievable with silver, and the high chemical resistance and biocompatibility of gold. Earlier in Chapter 2, the dispersion relation for SPs propagating on bulk metal, as well as the thickness dependence of a thin metal film for SPR were meticulously discussed. In order to relate SPs excitation on a thin two-layer metallic film, further deliberation is necessary. This chapter

outlines the mathematical modeling of the two-layer metallic film configuration, with specific focus on the SPR curve, evanescent field and penetration depth simulations.

3.3.1 Properties of silver film

Silver, a transition metal, is a chemical element in the periodic table with the symbol **Ag** and atomic number 49. Silver has an atomic mass of ~ 107.9 g/mol, density of 10.49 g/cm³ and boiling point of 2435 K. Silver is found in native form, in various ores such as argentite (Ag₂S) and horn silver (AgCl). Silver metal, in its pure state, has a brilliant white metallic lustre. It is strong (a little harder than gold), malleable, ductile, and can endure extreme temperature ranges. Although silver is stable in pure air and water, it tarnishes easily when exposed due to reactions with sulphur compounds in the atmosphere forming silver sulphide. It has the highest electrical and heat conductivity of all metals known, with the lowest contact resistance. The silver pellets used in our deposition processes, with a purity of 99.999%, are purchased from Super Conductor Materials, Inc. The refractive index (n) and extinction coefficient (k) of silver, ranging from 100 nm to 800 nm in optical wavelength, is plotted in Figure 3-1 [98].

Figure 3-1: Refractive index, n , and extinction coefficient, k , of silver, ranging from 100 nm to 800 nm in optical wavelength [98]

3.3.2 Properties of gold film

Gold is a metallic element in the periodic table with the symbol **Au** and atomic number 79. Gold has an atomic mass of ~ 197.0 g/mol, density of 19.30 g/cm³ and boiling point of 3129 K. Due to its relative chemical inertness, gold is usually found as the native metal or alloy. Gold has characteristic yellow color and it is the most malleable and ductile metal known. Gold is a good conductor of heat and electricity, and is not affected by air and most reagents. Heat, moisture, oxygen, and most corrosive agents have very little chemical effect on gold. Although gold material is more expensive and has higher resistance than silver, the use of gold over silver in electronics and bio-related applications is a matter of longevity weighed against material cost. The gold pellets used in the deposition processes subsequently for SPR experiments have a purity of 99.999%. The refractive index (n) and extinction coefficient (k) of gold, ranging from 100 nm to 800 nm in optical wavelength, is plotted in Figure 3-2 [98].

Figure 3-2: Refractive index, n , and extinction coefficient, k , of gold, ranging from 100 nm to 800 nm in optical wavelength [98]

3.3.3 Functionalization of gold surfaces

The selectivity of SPR immunoassay is provided by a biological recognition element (either antibody or antigen depending on the assay) immobilized on the metal film surface. The versatility of this direct immunoassay is thus only limited by the user's discretion. However, successful immobilization of the recognition element on the transducing surface without disrupting the sensor's performance is vital. Thus far, there have been numerous methods for the functionalization of gold surfaces. For instance, tremendous study has been on-going and developed for (1) adsorption of antibodies on gold film [99, 100], (2) covalent attachment of biological recognition element to the metal film via a linker layer [101, 102], (3) utilizing a flexible hydrogel matrix composed of carboxymethylated dextran chains (100 – 200 nm) to form a porous three-dimensional linking matrix [103, 104], (4) employing organic silica compounds to attach small amounts of antibodies on gold surface [105, 106], (5) using gold-binding repeating polypeptide [107], and last but not least, (6) depositing a thin (~ 100 nm) active polymer film for further modification [108, 109]. The availability of many methods to functionalize gold surfaces broadens the viable applications of SPR.

3.4 Multiple-beam interference matrix method for reflection and transmission coefficients calculation

As discussed in Chapter 2, two popular configurations for SPs excitation employ prism and waveguide in-coupling. In these methods, a thin film is deposited on the prism or waveguide surface to achieve resonance between the incoming wave and SPs. The theory between the prism- or waveguide-based SPR configurations is somewhat similar as light is guided along a waveguide via total-internal-reflection as well. Therefore, the modeling and simulation discussed hereupon can be applied for both configurations. The only difference between the two is that the vertical axis of the SPR curve obtained with the prism setup is “reflectivity” whereas the waveguide setup yields “transmission” instead. In situations where SPs are excited on a thin metal film, a change in the bulk SPs dispersion relation (Equation 2-4) occurs due to an additional radiation damping term, as shown in Equation 2-12. In a simple three-layer system, prism (waveguide) / metal / sample for instance, the SPR reflectivity equation can be obtained simply by summing all the light reflected back into the prism (waveguide). However, in the case of a four-layer system consisting prism (waveguide) / silver / gold / sample, the reflectivity is not just a simple sum to infinity of a geometric progression. In order to analyze and compare the resolution of the minimum reflectivity and the degree of the evanescent field enhancement for the single and double-layer SPR configurations, the reflection and transmission coefficients are computed by using the multiple-beam interference matrix method [110]. The reflection coefficient yields the reflectance at various angles of incidence, while the transmission coefficient yields the respective evanescent field enhancement. As the excitation of SPs requires the incident electric field to be in plane while the magnetic field is perpendicular to the plane of incidence, only the magnetic field component of the incident beam is continuous across all boundaries. Hence, in the following sections, our analysis and simulations will be based on

the multiple reflections and transmissions of the magnetic fields, instead of the commonly used electric fields.

Figure 3-3: Schematic of reflections and transmissions for a N -layer system

For a N -layer system (layer 1: prism (waveguide), layer N : analyte) as shown in Figure 3-3, taking into account the combined effects of the multiple reflections between all the layers, the fields in the first ($H_{1,i}$: incident light; $H_{1,r}$: reflected light) and final ($H_{N,t}$: transmitted light) mediums are related by:

$$\begin{pmatrix} H_{1,r} \\ H_{1,i} \end{pmatrix} = \begin{pmatrix} S_{11} & S_{12} \\ S_{21} & S_{22} \end{pmatrix} \begin{pmatrix} 0 \\ H_{N,t} \end{pmatrix} \quad \dots\dots\dots (3-1)$$

$$\text{where } \begin{pmatrix} S_{11} & S_{12} \\ S_{21} & S_{22} \end{pmatrix} = S_{1,N} = T_{1,2} P_2 T_{2,3} P_3 \dots P_{N-1} T_{N-1,N} \quad \dots\dots\dots (3-2)$$

$T_{i,j}$ represents the transition matrix between the i - j interface, while P_j represents the propagation matrix in a medium, and they are given by:

$$T_{i,j} = \frac{1}{t_{ij}} \begin{pmatrix} 1 & r_{ij} \\ r_{ij} & 1 \end{pmatrix} \quad \dots\dots\dots (3-3)$$

$$P_j = \begin{pmatrix} \exp(ik_{z,j}d_j) & 0 \\ 0 & \exp(-ik_{z,j}d_j) \end{pmatrix} \quad \dots\dots\dots (3-4)$$

r_{ij} and t_{ij} are the Fresnel reflection and transmission coefficients at the i - j interface, and $\text{Re}(k_{zj}d_j)$ is the phase and $\text{Im}(k_{zj}d_j)$ is the absorption introduced to the field as it propagates through medium j of thickness d_j . Therefore, we obtain the reflection (r_{1N}) and transmission (t_{1N}) coefficients for the entire stack:

$$r_{1N} = \frac{H_{1,r}}{H_{1,i}} = \frac{S_{12}}{S_{22}} \quad \dots\dots\dots (3-5)$$

$$t_{1N} = \frac{H_{N,t}}{H_{1,i}} = \frac{1}{S_{22}} \quad \dots\dots\dots (3-6)$$

3.4.1 SPR reflectivity curves simulation

For p -polarized light, the Fresnel reflection coefficient is defined as:

$$r_{jk}^p = \left(\frac{k_{zj}}{\epsilon_j} - \frac{k_{zk}}{\epsilon_k} \right) / \left(\frac{k_{zj}}{\epsilon_j} + \frac{k_{zk}}{\epsilon_k} \right) \quad \dots\dots\dots (3-7)$$

$$\text{where } k_{zj} = \frac{\omega}{c} (\epsilon_j - \epsilon_1 \sin^2 \theta_1)^{1/2} \quad \dots\dots\dots (3-8)$$

and the boundary continuity condition is given as:

$$t_{jk}^p = 1 + r_{jk}^p \quad \dots\dots\dots (3-9)$$

Solving Equations 3-1 to 3-9 for a 3-layer system, the reflected intensity comes out as:

$$R_{123} = |r_{123}^p|^2 = \left| \frac{E_r^p}{E_i^p} \right|^2 = \left| \frac{H_{yr}}{H_{yi}} \right|^2 = \left| \frac{r_{12}^p + r_{23}^p \exp(i2k_{z2}d_2)}{1 + r_{12}^p r_{23}^p \exp(i2k_{z2}d_2)} \right|^2 \quad \dots\dots\dots (3-10)$$

which is similar to Equation 2-9 described earlier. The reflected intensity for a 4-layer system is:

$$R_{1234} = \left| \frac{r_{12}^p + r_{23}^p \exp(i2k_{z2}d_2) + r_{34}^p \exp(i2k_{z2}d_2) \exp(i2k_{z3}d_3) + r_{12}^p r_{23}^p r_{34}^p \exp(i2k_{z3}d_3)}{1 + r_{12}^p r_{23}^p \exp(i2k_{z2}d_2) + r_{23}^p r_{34}^p \exp(i2k_{z3}d_3) + r_{12}^p r_{34}^p \exp(i2k_{z2}d_2) \exp(i2k_{z3}d_3)} \right|^2 \dots\dots\dots (3-11)$$

3.4.2 SP evanescent field enhancement simulation

In addition to reflection coefficients, the transmission coefficients are very important too as they tell us the theoretical enhancements that are possible with the single gold film, single silver film and two-layered silver-gold film configuration. As mentioned earlier, an expression for the magnetic field intensity enhancement is first derived since the magnetic field, H_y , is continuous across all boundaries for p -polarized light. To avoid confusion, as the electric field intensity is not continuous across interfaces for p -polarized light, the analyte-metal interface is referred to the analyte side of this interface and vice-versa. When the reflected intensity, R , reaches its lowest value, the intensity of the electromagnetic field is greatly increased on the metal surface due to the enhancement by surface plasmons. The value of this enhancement is given as the ratio of the field intensity on the analyte-metal interface, $|H_y(N,N-1)|^2$, where $H_y(N,N-1)$ is the magnetic field of the SPs, to the incoming field intensity at the prism-metal interface, $|H_y(1,2)|^2$, for p -polarized light. Using Equations 3-1 to 3-9 and solving the multiple-beam interference for a 3-layer system, we obtain:

$$\left| \frac{H_y(3/2)}{H_y(1/2)} \right|^2 = |t_{123}^p|^2 = \left| \frac{t_{12}^p t_{23}^p \exp(ik_{z2}d_2)}{1 + r_{12}^p r_{23}^p \exp(i2k_{z2}d_2)} \right|^2 \dots\dots\dots (3-12)$$

Similarly, the enhancement of the magnetic field intensity for a 4-layer system is found to be:

$$\left| \frac{H_y(4/3)}{H_y(1/2)} \right|^2 = \left| \frac{t_{12}^p t_{23}^p t_{34}^p \exp(ik_{z2}d_2) \exp(ik_{z3}d_3)}{1 + r_{12}^p r_{23}^p \exp(i2k_{z2}d_2) + r_{23}^p r_{34}^p \exp(i2k_{z3}d_3) + r_{12}^p r_{34}^p \exp(i2k_{z2}d_2) \exp(i2k_{z3}d_3)} \right|^2 \dots \dots \dots (3-13)$$

Therefore, the electric field intensity enhancement in the analyte-metal interface can be obtained by relating the electric and magnetic fields according to this equation [73]:

$$\left| \frac{E_{\parallel}(N/N-1)}{E_{\parallel}(1/2)} \right|^2 = \frac{\epsilon_1}{\epsilon_N} \left| \frac{H_y(N/N-1)}{H_y(1/2)} \right|^2 \dots \dots \dots (3-14)$$

Although the evanescent field intensity enhancement factor can yield a value ranging from tens to a hundred during SPs resonance, this field has an imaginary Poynting vector and does not possess any real power if the analyte medium is a pure dielectric. The transmittance, defined by $T = \frac{n_N \cos \theta_N}{n_1 \cos \theta_1} |\tau|^2$, yields a zero value during total internal reflection, as θ_N will be right-angled.

3.4.3 Penetration depth simulation

The electromagnetic field, greatly enhanced by a metallic film in the event of SPR, rises to its peak value at the surface of the metal film. As this field penetrates into the adjacent sample medium, it starts to decay exponentially. The evanescent field intensity, I_z , at a distance z from the interface is defined as:

$$I_z = \left| \frac{E_{\parallel}(3/2)}{E_{\parallel}(1/2)} \right|^2 \exp(i2k_{z4}z) \dots \dots \dots (3-15)$$

The evanescent field penetration depth (d_p), defined as the distance where the electric field falls to $1/\exp(1)$ of its initial value, can also be expressed as the distance where the electric field intensity decays to $1/\exp(2)$ of its initial value:

$$\left| \frac{E_{\parallel}(3/2)}{E_{\parallel}(1/2)} \right|^2 \exp(i2k_{z4}d_p) = \frac{1}{\exp(2)} \left| \frac{E_{\parallel}(3/2)}{E_{\parallel}(1/2)} \right|^2 \dots\dots\dots(3-16)$$

When exploiting SPR for biosensing, one key issue is that the bio-molecules of interest must be immobilized on the metal surface. Usually, a detecting molecule (the ligand) is first immobilized on the metal surface, and its binding partner (the analyte), which is usually protein, is injected in aqueous solution through the flow cell under continuous flow. As the analyte binds to the ligand, the accumulation of protein results in an increase in the refractive index which is measured in real time. Knowledge of the penetration depth is of paramount importance as it gives us information on the effective sensing region the sensor encompasses. The overall length of the ligand – analyte pair or antibody – antigen – antibody assembly must be within the sensing range of the SP evanescent field.

3.5 Terminology

Shown in Figure 3-4 are the SPR curves and respective electric field intensity enhancement simulated for the case of a 1.51509 refractive index prism, 55 nm thick gold coating, and analyte refractive indexes of 1.33 and 1.36. It is evident that a lower SPR resonance dip yields stronger evanescent field enhancement, as depicted in the 1.33 refractive index case. This is in agreement with the law of conservation of energy as one would expect that the higher energy loss in reflectivity would translate to more SPs oscillations and hence a stronger SP evanescent field.

Figure 3-4: Top: Simulated SPR curve, considering a 55 nm thick gold coating and analyte refractive indexes of 1.33 and 1.36; Below: Corresponding simulated electric field intensity enhancement

With reference to Figure 3-4, we will like to define several terminologies used throughout the text for consistency.

Resonance width: The full width of the SPR minimum in the reflectivity (or transmission for waveguide) spectrum measured at the half maximum, in units of measurement parameter (typically angle or wavelength).

Resonance depth (dip): The lowest value of the normalized TM polarized reflectivity (or transmission) at full resonance.

Shift in resonance depth: The change in the position of the resonance dip for a specific change in the refractive index of the analyte, typically in units of angle or wavelength per refractive index change.

Sensing region: The region in which the sensor is capable of monitoring any binding interactions or changes in refractive indexes, generally proportional to the penetration depth of the SP electromagnetic field.

Sensitivity: In some publications, sensitivity is defined as the change in angle or wavelength per unit change in the refractive index of the sample ($d\theta/dn$ or $d\lambda/dn$). However, sensitivity carries a wider definition as it is dependent on many parameters and various situations. For instance, improved sensitivity can be attributed to a wider sensing region as well since the sensor will be more sensitive to binding interactions that occurs further from the metallic surface. Therefore, in this text, we will emphasize sensitivity in a more specific manner, i.e. sensitivity in terms of $d\theta/dn$ or $d\lambda/dn$, and sensitivity in terms of a wider sensing region. Additionally, another type of sensitivity, known as detection sensitivity, has also been defined by the scientific community [111]. This sensitivity refers to the change in reflectance for a given change in analyte refractive index (dR/dn). However, to avoid further complicating the use of “sensitivity”, we will term detection sensitivity as signal-to-noise ratio in this thesis instead.

Signal-to-noise-ratio (SNR): It is pretty straightforward to deduce that SNR is inversely related to FWHM since a narrower FWHM implies a stronger contrast between signal and noise. A sharper dip ensures that there will be lesser confusion when identifying the exact resonance point. From another perspective, it is worthwhile to notice that for a particular SPR curve, the lower the resonance dip, the wider the FWHM. However, the widening of the FWHM in this situation does not indicate a poorer SNR, but the contrary instead. Therefore,

the SNR of an SPR sensor holds an inverse relationship with the values of FWHM and resonance depth. Determining the exact degree of relatedness requires numerous statistical data and analytical software, which is not within the scope of this thesis.

For a SPR sensor, there is no general consensus on which is more important between sensitivity and SNR. Better sensitivity seemingly indicates improved resolution of the sensor. However, a narrower FWHM and sharper dip at the resonance depth allows the distinct determination of the concentration of the analyte, in comparison to a sensor which possesses large $d\theta/dn$ or $d\lambda/dn$ but yields a wide FWHM and broad spectrum at the resonance depth. Hence, a compromise between these two parameters is always sought after, tailoring to the specific needs of a particular SPR application.

3.6 Conclusion

The two-layer silver-gold configuration for SPs excitation is introduced in this chapter. Potentially, this configuration integrates the better evanescent field enhancement of silver, the narrower FWHM of the SPR curve achievable with silver, and the high chemical resistance and biocompatibility of gold. A multi-layer interference model based on magnetic field continuity has been presented and discussed thoroughly. This model can be applied for the theoretical study of reflectivity and transmission for multi-layer systems, enabling the comprehensive study of the SPR curve and SP evanescent field penetration depth.

CHAPTER 4

BIMETALLIC FILM SPR CONFIGURATION BASED ON PRISM GEOMETRY

4.1 Overview of Chapter 4

As described earlier in Chapter 2, surface plasmons (SPs) are charge-density oscillations propagating at the interface of plasma and a dielectric medium. Since these SPs are p -polarized (magnetic vector is perpendicular to the direction of SPs' propagation and the plane of incidence), only a transverse magnetic (TM) optical light can be used to excite the SPs. The Kretschmann prism coupling geometry (shown in Figure 4-5), being the most straightforward technique in achieving the SPR phenomenon, is employed in our experiments.

To exhibit surface plasmon oscillations, a metal must have a negative real part of the dielectric constant and the positive imaginary part should be very small to keep damping to a minimum. The width of a resonance is due to the intrinsic and radiation damping of the SPs and it increases with an increase in the imaginary part of the dielectric constant of the metal.

The choice of metal used is critical since the metal must exhibit free electron behaviour as described by the free electron model. To be useful for SPR, a metal must have conduction band electrons capable of resonating with light at a suitable wavelength. Silver and gold are two such metallic elements that satisfy this condition and they are more popularly used in research and commercial applications because of their sensitivity and stability respectively. Note that at wavelengths shorter than approximately 500 nm ($\sim$ surface plasmon cutoff wavelength), the conservation of energy condition may not be satisfied (as depicted in Figure 2-2) and surface plasmons will not be excited. Therefore, to ensure the occurrence of SPR, red LEDs or lasers are usually the preferred excitation sources used in experiments.

It is well known that employing a thin silver layer for SPR sensing achieves a sharper SPR reflectivity dip (narrower FWHM) and stronger evanescent field enhancement as compared to using gold. However, silver's high susceptible to oxidation and bio-incompatibility are its major limitations, especially for biosensing applications. Hence, the proposed bimetallic film configuration is able to integrate the individual advantages achievable with single silver and gold film. In this chapter, the SNR and sensitivities realizable with various metallic film configurations are reviewed in detail.

4.2 SPR reflectivity curves: Comparison of FWHMs between the different metallic film configurations

4.2.1 Thickness optimization of metallic film

Before we proceed to demonstrate the experimental SPR phenomenon with the prism configuration, the optimum thicknesses of the different metal films are first determined theoretically from Equations 3-10 and 3-11. The optimum thickness is deemed to be the thickness which results in minimum SPR reflectivity and hence, achieving the maximum

evanescent field enhancement. An SPR sensor optimized to obtain the lowest reflectivity yields improved signal-to-noise-ratio. Additionally, an enhanced evanescent field possesses longer penetration depth, resulting in better sensitivity in terms of a wider sensing region.

We make use of Matlab software to facilitate the process of solving the equations numerically. The following parameters are used in our simulations: laser wavelength: 633 nm; prism refractive index: 1.51509; analyte refractive index: 1.3501; dielectric constant of silver: $-17.81 + 0.676i$; dielectric constant of gold: $-10.98 + 1.464i$ [73]. Using Equation 3-10, 51 SPR curves (reflectivity against angle of incidence) are simulated for the single silver and single gold SPR configuration, while increasing the film thickness from 30 to 80 nm at 1 nm step. The reflectivity values at the respective minimum SPR dip are extracted from all these curves and plotted in Figure 4-1. We can observe that at different thicknesses, the minimum SPR dip possesses different reflectivity values and it is closest to zero when the film thickness is 52 nm for the single silver film and 44 nm for the single gold film SPR configuration. Hence, these are the optimum metal thicknesses that we have to deposit on our prism in order to achieve maximum evanescent field enhancement.

Figure 4-1: Reflectivity at resonance dip of the angle-modulated SPR curves simulated with various thicknesses of silver and gold in single metal film SPR configuration, considering an analyte with refractive index of 1.3501

Figure 4-2 depicts the reflectance values at various silver-gold thickness combinations. The thickness of the underlying silver is varied between 25 nm to 65 nm while that of the overlying gold is varied between 0 nm to 20 nm. It is observed that close to zero reflectance can be achieved with various optimum silver-gold thicknesses. Table 4-1 shows the optimum thickness combinations of silver and gold that yields lowest reflectivity in Figure 4-2.

Figure 4-2: Reflectance at various silver-gold thickness combinations for a two-layer metallic film SPR configuration, considering an analyte with refractive index of 1.3501

Table 4-1: Optimum thickness combinations of silver and gold that yields lowest reflectivity in Figure 4-2

Ag	49	47	45	44	42	40	39	37	36	35	33	32	31	30	29	28	26
Au	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17

4.2.2 Simulation of SPR curves

The SPR curves for several optimum thickness combinations, obtained from Table 4-1, are plotted in Figure 4-3. As interpreted from Figure 4-3, a thicker gold - thinner silver coating

results in a broader SPR curve, hence lower sensitivity in terms of FWHM. Therefore, we choose 42 nm of silver with 5 nm of gold, as we believe that 5 nm of gold will be sufficient to bury the silver. In addition, the reflectivity measurement that we will conduct subsequently is the first experimental verification of the enhancement achievable with the proposed configuration and we hope that a thicker silver – thinner gold layer will yield more prominent results in this proof-of-principle experiment.

Figure 4-3: SPR curves generated with various optimum silver-gold thicknesses for a two-layer metallic film SPR configuration, considering an analyte with refractive index of 1.3501

Upon determining the optimum metallic film thicknesses, the theoretical SPR reflectivity curves, with angle modulation from 50° to 90°, are plotted in Figure 4-4.

Figure 4-4: Theoretical SPR curves for the three metallic SPR configurations, considering an analyte refractive index of 1.3501

4.2.3 Experimental procedures

4.2.3.1 Prism preparation and metallic film deposition

Three prisms made of BK7 material, with a refractive index of 1.51509 at 633 nm, are used in our experiments. The prisms are first washed with IPA and then immersed in a beaker of acetone, which is placed in ultrasonic bath. In doing so, microscopic particles are removed by the vibrant movement of air molecules. After the clean prisms are removed from the beaker, they are washed with de-ionized water before blowing with a stream of the nitrogen gas and placed on top of a hotplate at 120°C to remove the moisture on their surface. Next, three metallic films are deposited on the prism hypotenuse surface by using the E-beam evaporator (Edwards) in three separate deposition processes and their thicknesses are monitored with a crystal deposition monitor. The first prism is coated with 52 nm of silver, the second with 44 nm of gold, and the third with 42 nm – 5 nm of silver-gold. To ensure

that the metal surfaces are smooth, the depositions are performed at a pressure of $\sim 5 \times 10^{-6}$ torr and a deposition rate of ~ 0.1 nm/s. As silver degrades over time when exposed to air, measurements with the single silver film SPR configuration are performed immediately after the film deposition process.

4.2.3.2 Analyte preparation

Glucose, a simple sugar, is one of the most important carbohydrate nutrient sources and is fundamental to almost all biological processes. Quantification of glucose concentration is critical in monitoring and analysis of agricultural products, control and regulation of cell culture processes, and diagnosis and control of human diseases, for instance diabetes. Homogenous glucose solutions with various refractive indexes are used as the analyte for our experiments. D-(+)-Glucose purchased from Sigma is dissolved in de-ionized water and its refractive index is measured with a refractometer (Kyoto Electronics, model: RA-130). Glucose, being highly soluble in water due to their many hydroxyl groups, is a suitable sample for this experiment due to its simplicity in preparation. Furthermore, the fact that carbohydrates are broken down into glucose by our digestive system makes this simple sugar a relatively reasonable sample to represent certain components within blood. The refractive index that is achievable by different concentration of glucose solution spans across a wide range, from ~ 1.33 to ~ 1.47 . In the experiments described in this chapter, the refractive of the glucose solution is 1.3501.

4.2.3.3 Instrumentation

The setup which we will employ for our experiments (shown in Figure 4-5) is as such: a red HeNe laser source, with a wavelength of 633 nm, passes through a polarizer and illuminates the hypotenuse surface of a BK7 glass ($n = 1.51509$) prism. The prism is placed on a high

precision rotary stage and a homogenous glucose solution is used as the analyte. The glucose solution is contained in a funnel sealed firmly to the prism's surface. Next, the reflected beam passes through a collector lens and the optical power is measured by a power meter. The dielectric constants of silver and gold at this wavelength are $-17.81 + 0.676i$ and $-10.98 + 1.464i$, respectively [73].

Figure 4-5: Two-layered metallic film SPR configuration and experimental setup for measuring the angle-modulated SPR curves

4.2.3.4 Experimental SPR reflectivity curves

Using the experimental configuration as depicted in Figure 4-5, the experimental reflectance values at various modulated angles for the three configurations are plotted in Figure 4-6. It is evident that the experimental results agree reasonably well with the simulated values plotted in Figure 4-4. However, we do notice that the experimental reflectance minimums do not drop to zero, and the experimental resonance angles are slightly off the simulated values.

Some discrepancies can be attributed to the fact that the dielectric constant values of the metal films we used in our simulation are close estimates, but certainly not the true exact values. Besides, it is assumed that the laser source is a single ray in the simulation, when in actual fact, the laser source has a finite beam waist of Gaussian profile.

Figure 4-6: Experimental SPR curves for the three metallic SPR configurations, with glucose solution of refractive index 1.3501 as analyte

4.2.4 Discussion

The SPR angles and FWHM of the SPR curves obtained theoretically and experimentally are summarized in Table 4-2. Though the bimetallic layer configuration achieves an experimental FWHM which is approximately 1.60× broader than that obtained with the single silver film SPR sensor, its FWHM is 2.96× narrower than what can be achieved with the single gold film design. As such, a much better resolution and thus higher SNR optical sensor can be achieved with the double layer arrangement as compared to the single gold film SPR sensor.

Table 4-2: Theoretical and experimental SPR angles and FWHM of SPR curves for various metallic film SPR configurations

Configuration	Single layer	Single layer	Bimetallic Layer
Metal	Silver	Gold	Silver - Gold
Thickness (nm)	52	44	42 – 5
Simulated SPR angle (°)	70.13	75.58	71.58
Simulated FWHM	1.37	8.79	2.80
Experimental SPR angle (°)	69.94	75.07	71.23
Experimental FWHM (°)	1.93	9.12	3.08

4.3 SPR reflectivity curves: Comparison of sensitivity ($d\theta/dn$ and $d\lambda/dn$) between the different metallic film configurations

In addition to comparing the SNR achievable with the various metallic film configurations, it will be of great value to know more about their sensitivity in terms of $d\theta/dn$ and $d\lambda/dn$. Theoretically, it will be relatively simple to obtain these two parameters using the model presented earlier in Chapter 3. Experimentally, the value of $d\theta/dn$ can be obtained with ease since the incident angle can be adjusted simply by turning the rotary stage. However, attaining the value of $d\lambda/dn$ involves more difficulty as lasers are only available in discrete wavelengths, commonly in 532 nm and 633 nm for instance. Though white LED with a broad spectral may be employed, it is certainly not an easy task to collimate, focus and polarize the incident light such that minimal distortion occurs as the light enters and leaves the prism.

4.3.1 A look into sensitivity in terms $d\theta/dn$

To obtain the value of $d\theta/dn$, the SPR curves for a variety of samples with different refractive indexes have to be first obtained. Analytes of random refractive indexes ($n = 1.3330, 1.3406, 1.3505, 1.3598$) are input into the simulation program used earlier in Section 4.2.2, *ceteris paribus*. Concurrently, glucose solutions of the same refractive indexes are measured with the SPR sensors. Shown in Figures 4-7 to 4-12 are the theoretical and experimental angle-modulated SPR curves for the three metallic configurations. As observed, the resonance dip shifts most for a given change in refractive index with the single gold film SPR configuration, whereas the least shift occurs for the single silver film arrangement. Based on the experimental values, the shift in resonance angle with refractive index changes is plotted in Figure 4-13.

Figure 4-7: Theoretical angle-modulated SPR curves for various samples of different refractive indexes, considering a single silver film (52 nm) SPR configuration

Figure 4-8: Experimental angle-modulated SPR curves for glucose solutions of different refractive indexes, measured with a single silver film (52 nm) SPR configuration

Figure 4-9: Theoretical angle-modulated SPR curves for various samples of different refractive indexes, considering a single gold film (44 nm) SPR configuration

Figure 4-10: Experimental angle-modulated SPR curves for glucose solutions of different refractive indexes, measured with a single gold film (44 nm) SPR configuration

Figure 4-11: Theoretical angle-modulated SPR curves for various samples of different refractive indexes, considering a two-layer silver-gold film (42 nm – 5 nm) SPR configuration

Figure 4-12: Experimental angle-modulated SPR curves for glucose solutions of different refractive indexes, measured with a two-layer silver-gold film (42 nm – 5 nm) SPR configuration

Figure 4-13: Linearly fitted experimental shift in resonance angle with refractive index changes ($d\theta/dn$) for the single silver, single gold and silver-gold SPR configurations

From Figure 4-13, the sensitivities for the single silver film (52 nm) is ~ 120.2 $^{\circ}$ /RIU, the single gold film (44 nm) is ~ 167.3 $^{\circ}$ /RIU and the silver-gold film (42 nm – 5 nm) is ~ 143.5 $^{\circ}$ /RIU. Clearly, the gold film SPR configuration possesses the greatest sensitivity in terms of $d\theta/dn$. Nonetheless, this does not translate linearly to better sensor performance as the improved sensitivity arises at the expense of SNR, as seen from the wider FWHM and broader reflectivity dip.

4.3.2 A look into sensitivity in terms $d\lambda/dn$

To obtain the value of $d\lambda/dn$ theoretically, slight modifications will have to be made to the setup considered earlier. In this section, only theoretical analyses are possible as spectral interrogation with the prism in-coupling scheme is much more difficult to realize. Considering wavelength modulation at a fixed angle of incidence at 75° , the SPR curves for a variety of samples with different refractive indexes ($n = 1.3330, 1.3406, 1.3505, 1.3598$) are simulated and shown in Figures 4-14 to 4-16.

Figure 4-14: Theoretical wavelength-modulated SPR curves for various samples of different refractive indexes, considering a single silver film (52 nm) SPR configuration

Figure 4-15: Theoretical wavelength-modulated SPR curves for various samples of different refractive indexes, considering a single gold film (44 nm) SPR configuration

Figure 4-16: Theoretical wavelength-modulated SPR curves for various samples of different refractive indexes, considering a two-layer silver-gold film (42 nm – 5 nm) SPR configuration

Figure 4-17: Linearly fitted theoretical shift in resonance wavelength with refractive index changes ($d\lambda/dn$) for the single silver, single gold and silver-gold SPR configurations

From Figure 4-17, it is evident that the silver film SPR configuration achieves the highest sensitivity in terms of $d\lambda/dn$, on the contrary with the results for angular modulation. A somewhat surprising observation is that the sensitivity for the bimetallic film arrangement is not sandwiched between that of pure silver and pure gold, but slightly lower than either of them. The simulated sensitivity is ~ 2027 nm/RIU for silver, ~ 1923 nm/RIU for gold and ~ 1815 nm/RIU for silver-gold configurations. The slopes for the three curves deviate only up to 10 %, indicating that the choice of films will not be the prime consideration for prism in-coupling SPR sensors employing wavelength interrogation. To grasp a more in-depth understanding on the sensitivity, more considerations are needed, for instance the frequency-dependent-refractive indexes of the prism and sample. In view of the model proposed in this context, the results presented are fruitful for preliminary studies and the discussed model can be employed to tailor the design for a specific SPR application.

4.4 SP field enhancement and penetration depth: Comparison of sensitivity (sensing region) between the different metallic film configurations

4.4.1 SP evanescent field enhancement on metal surface

When employing the SPR technique as a biosensor, ligands that are several tens of nanometer thick are immobilized on the metal surface in order to capture the desired analyte for sensing. Therefore, it is essential that the SP evanescent field, which penetrates into the biological sample, is strong enough to sense the analyte that is bounded to the ligand. Since the evanescent field decays exponentially into the biological sample, it is highly desirable that the size of the ligands immobilized on the metal surface is minimized so as to achieve the maximum sensitivity. To achieve stable test results as well as ride on the more matured ligand immobilization techniques on gold surfaces, most commercial SPR sensors use gold as the metal film [3]. However, the tradeoff as compared to using silver is that the evanescent field enhancement is much weaker. By employing the two-layered metallic configuration, high evanescent field enhancement (close to silver's enhancement) can be achieved. A stronger evanescent field implies a larger sensing region for monitoring cells interactions. Extension of the evanescent field might not improve the sensitivity substantially in the monitoring of cells that are small (several nanometers). However, as the cells get larger (and usually the ligands as well), the enhanced sensitivity due to field extension will be more pronounced, as a weak evanescent field might have decayed too severely to sense the biological binding. Therefore, the bimetallic SPR biosensor can be employed for more biological sensing applications as longer ligands may be used to monitor bigger analytes. The bimetallic configuration improves the sensitivity of conventional single gold layer SPR sensors in terms of having a larger sensing region.

In the following section, we will use the multiple beam interference matrix approach described in Chapter 3 to simulate the theoretical enhancements that is possible with the single gold film, single silver film and two-layered silver-gold film configuration. Using Equations 3-10 to 3-14, the SPR reflectivity curves and electric field intensity enhancement factors for single silver, single gold and bimetallic SPR configurations are plotted for various angles of incidence and shown in Figures 4-18 to 4-20. For comparison, the reflectivity curve and enhancement factor during total internal reflection, i.e. no metallic film, are plotted in Figure 4-21.

Figure 4-18: SPR reflectivity and corresponding electric field intensity enhancement factor curves for a single silver film SPR configuration, considering analyte refractive index of 1.3501

Figure 4-19: SPR reflectivity and corresponding electric field intensity enhancement factor curves for a single gold film SPR configuration, considering analyte refractive index of 1.3501

Figure 4-20: SPR reflectivity and corresponding electric field intensity enhancement factor curves for a bimetallic silver-gold film SPR configuration, considering analyte refractive index of 1.3501

Figure 4-21: Total-internal-reflection reflectivity and corresponding electric field intensity enhancement factor curves, considering analyte refractive index of 1.3501

The main motivation behind optimizing the metallic thickness is to achieve ~zero SPR reflectivity and ensure that the evanescent field intensity enhancement is maximum, in comparison to situations where the metallic thickness hasn't been optimized to achieve ~zero reflectivity. However, we can observe from Figures 4-18 to 4-20 that the maximum evanescent field intensity enhancement (MEFIE) factor does not occur at the angle of incidence with minimum SPR reflectivity. Instead, the MEFIE angle is slightly smaller than the SPR angle. Shown in Table 4-3 are the theoretical SPR angle, corresponding minimum reflectivity and evanescent field intensity enhancement at this angle; angle of incidence where MEFIE occurs and the corresponding enhancement factor; and the difference between the SPR angle and MEFIE angle.

Table 4-3: Theoretical SPR angles, reflectivity and corresponding enhancement factor; MEFIE angles and corresponding enhancement factor; and the difference between the SPR and MEFIE angles for the three SPR configurations

Configuration	Single silver	Single gold	Silver-gold
Thickness (nm)	52	44	42 – 5
SPR angle, θ_{SPR} (°)	70.13	75.58	71.58
Reflectivity at θ_{SPR}	1.1829 e-004	3.7086 e-004	1.0480 e-005
Enhancement factor at θ_{SPR}	54.31	9.54	28.55
Angle where enhancement is maximum, θ_{MEFIE} (°)	70.03	74.37	71.40
MEFIE factor	55.02	10.52	29.14
$\theta_{\text{SPR}} - \theta_{\text{MEFIE}}$ (°)	0.10	1.21	0.18

The rationale for θ_{MEFIE} to be slightly lower than θ_{SPR} is related to the resonance character of surface plasmons. As we sweep through the angles of incidence, the reflection we observed is a coherent superposition of a partial wave reflected from the prism/metal interface with the surface mode radiated via the prism. For an ideal loss free metal, the phase would change from 0° below the resonance to 180° above it, with a discrete step increase at the resonance. A minimum in the reflectivity occurs when the two partial waves differing in phase by 180° undergo destructive interference. However, any damping in the system broadens and smears this phase change. Hence, the angle of minimum reflectivity is reached slightly above the angle for maximum evanescent field enhancement [53]. The higher the loss of the metal, the more smeared out is the phase change of the resonance mode. This indicates a larger angular

difference between the surface mode peak intensity enhancement and the reflectivity minimum. The data presented in Table 4-2 clearly reinforce our analysis as it is very well known that the imaginary dielectric constant of gold is larger than silver's ($\epsilon''_{Au} > \epsilon''_{Ag}$).

Additionally, the electric field intensity enhancement factors for single silver, single gold and bimetallic SPR configurations are found to be 54.31, 9.54 and 28.55 respectively. This implies that the single silver layer and bimetallic SPR configurations are capable of enhancing the electric field intensities at the analyte-metal interface by a factor of 5.7 and 3.0 times stronger than that of single gold.

4.4.2 Electric field intensity in metal and SP field penetration depth at lowest SPR reflectivity

Although the maximum evanescent field intensity enhancement does not occur at the point of minimum reflectivity, θ_{SPR} is still a very useful parameter for obtaining an enhancement close to MEFIE. This is because MEFIE occurs at an angle slightly less than θ_{SPR} , and this difference varies depending on the choice and combination of metals used. Hence, locating θ_{MEFIE} in practical sensors will be an uphill task. The most straightforward method to accurately monitor the refractive index of analytes and achieve high evanescent field enhancement will be to locate θ_{SPR} instead. In this section, we analyzed the evanescent field penetration depth at the point of lowest SPR reflectivity.

Plotted in Figure 4-22 are the electric field intensities for all the metallic configurations when the respective SPR reflectivity is minimum. It is evident that the electric field increases in the metal film during resonance and decay exponentially in the analyte medium.

Figure 4-22: Electric field intensities within the various metallic films during SPR

The evanescent field penetration depths for all the metallic configurations are shown in Figure 4-23. From Figure 4-23, the electric field penetration depth from a gold surface is found to be 175 nm. However, the evanescent fields from the single silver and bimetallic SPR configurations have longer probing depths. It takes 414 nm and 316 nm respectively for the electric field intensities of the silver and double-layer to decay to $1/e^2$ of gold's initial electric field intensity, which works out to be $2.36\times$ and $1.81\times$ longer than the single gold layer SPR sensor. These values are presented in Table 4-4.

Figure 4-23: Electric field intensity of the SP fields from various metallic films penetrating into glucose solution. The horizontal solid line represents the enhancement factor at the penetration depth of the gold film SPR sensor. The region where this solid line intersects all the enhancement factor curves is blown up and inserted

Table 4-4: Electric field intensity enhancements at analyte-metal interface

Configuration	Single layer	Single layer	Bimetallic Layer
Metal	Silver	Gold	Silver - Gold
Thickness (nm)	52	44	42 - 5
Distance (nm) where enhancement factor = $\frac{1}{e^2} \left \frac{E_{ }(3/2)}{E_{ }(1/2)} \right _{gold}^2$	413.62	175.29	316.09

4.4.3 Effect of penetration depth on sensitivity

To illustrate the advantage of an enhanced optical field for biosensing, we study the SPR response for a thin layer of material inserted in ambient water at varying distances from the metal surface, as shown in Figure 4-24. A 10 nm layer of index 1.3501, in ambient water of index 1.3330, is slowly moved away from the metal surface and the corresponding shift in the SPR resonance dip is evaluated. In real biosensing applications, this thin layer may represent analytes floating near the metal surface or even biological bindings that are taking place at various distances from the metal surface [112].

Figure 4-24: Influence of distance on resonance dip for a thin layer at various distances from the metal surface

The multilayer beam interference technique described earlier is extended to a six-layer model to be suitable in this context. Plotted in Figure 4-25 are the simulated angular changes of the resonance dip with respect to the distance of the thin layer, for the single silver, single gold and two-layer silver-gold SPR configurations. When the thin layer is in the immediate vicinity of the gold metal surface, any changes to the distance triggers a larger shift in resonance dip, in agreement with the better sensitivity discussed earlier for the angular-

modulated gold film SPR sensor. However, the sensitivity of the gold film SPR configuration decreases at a higher rate as the thin organic layer is moved further. Above a critical distance of ~ 97 nm, its sensitivity has plunged lower than that of silver and the two-layer silver-gold films. Evidently, for assays which requires monitoring long antibody – antigen assembly; microscopy of bigger cells / particles; or fluorescence applications where labels are tagged to a protein a distance away from the metal surface, an enhanced SP field is certainly required. Utilizing the bimetallic configuration is indeed an improved option in these circumstances.

Figure 4-25: Simulated angular changes of the resonance dip for a thin layer of material inserted in ambient water at varying distances from the metal surface, considering the single silver, single gold and two-layer silver-gold SPR configurations (the region where intersection occurs is blown up and inserted)

4.5 Experimental results of the two layered metallic film SPR configuration using a green incident beam

Usually, green lasers are not commonly used in SPR experiments as this wavelength is quite close to the minimum wavelength for plasmon excitation. Potentially, the employment of green light sources to achieve SPR extends the use of the two-layered metallic film configuration. Therefore, we attempt to employ a green laser excitation source to validate the SPR phenomenon on the single silver layer, single gold layer and bimetallic film configuration. The simulated and measured SPR reflectivity curves for a modified experimental setup employing green laser at 532 nm, high index prism of 1.785, and various analytes (acetone, water and air) are shown in Figures 4-26 to 4-28. The metallic thicknesses for the single silver, single gold and bimetallic silver-gold film configurations are 50 nm, 48 nm and 23 nm – 21 nm respectively.

Figure 4-26: Simulated and experimentally SPR curves for the single silver film SPR configuration with air, water and acetone as analytes (Simu refer to simulation results while Exp refer to the experimental results)

Figure 4-27: Simulated and experimental SPR curves for the single gold film SPR configuration with air, water and acetone as analytes

Figure 4-28: Simulated and experimental SPR curves for the two-layer metallic film configuration with air, water and acetone as analytes

As shown in the figures, two-layer metallic film configuration shows narrower FWHM and hence better SNR. The measured FWHM of the reflectivity curve for the bimetallic layer configuration is 4.8 times narrower than the single gold film configuration [113].

4.6 Stability of metallic film

There are two critical limitations in the selection of a metal for stable sensor construction. Firstly, the surface exposed to light must be a pure metal. Oxides, sulfides and other films formed by atmospheric exposure interfere with SPR. Next, the metal film must also be compatible with the chemistries needed to perform assays.

From the list of possible metals, using silver as a sensing metal has a disadvantage as it is highly susceptible to oxidation. In terms of practicality, gold is a good option as it is very resistant to oxidation and other atmospheric contaminants, but sufficiently reactive to accommodate coating with a wide variety of binding molecules [114]. One main reason for the wide use of gold in many commercial SPR biosensors is its high stability and good adhesion to biomolecules. In the proposed bimetallic SPR configuration, stability of the sensor is not compromised as silver, which oxidizes easily, has been buried under the more inert gold material. Practically, at the silver-gold interface, diffusion of gold atoms into silver film might occur during the deposition process, causing the formation of a very thin layer of silver-gold alloy. The presence of this thin layer might contribute to marginal changes to SPR reflectivity curves. Nonetheless, the influence of this thin alloy layer on the performance of our sensor is minor, as depicted in the good agreement between experimental and simulation results. Besides, this problem can be overcome easily through proper characterization in commercial and research applications.

4.7 Conclusion

In conclusion, this chapter discusses and compares comprehensively the SNR and sensitivities between the single silver, single gold and two-layer silver-gold film prism-based SPR configurations, with specific focus on the SPR reflectivity curves and SP electromagnetic fields penetration depths. Theoretical simulations for the single silver, single gold and bimetallic SPR configurations have been performed to obtain the optimum metal thickness to achieve the minimum reflectance for the various configurations. Experiments performed produced the results that are in good agreement with the simulated values. The SPR reflectivity minimum obtained with the bimetallic setup is sharper and has a FWHM which is $3.0\times$ or $4.8\times$ narrower than the single gold setup, with red or green incident sources respectively. However, the single gold SPR arrangement provides the highest sensitivity in terms of $d\theta/dn$ when interrogated angularly, although the wider FWHM and broad reflectivity dip is another consideration. When the wavelength of the incident beam is interrogated, the three configurations demonstrate only slight deviations in sensitivity ($d\lambda/dn$) theoretically. Further analyses of the electric field intensities that penetrate into the analyte are also performed. It is demonstrated that the intensity enhancement of the bimetallic configuration at the analyte-metal interface is $3.0\times$ greater and its probing depth is $1.81\times$ longer than that of the single gold SPR configuration. Finally, the higher stability and better biomolecules adhesion of this configuration makes it be a much better option to be employed in biosensing applications as compared to the single silver film SPR sensor. Hence, the two-layer silver-gold SPR configuration realizes an overall integration of the longer probing field of silver, the better SNR of silver, and the high chemical resistance and biocompatibility of gold.

CHAPTER 5

BIMETALLIC FILM SPR CONFIGURATION BASED ON WAVEGUIDE GEOMETRY

5.1 Overview of Chapter 5

In Chapter 4, the enhancement possible with the two-layer silver-gold film had been discussed thoroughly, and the experimental results are in good agreement with simulated analyses. Though the theoretical discussions were very comprehensive, experimental data yields comparison only on the SNR and sensitivity ($d\theta/dn$). In this chapter, we seek to compare experimentally another parameter, i.e. the sensitivity in terms of $d\lambda/dn$, using the waveguide in-coupling scheme for SPR. As compared to the prism SPR setup, waveguide SPR is an easier alternative for wavelength interrogation of the incident beam. White light can be easily launched into and guided through the waveguides, offering experimental simplicity to achieve wavelength-interrogated SPR.

Moreover, the employment of waveguide SPR offers another advantage. Rapid technological advancement in device miniaturization, material science, fluid mechanics, optics and a host

of other fields is spearheading surging demands for portable, accurate, sensitive and affordable diagnostic tools. This driving force is far greater than ever before with the aging world population and rising health consciousness of the public. In particular, the investigation of waveguide SPR is of significant research value, especially in recent years where lab-on-a-chip, decentralized-diagnostics-home-healthcare and point-of care diagnosis initiatives are spearheading research efforts globally. The exploitation of waveguide SPR offers portability, compatibility, multiple sensing, and mass-production possibility [115]. During resonance, a significant enhancement of the evanescent field will be possible, primarily due to the excitation of SPs on the surface of the thin metal film.

To begin, linear waveguides are fabricated using commercially available SU-8 (MicroChem) photoresist by standard lithographic procedures. Next, three different metallic coatings (single-layer silver, single-layer gold and two-layer silver-gold) are deposited on the surface of the waveguides. Various homogeneous samples of different refractive indexes were monitored using wavelength interrogation, and the sensitivities of the different configurations are analyzed.

5.2 Experimental consideration and preparation

The theory on waveguide SPR has been well discussed earlier in sub-section 2.3.5.2, encompassing the SPs dispersion relation and feasible modulation techniques that can be employed in an optical waveguide. The wavelength-modulated waveguide SPR geometry, being the most compact technique for achieving SPR, is employed in our experiments. Similar to Chapter 4, we seek to compare the sensitivity of the two-layer metallic film configuration with single silver and gold films.

5.2.1 SU-8 photoresist as a waveguide material

Produced by *MicroChem Corp.*, NANOTM SU-8 2000 (formulated in Cyclopentanone) is an epoxy-based negative tone and chemically amplified near UV photoresist. SU-8 has extensively been used to fabricate many photonics devices as it exhibits high optical transparency to wavelengths greater than 360 nm and a low propagation loss of 0.36 dB/cm at 830 nm [116] has recently been demonstrated. In addition to being biocompatible [117] and mechanically stable, SU-8 possesses high aspect ratios of greater than 18 [118] and is available in various viscosities. Hence, microstructures and devices of varying thicknesses (from <1 μm to >200 μm) can be easily fabricated. Currently, the family of SU-8 resists have found wide application in the areas of soft-lithography [119], nano-imprinting [120], optical fluidic devices [121, 122], optical waveguides [123], micro-optical devices [124, 125], etc.. Like most polymers, SU-8 possesses wavelength-dependent optical properties. Shown in Figure 5-1 are the refractive index and extinction coefficient of SU-8, as detailed in the datasheet provided by the supplier.

Figure 5-1: Refractive index and extinction coefficient of SU-8 material (taken from ref [126])

As observed from the dispersion curve, the refractive index range of SU-8 within the visible spectrum from blue to red varies quite substantially by ~ 0.03 RIU. However, the extinction coefficient has a very low value throughout the visible range, indicating the high optical transparency of this material. This will be extremely vital for waveguide applications.

5.2.2 Biocompatibility of SU-8 photoresist

As SU-8 material has popularly been employed in micro-fluidics and MEMS applications, studies on its biocompatibility is of paramount importance. Recent studies by several groups have indicated that SU-8 demonstrates good biocompatibility and reduced biofouling when employed for drug delivery devices [117], and cells cultured on SU-8 surfaces showed no negative response [127]. Naturally, it will be a prudent option to employ SU-8 material for our waveguides, laying a platform for biological applications in future.

5.2.3 SU-8 photoresist thickness calibration

Figure 5-2 shows the calibrated film thicknesses of SU-8 2005 and SU-8 2025 resist with respect to spin-speed on silicon dioxide (500 nm) – on – silicon substrates. From the calibration curves, we can achieve the resist thickness we desire simply by modulating the spin-coating speed.

Figure 5-2: Calibrated film thicknesses of SU-8 2005 and SU-8 2025 resist with respect to spin-speed on silicon dioxide – on – silicon substrates

5.2.4 Waveguide fabrication

The waveguide fabrication process starts by spinning SU-8 2025 on a pre-treated 500 nm thick silicon dioxide – on – silicon substrate at a spin speed of 3700 rpm, achieving a final film thickness of ~ 20 μm following calibration curve. Next, the resist on substrate has to be soft-baked on a level hotplate, to evaporate the solvent and densify the film. In this soft-bake process, the resist was placed on a hotplate at 65°C for 2 minutes followed by 95°C for another 3 minutes. After 10 minutes of cooling, the SU-8 photoresist is exposed to 250 mJ/cm² of UV radiation under a patterned mask to trigger the formation of acid catalyst in the exposed regions. The mask is designed to fabricate linear waveguides with 50 μm width. The UV exposure has been implemented on a Q 2001CT UV mask contact aligner (Quintel Corporation), with a peak emission at 365 nm wavelength and an irradiance of 15 mW/cm². The film then undergoes post-exposure-baking at 65°C for 2 minutes and 95°C for 5 minutes to initiate cross-linking of the exposed region. During this phase, exposed SU-8 begins to

cross-link in the presence of acid catalyst and pulls in from the unexposed regions, forming gentle surface relief structures which are clearly visible. After post-exposure-baking, the sample was immersed in a beaker of MicroChem's SU-8 developer for 5 minutes and rinsed briefly with Isopropyl Alcohol (IPA), before blowing it dry with a stream of nitrogen gas. Finally, we hard-baked the waveguides at 200°C for 30 minutes to further cross-link the resist, evaporate all the solvents and ensure that the refractive index is stable over time. The optical and SEM images of the fabricated waveguide are shown in Figure 5-3.

Figure 5-3: (left) Edge of waveguide as viewed under a 50 $\times$ optical microscope; (right) Cross-section of waveguide under SEM

5.2.5 Metallic film deposition

Various metallic films, all 2 mm long, are deposited on the waveguides using the electron beam evaporator (Edwards) and their thicknesses are monitored with a crystal deposition monitor. Both the single-layered silver and gold films are 45 nm thick, whereas the bimetallic film constitutes a 45 nm thick silver with a 5 nm thick overlying gold film. In contrast to Chapter 4, metallic film thickness optimization was not performed in this situation for several reasons. Firstly, the waveguides used for SPR are multimodal and exact theoretically simulation of the optimum metallic film thicknesses may only be possible with memory-intensive software like finite-difference-time-domain. Secondly, all the dielectric

materials used in this experiment, for instance SU-8 waveguide and analyte, exhibit frequency-dependent optical characteristics, which are extremely difficult to model up to high accuracy. Figure 5-4 shows the schematic of the bimetallic layer waveguide SPR configuration.

Figure 5-4: Schematic of the bimetallic layer waveguide surface plasmon resonance sensor

5.2.6 Analyte preparation

The analytes we employed for waveguide SPR sensing will be similar as before, i.e. homogenous glucose solutions. Glucose power is slowly added into de-ionized water which under constant stirring by a magnetic stirrer, yielding refractive indexes of 1.41, 1.42, 1.43, 1.44 and 1.45. The refractive indexes of the five solutions are measured with a refractometer.

5.2.7 Instrumentation

In our experimental setup as shown in Figure 5-5, white light from an LED source of 8000 mcd passes through a polarizer and into a microscopic objective lens. In order to focus the incoming white light into a small spot, we employed a 100× objective lens to couple light directly into the waveguide. We have opted for a direct coupling technique for three reasons.

Firstly, maximum power from our LED can be launched into the waveguide using minimal optical components. Secondly, we are able to polarize the incident beam vertically (*p*-polarized) to maximize the excitation of SPs. Finally, the in-coupled angle of the tightly focused incident beam exceeds the acceptance angle of the waveguide, ensuring that all possible propagation modes are generated. Our fabricated waveguide, having a length of 15 mm, width of $50\ \mu\text{m}$ and height of $20\ \mu\text{m}$, sits on a sub-micrometer precision stage (Newport ULTRAlign, model: 561D) for ease of alignment. In- and out-coupling of light into and from the waveguide is facilitated with a zoom stereo optical microscope (Olympus, model: SZ11). The waveguide output is first viewed with a CCD camera mounted with a $20\times$ objective lens, and the waveguide position is translated to achieve optimal coupling. Thereupon, the CCD camera is removed and replaced with an optical fibre. The light output from the waveguide is butt-coupled into the optical fibre and the spectrum is measured using a spectrometer (Ocean Optics, model: USB2000).

Figure 5-5: Experimental setup to measure the spectral of output beam from the waveguide. (1: White LED, 2: Polarizer (vertical transmission axis), 3: $100\times$ objective lens, 4: Waveguide ($50\ \mu\text{m} \times 20\ \mu\text{m}$) coated with metallic film, 5: Optical fibre (50/125), 6: Ocean Optics USB2000 spectrometer)

5.3 Results and discussion

5.3.1 Normalized SPR intensity curves

Experiments are performed on waveguides coated with the three metallic configurations using glucose solutions with refractive indices ranging from 1.41 to 1.45. After the setup has been aligned to achieve a relatively stable maximum output from the waveguide, the spectral data of the output intensity without any sample is first measured. Subsequently, a drop of sample is dripped on top of the waveguide using a needle syringe to completely cover the metal sensing surface. As expected, upon dripping the glucose sample on the sensing surface, a distinct drop in the spectral plot within a short range of wavelengths can be observed. The occurrence of such a phenomenon is largely attributed to SPR whereby the evanescent wave of the propagated mode within the waveguide has tunneled through the metal to excite SPs on the metal-sample interface. We understand that the drop in intensity can also be attributed to the poor confinement of light as the refractive index of the top medium (sample) increases. However, this power drop due to poorer confinement is only a small fraction of total power and it will not result in the distinct drop in intensity at a particular central wavelength. When a particular waveguide mode fulfills the SPs resonance condition, energy from this mode is absorbed by the metallic film, inducing collective oscillations of electrons. Prolong SPs excitation induces a thermal gradient vertically upwards, normal to the waveguides. Although our light source is a low-power LED, we keep our measurement process as short as possible and switch on the LED only when necessary to minimize the temperature dependent effects on our results. Figure 5-6 shows the normalized spectral plots when glucose solutions of refractive indices of 1.410, 1.420, 1.430, 1.440 and 1.45 are monitored with the bimetallic film waveguide SPR sensor.

Figure 5-6: Surface plasmon curves for increasing refractive indexes, monitored with the bimetallic film waveguide SPR sensor

As depicted in Figure 5-6, the resonance wavelength shifts right as the refractive index of the sample is increased. It is noted that the minimum intensity at the resonance wavelength does not drop till zero. This occurs as the waveguide possesses multi-modal guiding characteristics across the range of wavelengths. The resonance condition is not fulfilled at certain modes and the output intensities from these modes are unchanged. In addition, though we polarized the light before launching into the waveguide, polarization conversion within the waveguide occurs. Hence, the propagating beam consists of some TE portions which do not lead to SPs excitation. To improve the sensitivity of the system, we can opt to employ a smaller waveguide. However, with a smaller waveguide, the in-coupling of light via the direct coupling method poses a problem as the noise level will increase. In addition, the power delivery of the waveguide will be weaker and it will be harder for the detector to measure the signal. We have decided on the current dimensions as we feel that this setup can easily be miniaturized into a compact system in future and yet provide a reasonable sensitivity for diagnostic applications.

Samples of various refractive indexes are monitored on the three metallic configurations and their respective resonance wavelengths are plotted in Figure 5-7. As observed from the figure, the proposed bimetallic configuration provides a compromise of the sensitivity between silver and gold film. This sensitivity is better than that achievable with the single gold film, while not deviating much from that attainable with the single silver film. On a broader note, the intensity minimums have an unpredictable trend, making it difficult to quantify correctly the FWHMs of all the curves. The irregular intensity minimum and broad FWHM can be attributed to the large waveguide that we employ. Two possible consequences arises: supported modes that fulfill the SPR requirement resonate at different wavelengths with different resonance depths [128] while modes that do not fulfill the strict condition for SPR contribute to the noise level instead. Additionally, multimode waveguides may permit modal and polarization conversions due to perturbations within the waveguide [129]. A clearer picture will be possible if a single modal waveguide is used instead [130, 131]. The current configuration is adopted for ease of experiment and proof-of-principle purposes only. Moreover, the current resonance wavelength analysis is already very indicative of the sensing edge achievable with the bimetallic configuration.

Figure 5-7: Resonance wavelength for various refractive indices (in bimetallic, silver and gold configurations)

5.3.2 Sensitivity improvement in terms of $d\lambda/dn$

Figure 5-8 shows the corresponding shift in resonance wavelength for the three configurations when the refractive indices of the samples are increased from 1.41 to 1.45. In the single silver film configuration, the change in resonance wavelength with refractive index ($d\lambda/dn$) is approximately 1548 nm/RIU, much greater than the 594 nm/RIU achievable with the single gold film configuration. The bimetallic silver-gold layer provides a compromise with a reasonably good sensitivity of 1232 nm/RIU, which is $\sim 2.07\times$ improvement than the single gold film configuration. By simply changing the metallic layer from gold to silver-gold on any SPR sensors, the smallest refractive index change detectable will be reduced by half. Though the sensitivity improvement comes at the expense of dynamic range, the reduced range is still sufficient for most applications. For instance, a polychromatic incident beam with a spectrum width of 200 nm offers a refractive index

sensing range ~ 0.16 RIU, potentially adequate to differentiate between large differences in analyte concentration. The sensitivities for the three metallic configurations measured with our experimental configuration are different from that reported in several works. In previously reported publications, waveguide SPR sensors based on (1) an intermediate buffer layer of MgF₂ for water sensing; (2) silica-on-silicon channel waveguides for aqueous sensing and; (3) $K^+ \leftrightarrow Na^+$ ion exchanged waveguide in BK7 optimized for aqueous environment sensing yield sensitivities of 150 nm/RIU [132], 683 nm/RIU [128] and 2100 nm/RIU [82] respectively. Technically, the difference in measure sensitivities is ascribed to the different configurations employed. For instance, any differences in the refractive index of the waveguide and analyte sample, as well as the thicknesses of the metallic films deposited will contribute to disparity in results. The main objective of this chapter is to analyze the sensitivities attainable with the various metallic film SPR configurations, instead of challenging the highest sensitivity achievable. Therefore, detailed analysis on the different sensitivities measured will not be discussed further. It is worthwhile to compare between the different conclusions on wavelength-dependent-sensitivity discussed in this chapter and in Section 4.3.2. In the earlier section, discussions focus mainly on the attenuated-total-reflection configuration, assuming constant prism and sample refractive index. The material frequency-dependent characteristics would have been negligible if the resonance dips for all the configurations occur around the same region. On the contrary, it is obvious that the resonance point for the silver SPR sensor is in the 500 – 570 nm range, gold SPR sensor in the 600 – 670 nm range, and bimetallic film sensor in the 550 – 610 range. The different ranges for SP excitation with the various metallic configurations amplify the effect of wavelength-dependency. Secondly, the propagation of a guided wave is much more complicated than that of free space optical propagation. Modal characteristics, dispersion and optical confinement are few of the many considerations associated with guided wave transmission.

Figure 5-8: Linearly-fitted shift in resonance wavelength for a given change in refractive index, $d\lambda/dn$.

Despite the fact that silver is the more sensitive metal for sensing a smaller change in the refractive index of the analyte, its disadvantage lies in its high susceptibility to oxidation. Gold is a good option in terms of practicality as it is very resistant to oxidation and other atmospheric contaminants but sufficiently reactive to accommodate coating with a wide variety of binding molecules [114]. Therefore, the bimetallic configuration is capable of integrating the higher sensitivity of silver with the better film stability and molecular adhesion of gold to realize a highly responsive, better precision and longer life-time SPR sensor. The problems of silver-gold alloy and the diffusion of silver atoms into the gold film at the silver-gold interface have not been considered evidently in this chapter. Nonetheless, the SPR curves obtained are reasonably good enough to illustrate the SPs phenomenon vividly and demonstrate the advantage of employing the two-layered metallic film waveguide SPR configuration.

In contrast to the simulated results presented earlier in Chapter 4, where simulated wavelength-modulated sensitivity with the prism geometry yields no significant differences for all the configurations, our experimental results employing the waveguide SPR configuration showed huge disparity in sensitivity ($d\lambda/dn$). The distinction between the two results can be attributed to several reasons. Firstly, the simulated $d\lambda/dn$ is based on the prism scheme where the illuminating source is a highly collimated p -polarized laser beam. However, the experimental data presented was measured with multi-modal waveguides, whose optical propagation is much more complex. Additionally, almost all materials in the world possess intrinsic frequency-dependent optical properties, including the waveguide material, i.e. SU-8. As presented earlier in 5.2.1, the refractive index of SU-8 varies considerably in the visible spectrum. The discrepancies in results might be attributed to the exclusion of wavelength-dependent dielectric constant for the prism and sample in our earlier simulation.

5.4 Conclusion

Employing the waveguide SPR setup with three different metallic configurations, various SPR curves were obtained and analyzed. In comparison to the single gold film waveguide SPR configuration, our experimental results indicated that the bimetallic film setup possess greater than two-fold improvement in sensitivity ($d\lambda/dn$), from 594 nm/RIU to 1232 nm/RIU. Gold material, popularly used for many applications due to its inertness and good bio-adhesion, protects the underlying silver which provides huge enhancement of the evanescent field. In addition to the ease of achieving wavelength interrogation, the employment of a low cost and low power consumption polychromatic LED source demonstrates the feasibility of the proposed configuration for point-of-care diagnostic applications. Hence, less sensitive detectors can be used whilst achieving high sensitivity. In addition, the increased evanescent field implies that longer ligands can be immobilized on

the sensing surface and less concentrated analyte solutions can be monitored. The higher sensitivity, compact nature and better evanescent field enhancement of this configuration makes it an extremely attractive technique for bio-sensing applications, with a capability of massively parallel processing for high throughput screening.

CHAPTER 6

FABRICATION OF EMBEDDED WAVEGUIDES

6.1 Overview of Chapter 6

When applying the waveguide SPR setup for bio-molecular sensing or fluorescence excitation, integration with a micro-fluidic flow cell or fluorescence chamber will be essential. However, the waveguides presented earlier in Chapter 5 are edge relief structures fabricated using standard contact-mask-lithographic procedures. Therefore, integration with an enclosed chamber for fluorescence excitation is not possible due to the undulating cross-sectional profile of our waveguides and finding a new method for fabricating planar embedded waveguides is especially critical. In this chapter, we review and compare two recently proposed techniques to fabricate embedded step-index waveguides.

6.2 A synopsis of waveguide fabrication techniques

There are many approaches to waveguides fabrication in literatures. One common method is to employ UV irradiation for refractive index modulation in materials such as sol-gel [133] and PMMA (polymethyl methacrylate) polymers [134]. In addition, ion-exchanged [135]

optical waveguides can be fabricated too via the diffusion of mono-valent atoms of higher polarizability into glass matrix. However, one constrain of the waveguides fabricated with these processes is that their thicknesses cannot be varied over a large range (typically $< 5\mu\text{m}$). Therefore, the insertion loss might be quite substantial when launching light into the waveguides and high precision coupling systems are required for optimal coupling.

Another waveguide fabrication method will be to employ photosensitive photoresists in contact-mask-lithography for the fabrication of edge relief waveguides. In particular importance for bio-related applications, SU-8 photoresist (MicroChem) is popularly used as it has been shown to demonstrate good biocompatibility and reduced bio-fouling [117]. Additionally, its availability in various viscosities facilitates the fabrication of devices with varying thicknesses. Usually, an additional development step is required to remove the unexposed portions which has not cross-linked, and reveal the fabricated edge-relief waveguides. As the index difference at the film-SiO₂ (~ 0.10) and film-air (~ 0.57) boundaries are large, such waveguides supports many propagation modes (10-30) even at a thickness of $5\mu\text{m}$. Recently, two new approaches to fabricate index-modulated elements with SU-8 material have been demonstrated, namely the photo-thermal [136] and the dual UV-exposure [137] lithographic techniques. In the first approach, SU-8 portions exposed to UV under a patterned mask cross-linked via photo-initiation, whereas the unexposed portions polymerized due to high thermal treatment. Since the unexposed portions undergo more thermal densification prior to cross-linking, their refractive is $\sim 7.2 \times 10^{-3}$ higher than the UV-initiated regions. In the second approach, refractive index modulation is realized as different regions undergo different thermal densification prior to UV-induced polymerization. Therefore, a small refractive index contrast of $\sim 8 \times 10^{-4}$ or lower can be achieved. In the following sections, we will discuss the theory of index modulation for the two techniques and describe the respective waveguide fabrication processes.

6.3 Refractive index modulation techniques

6.3.1 Photo-thermal lithography

As SU-8 is an epoxy-based photoresist, we are able to alter its refractive index marginally by varying several processing parameters. Differential Scanning Calorimeter measurements have revealed that SU-8 possesses a minor reaction exotherm at an onset temperature of 137°C and a major exothermic reaction at a higher temperature of 167°C [138]. This suggests that the unexposed areas in SU-8 will begin to cross-link if it is subjected to an onset temperature of 137°C, and experimental data has shown that development will no longer be effective if the resist is baked at 155°C for 10 minutes [139]. Hence, we attempt to characterize the refractive index of fully cross-linked SU-8 that is fabricated either by standard UV-lithography or sole thermal treatment.

The characterization process starts by spin-coating two samples of SU-8 2005 on silicon dioxide – on – silicon substrates. As we hope to modulate the refractive indices at different portions of a single SU-8 film eventually, the process steps between the two samples are similar except for UV exposure. Figure 6-1 shows the process flow chart of the two samples.

Figure 6-1: Process flow chart to cross-link two samples of SU-8 2005 via different fabrication procedures

During UV exposure of sample #1, acids are formed and they catalyze polymerization during post-exposure-baking of the resist. On the contrary, the final hard-baking step at 170°C for 30 minutes ensures that sample #2 cross-linked fully due to sole thermal treatment. We consider the polymerization process in sample #1 to be UV initiated and sample #2 to be thermally triggered. As measured using a prism coupler (Metricon Corporation, Model 2010), the refractive indices of fully cross-linked SU-8, UV initiated or thermally triggered, are found out to be 1.5858 or 1.5930 respectively at a wavelength of 633 nm. We believe that the explanation for the lower refractive index of sample #1 is as such. After pre-baking and UV exposure of SU-8 photoresist, a residual amount of solvent is still left in the film. During post-exposure-baking, the exposed SU-8 starts to cross-link and its height is set in the polymerization process. As the pre- and post- exposure-baking temperatures are low (95°C), some solvents still remain within the film. Upon further baking, these solvents

within the cross-linked SU-8 evaporate, resulting in an optically looser medium. Even when the film is hard baked at 170°C to evaporate all the solvents, the shrinking of SU-8 due to more complete cross-linking of the resist is marginal. On the contrary, sample #2 experiences increasing thermal treatment as the temperature is slowly ramped to 170°C. Therefore, more solvents are evaporated just before the film completely cross-linked, resulting in a denser film. The increase in refractive index n is due to increase in the density ρ of the resist after thermal treatment, as indicated by the Lorentz-Lorentz equation [140, 141].

$$\frac{n^2 - 1}{n^2 + 2} = \frac{4\pi N}{3M \epsilon_0} \rho \beta \quad \dots\dots\dots (6-1)$$

where N is the Avogadro number, M is the molecular weight of the polymer repeat unit, ϵ_0 is the permittivity of free space and β is the polarizability of the molecules, which increases with density during shrinkage of the resist ($\beta \propto \rho$). For ease of understanding, if we assume that all the variables are constant except n and ρ ($\rho \propto \beta$ anyway), we will obtain a positive value for $d\rho/dn$.

6.3.2 Dual UV-exposure lithography

In standard UV lithography processes, pre-baking of the resist is always performed prior to UV exposure to evaporate the solvent and densify the film. However, as the pre-exposure-baking temperature is low (typically at 95°C), residual amount of solvent is still left in the film. The amount of solvent within the SU-8 film prior to UV exposure will determine the looseness and height of the final film. Therefore, we believe that the degree of thermal densification prior to UV exposure will affect the refractive index and thickness of the fabricated film. In our characterization process to determine the influence of pre-exposure-

bake time on refractive index, we have spin-coated SU-8 2005 on ten silicon substrates with a spin speed of 1400 rpm for 30 s. All the samples are first pre-baked at 65°C for 2 min followed by 95°C for ten different timings (0, 1, 2, 3, 5, 10, 15, 20, 30, and 40 min). The samples are then left to cool naturally for 10 min before flood exposing to UV radiation. Next, the SU-8 samples are subject to post-exposure baking at 65°C for 5 min and 95°C for 5 min to initiate cross-linking. A hard-baking step at 200°C for 30 min is performed to further cross-link the resist, evaporate all the solvents and ensure that the refractive index is stable over time. Figure 6-2 shows the refractive indices of the ten samples, measured using a prism coupler, for different pre-exposure-bake timings.

Figure 6-2: Refractive index variation with different pre-exposure-bake timings

The refractive index of the final cross-linked SU-8 film increases sharply after 2 min of pre-exposure-baking and reaches saturation when the film is pre-baked for approximately 20 min. This indicates that a large amount of solvents are evaporated within the first 2 min of pre-baking at 95°C, and almost all the solvents would have been evaporated after 20 min. The increase in refractive index is attributed to more thermal densification of SU-8

photoresist prior to cross-linking, similar to that described earlier in Equation 5-1.

From Figure 6-2, we can observed that the final refractive index difference is approximately 0.0008 if the film is subject to 2 and 20 min of pre-exposure-baking respectively, and this index difference can be reduced further if we fluctuate the pre-exposure-baking times between 2 and 20 min. Riding on the index modulation principles, we employ a simple dual-UV-exposure lithographic technique to fabricate waveguides without the development process. As the measured index contrast is near the resolution limit of our equipment, we seek to demonstrate the refractive index modulation by observing light propagation and measuring the absorption loss through embedded waveguides fabricated with this technique.

6.4 Index-modulated waveguide fabrication processes

6.4.1 Waveguide fabricated with photo-thermal lithography

The waveguide fabrication process starts with the deposition of SU-8 2005 on a silicon dioxide (500 nm) on silicon substrate by spin coating. The resist was spun at a spinning speed of 1400rpm, achieving a final film thickness of 7 μm following the calibration curve. Silicon dioxide, with a refractive of ~ 1.457 , acts as the lower index cladding for mode propagation through the waveguide. Next, the SU-8 film goes through pre-exposure-baking at 65°C for 2 minutes and 95°C for 2 minutes on a hotplate to evaporate most of the solvent and densify the film. The film is left to cool naturally for 10 minutes, and then exposed to 100 mJ/cm^2 of UV radiation under a patterned mask. The mask is designed to fabricate linear waveguides of two different widths, namely 5 μm and 20 μm . The UV exposure has been implemented on a mask contact aligner, with a peak emission at 365 nm wavelength. After UV exposure, the film undergoes post-exposure-baking at 65°C for 5 minutes and 95°C for 5 minutes to initiate cross-linking of the exposed regions. During the initial phase of baking,

exposed SU-8 begins to cross-link and pulls in from the unexposed regions, forming surface relief structures which are clearly visible. Upon further baking, the exposed SU-8 undergoes more cross-linking and its height is “set” in the polymerization process. However, in the unexposed regions, more solvents are evaporated during baking. This results in a larger height difference between the exposed and unexposed regions. Next, we trigger the cross-linking of the unexposed SU-8 thermally by baking the substrate on a hotplate at a temperature of 170°C for 30 minutes. To avoid introducing stress instantly, we ramp the temperature from 95°C to 170°C at a rate of 10°C/min. When unexposed SU-8 are subjected to high thermal treatment, it starts to shrink inwards hence resulting in an increase of its height. On the other hand, the thermal densification process results in marginal height reduction of the resist due to evaporation of the residual solvents. These two counter-processes act on the film during thermally initiated polymerization of SU-8 and result in undulating surface quality of the final cross-linked film. As measured by a Detak stylus-based surface profiler (Veeco Metrology), the height difference between the two regions reaches a peak value of approximately 400 nm. The fabrication process of the embedded waveguides is shown in Figure 6-3 and the SEM photograph of a 5 μm width waveguide is shown in Figure 6-4.

Figure 6-3: Schematic illustration of the photo-thermal waveguide fabrication process: (a) pre-exposure baking of film, (b) acids formed in UV exposed region, (c) exposed region cross-linked during post-baking while more solvents evaporate from the unexposed regions, (d) thermally triggered cross-linking of unexposed regions

Figure 6-4: SEM photograph of the surface of a photo-thermal fabricated waveguide tilted at 45° with the horizontal

6.4.2 Waveguide fabricated with dual UV-exposure lithography

Similar to the waveguides fabricated using the photo-thermal process, SU8 2005 is first spun on a silicon dioxide – on – silicon substrate at a spin speed of 1400 rpm. Next, the film is pre-baked at 65°C for 2 min and 95°C for 2 min on a hotplate. After 10 min of cooling, the SU-8 photoresist is exposed to 100 mJ/cm^2 of UV radiation under a patterned mask. In contrast with the photo-thermal fabricated waveguides, the film undergoes post-exposure-baking at 65°C for 2 min and 95°C for 20 min to initiate cross-linking of the exposed regions. However, the unexposed portions see this step as pre-exposure-baking which further densify the film and reduces its height. After post-exposure-baking, the film is left to cool for 10 min before undergoing a second UV radiation. This UV exposure creates acid catalyst in the initially unexposed regions, whilst not affecting the already cross-linked portions. The

film is baked again at 65°C for 5 min and 95°C for 5 min to cross-link the regions that are exposed to the second run of UV radiation. Finally, the film is hard-baked at 200°C for 30 min. The fabrication process of the embedded waveguides is shown in Figure 6-5. As the waveguides and claddings fabricated in this process undergoes standard soft-bake / UV exposure / hard-bake lithographic procedures, the surface quality of the waveguides are very good and cannot be imaged with a SEM machine. Measuring with an Atomic Force Microscope (AFM), the waveguide's surface has a peak-to-peak deviation of 23.1 nm and a root-mean-square surface roughness of 3.2 nm. We believe that the reason for the peak-trough phenomenon on the waveguide's surface is due to stretching of the film surface triggered by cross-linking competition between the differently treated regions. However, as compared to a film thickness of 7 μm , the surface roughness is still within an acceptable value. The two- and three-dimensional AFM images of the surface of a 5 μm wide waveguide are shown in Figure 6-6.

Figure 6-5: Schematic illustration of the dual-UV exposure waveguide fabrication process: (a) pre-exposure baking of film, (b) acids formed in UV exposed region, (c) exposed region cross-linked during post-baking while more solvents evaporate from the unexposed regions (d) second UV exposure, (e) cross-linking of the regions exposed to the second UV radiation, (f) Hard-

baking of the final structure to achieve mechanical hardness and refractive index stability

Figure 6-6: Two- and three-dimensional AFM images of the surface of a 5 μm wide waveguide

6.4.3 Comparison between waveguides fabricated with photo-thermal and dual UV-exposure lithographic processes

Summarize in Table 6-1 are the characteristic parameters for 5 μm wide waveguides fabricated with the photo-thermal and dual UV-exposure lithographic processes. For almost all applications, waveguides with a smaller core-cladding index difference, dn , and smoother surface profiles are preferred. This is because the small dn enables the fabrication of waveguides with larger core dimensions, whilst maintaining low level of propagation modes, thereby leading to better coupling efficiency and less stringent coupling alignments. Additionally, light energy can be better confined and guided in waveguides with smoother surface profiles. Therefore, it is apparent that dual UV-exposure lithography is a better process for fabricating waveguides, whereas photo-thermal lithography might be more suitable for fabricating phase elements such as diffractive-optical-elements.

Table 6-1: Characteristic parameters for 5 μm wide waveguides fabricated with the photo-thermal and dual UV-exposure lithographic processes

Comparison \ Process	Photo-thermal lithography	Dual-UV lithography
Index difference between core and cladding (dn)	0.0072	0.0008 (max)
Height difference between core and cladding (dh)	400 nm	23.1 nm (max)

6.5 Waveguide measurement

Figure 6-7 depicts the experimental configuration we employed to map the output beam profile of the waveguides fabricated with dual UV-exposure lithography. Light from a halogen lamp is focused into 9 μm /125 μm fibre pigtailed collimator via a 40X objective lens. The other end of this fibre is mounted on a fibre chuck and butt coupled into the fabricated waveguide. Another 40X objective lens is placed after the waveguide to expand the output beam and the beam profile is mapped using a CCD camera.

Figure 6-7: Experimental setup to map the output beam profile of the waveguide. (1: halogen lamp; 2: 40 $\times$ objective lens; 3: fibre pigtailed collimator (9/125); 4: embedded strip waveguide; 5: 40 $\times$ objective lens; 6: CCD camera)

The output beam profiles from a 5 μm wide – 7 μm thick waveguide and 20 μm wide – 7 μm

thick waveguide are shown in Figure 6-8. As measured using the cut-back method, the $5\ \mu\text{m}$ wide – $7\ \mu\text{m}$ thick waveguide has a loss of approximately 1.07 dB/cm at a wavelength of 633 nm. The refractive indices of the waveguide's core and cladding will remain stable even in different environmental conditions as the SU-8 photoresist has cross-linked fully via the two UV-induced polymerization processes.

Figure 6-8: Output beam profiles from waveguides with dimensions (a) $5\ \mu\text{m} \times 7\ \mu\text{m}$ and (b) $20\ \mu\text{m} \times 7\ \mu\text{m}$.

6.6 Conclusion

Two new techniques for fabricating index-modulated embedded waveguides using commercial photosensitive SU-8 material are proposed and discussed in this chapter. Both methods achieved refractive modulation through thermal densification of the photosensitive material, prior to polymerization. The first, known as photothermal lithography, involves high thermal treatment to densify and crosslink unexposed sections, hence achieving a refractive index contrast with UV exposed portions which are optically looser. The second technique, known as dual-UV exposure lithography, involves a UV expose – bake – UV expose procedure to marginally modulate the refractive index between the initially exposed and subsequently exposed regions. Measurements revealed that the dual-UV-exposure

lithographic technique is capable of achieving better quality waveguides, as compared to the photo-thermal process. In the former process, the refractive index of SU-8 increases with increasing pre-exposure-bake time and the refractive index difference is approximately 0.0008 if the film is subjected to 2 or 20 minutes of pre-exposure-baking. Embedded waveguides of different widths have been fabricated and the good output beam profiles demonstrate that light can be guided through these waveguides. A smaller refractive index contrast can be achieved if the difference in pre-exposure-bake timings is reduced. Hence, waveguides with larger cores can be fabricated, whilst maintaining low level of propagation modes, thereby leading to better coupling efficiency and less stringent coupling alignments. In addition, the large thickness flexibility of SU-8 photoresist facilitates the tailored design and fabrication of thicker multi-modal waveguides. For optical applications, the proposed technique reduces processing cost and improves fabrication reliability as no development is required. In the subsequent chapters, we make use of this lithographic procedure to fabricate embedded waveguides for waveguide SPR-fluorescence applications.

CHAPTER 7

BIMETALLIC FILM WAVEGUIDE SPR FOR FLUORESCENCE EXCITATION

7.1 Overview of Chapter 7

In the earlier chapters, the narrower SPR curves and higher sensitivity in terms of $d\lambda/dn$ achievable with the two-layer metallic configuration has been experimentally demonstrated with the prism and waveguide based SPR setup respectively. However, the enhanced SP evanescent field much discussed is only a theoretical prediction and has not been not verified experimentally. A straightforward method to quantify the SP field enhancement will be to employ fluorescence spectroscopy [54]. A longer probing field is capable of exciting fluorophores deeper into the sample solution, hence leading to stronger collective emission from the fluorescence particles.

In this chapter, we integrate the embedded waveguides (discussed in Chapter 6) with a fluorescence chamber for the experimental observation of enhanced emission achieved with bimetallic silver-gold layer induced SP wave extension. Rhodamine B (RhB) solution,

placed on top of metallic films with various silver and gold thickness combinations, are excited via SP evanescent fields. The intensity of the collected fluorescence presents a direct analysis of the SP field's penetration depth.

7.2 Concept of fluorescence emission on metal surfaces

Fluorescence is an optical phenomenon in which the molecular absorption of a photon triggers the emission of another photon with a longer wavelength. This occurs when a fluorescence molecule relaxes to its ground state after being electronically excited. Usually, fluorescence is induced by directly illuminating the fluorophores with an incident excitation beam. Additionally, it can be excited via an evanescent field, as demonstrated in total internal reflection fluorescence spectroscopy [142, 143] and surface plasmon resonance fluorescence spectroscopy [53, 54]. The intensity of the fluorescence emission can be used to quantify the penetrating properties of the evanescent field. An enhanced evanescent field, with an extended probing depth, is capable of exciting more fluorescence particles, as depicted in Figure 7-1.

Figure 7-1: (a) Excitation of fluorophores with an exponentially decaying SP evanescent field; and (b) Excitation of more fluorophores with an enhanced SP evanescent field

Before making full use of the field enhancement for the excitation of fluorophores, one needs to pay attention to the de-excitation channels that exist for fluorophores near a metal surface. In 1987, Hellen and Axelrod [144] presented a theoretical review on the fluorescence emission at dielectric and metal-film interfaces. Subsequently, W. Knoll *et al.* proceeded to discuss further the potential and applications of SPs for fluorescence spectroscopy [53]. At particular range of distances of the fluorophores from the metallic surface, several optical phenomena occur. In the immediate proximity of the metal surface, the metal acts as a very effective quencher for fluorescence particles. Dye molecules do not emit fluorescence photons as all the excitation energy is dissipated in the metal. This quenching phenomenon is operational for fluorophores within 5-10 nm from the metal, typical separation distance for Förster energy transfer processes. At intermediate distances between 10 – 20 nm, complex interactions occur. The evanescent field from a resonantly excited SP transfers energy to the fluorophores. The subsequent de-excitation of the dye excites a red-shifted SP on the metal, which can then re-radiate via the prism at an emission angle slightly different from the resonant excitation angle. At larger distances, fluorophores are located close enough to be excited by the largely enhanced but exponentially decaying SP field, yet not in the immediate vicinity of the metal to lose fluorescence intensity due to the metal quenching process. The evanescent field of SPs typically varies from ~ 100 – 300nm. Although the signal from fluorophores within 20 nm of the metal film is severely quenched, dyes located beyond 20 nm contribute much more significantly to the fluorescence intensity, dwarfing the quenching process.

7.3 Experimental preparation

7.3.1 Waveguide consideration

The resonance condition for SP excitation is unique for different metallic choices and thicknesses. In a single mode waveguide, the amplitude of the evanescent field is a discrete value and this will probably not coincide with the unique condition for SP excitation. Hence, a straightforward method to ensure that SP excitation is realized in our experiments will be to employ a larger waveguide that supports multimode propagation, as shown in Figure 7-2. The amplitude of the electric field at the core/metal interface increases as the order of the waveguide modes increases. As the propagation constants (photon wavevectors in the direction of light propagation) of all the waveguide modes are different [83], one of these modes is highly likely to satisfy (very close to even if not exact) the SP resonance condition. In addition, a large waveguide offers the convenience of easier coupling.

Our experiment is not designed to compare between the SP evanescent field on top of the metallic film and the TIR evanescent field of the bare waveguide. The TIR evanescent field of a multimode waveguide comprises the collective sum of every evanescent field from each mode, with an increasing strength for higher order modes. However, the resonance condition for SP excitation is only possible at one particular mode and the evanescent fields from the other modes will be suppressed due to absorption by the metallic film. Hence, it will not be a fair assessment if we compare the strength of the evanescent fields generated on bare waveguides with SP fields on metal-coated regions.

Figure 7-2: Waveguide propagation modes and the excitation of SP evanescent field at a particular mode

7.3.2 Waveguide fabrication

In Chapter 6, we propose a dual-UV lithographic method for the fabrication of low-loss embedded waveguides without the development process [137]. Small waveguides were intentionally fabricated to aid the characterization and loss measurements processes. However, in this chapter, larger waveguides are desirable to achieve multimodal waveguides for SPs excitation. We discuss the waveguide fabrication process hereupon as the intended waveguides are larger and hence several parameters are different.

SU8 2025 is first spun on a silicon dioxide (500 nm) on silicon substrate at a spin speed of 2600 rpm, achieving a final film thickness of $\sim 30 \mu\text{m}$ following a calibration curve. Next, the film is pre-baked at 65°C for 2 min and 95°C for 5 min on a hotplate. After 5 min of cooling, the SU-8 photoresist is exposed to $300 \text{ mJ}/\text{cm}^2$ of UV radiation under a patterned mask. The mask is designed to fabricate 5 linear waveguides of width $20 \mu\text{m}$. The film then undergoes post-exposure-baking at 65°C for 2 min and 95°C for 20 min to initiate cross-linking of the exposed regions. After post-exposure-baking, the film is left to cool for 5 min

before undergoing a second UV radiation. Finally, the film is baked again at 65°C for 2 min and 95°C for 5 min to cross-link the regions that are exposed to the second run of UV radiation. The refractive index difference between the SU-8 core and the SU-8 side claddings is ~ 0.0008 , whilst the difference between the SU-8 core and the SiO₂ lower cladding is ~ 0.13 .

7.3.3 Metallic film deposition

To coat various silver-gold film combinations onto our waveguides, we engraved onto a Mylar sheet (30 μm thick) a pre-designed mask (Mask #1) with a rectangular opening (2 mm $\times$ 15 mm) using a direct-write CO₂-laser cutting system (Epilog Legend, model: 24TT). As shown in Figure 7-3, Mask #1 determines the width of metallic film that will be deposited onto all the waveguides. Next, we engraved another mask (Mask #2), with a much smaller opening of 2.5 mm $\times$ 1.5 mm. This mask permits the metallic vapour to be deposited onto one waveguide only, while isolating all others (Figure 7-3c). Changing the position of Mask #2, four silver – gold film combinations (30nm – 20nm; 20nm – 30nm; 10nm – 40nm; 0nm – 50nm) were deposited onto the waveguides in 4 deposition processes, using an electron beam evaporator. One waveguide is left uncoated and this serves as a reference in our experiments. To ensure that the surfaces of the coated metal films are consistent and smooth, the depositions are performed at the same pressure of 5×10^{-6} torr with a rate of 0.1 nm/s. Subsequently, the film roughness for all the metallic film combinations are measured with an atomic force microscope (AFM), yielding mean roughness values between 0.706 nm and 1.074 nm. We did not adopt a much thinner gold – thicker silver configuration in this waveguide SPR – fluorescence experiment, on the contrary with the earlier chapters where SPR is employed for sensing glucose solutions, water, acetone *et cetera*. In this chapter, stability of the metallic films is greatly appreciated as we believe that fluorescence dyes will

be more sensitive to any chemical reactions or oxidation of the underlying silver. A thicker gold protective layer ensures film stability and eliminates any possible chemical reactions, no matter how minute it might be.

Figure 7-3: (7-3a – 7-3d) Procedures for depositing various metallic films on waveguides; and (7-3e – 7-3g) Fluorescence chamber and waveguide microchip integration

7.3.4 Reagent preparation

One important requirement for the choice of fluorophores is that the wavelength of the illumination laser must be within its absorption spectrum. Usually, the availability of dyes

increases as the wavelength decreases since a shorter wavelength possesses higher energy for fluorescence excitation. A prudent laser choice will be green (532 nm) as a variety of dyes can be excited with green illumination, and it is above the minimum wavelength for SP excitation. RhB particles (dye content ~ 95%) from Sigma, with an absorption peak of ~ 554 nm in water [145], are used for our experiments. In addition to direct laser excitation, fluorescence dyes can also be excited by the evanescent wave of propagating waveguide modes. 0.05 g of RhB, used without further purification, was dissolved in 500 mL of water to achieve a concentration of 1.04 μM . We have opted for a medium RhB concentration to improve the SNR of the measured fluorescence signal, ensure that the difference in signals collected from different regions will be distinct for analysis, and overcome the metal quenching effects.

7.3.5 Fluorescence chamber and waveguide microchip integration

There are many fabrication techniques that can be used to construct plastic and polymer on-chip devices. We employ direct-write CO_2 laser cutting of polymer thin films to realize our desired three-dimensional fluorescence chamber. CO_2 laser photoablation, which enables rapid prototyping, was recently well described [146]. The choice of polymer thin film for fluorescence chamber is driven by its physical and optical properties, availability of the material and ease of fabrication. Optical grade MylarTM (Biaxially-oriented polyethylene terephthalate or boPET) polyester film has represented the best compromise by these criteria. A rectangular fluorescence chamber of dimensions 4 mm $\times$ 15 mm is engraved onto a double-sided adhesive Mylar (100 μm thick) and pasted onto the planar waveguides (Fig 6-2e). RhB solution is injected into the chamber and a thin Mylar film (30 μm thick) is placed on top of the double-sided adhesive Mylar to seal up the chamber. To prevent scattered laser light propagating above the waveguide's surface from creating unwanted noises and undesirably exciting RhB dyes, a thick opaque wall is built on the waveguides surface before

the chambers, using correction fluid.

7.3.6 Instrumentation

Figure 7-4 schematically depicts the setup for coupling a laser beam into the waveguide and observing the fluorescence intensity of the RhB molecules. A vertically polarized 532 nm laser beam (max power 200 mW), attenuated to 0.5 mW to minimize photobleaching effects of RhB dyes before our experimental observations, is focused onto the edge of the fabricated waveguide through a 100× microscopic objective. Similar to the experiments in Chapter 5, we employed direct coupling technique for three reasons. Firstly, maximum power from our laser can be launched into the waveguide using minimal optical components. Secondly, we are able to polarize the incident beam vertically (*p*-polarized) to maximize the excitation of SPs. Finally, the in-coupled angle of the tightly focused incident beam exceeds the acceptance angle of the waveguide, ensuring that all possible propagation modes are generated. A 40× microscopic objective is placed at the output of the waveguide to observe the output beam, hence ensuring that our experiments have been performed when light propagation through the waveguide is optimal. Fluorescence of RhB within the close proximity of the waveguide is collected using a 40× microscopic objective. The imaging and spectral measurement setups described hereupon are all built using a versatile cage system (Thorlabs). Mounting a 532 nm notch filter (StopLineTM) after the objective lens, the fluorescence image is observed using a CCD camera (Sony, model: SSC-DC58AP). Before our measurements, we first focus onto the bare reference waveguide to locate the correct focusing plane. Next, we switch off the white light illumination and move the stage such that light is coupled into the adjacent waveguide with metallic coating. For fluorescence measurement of RhB, the CCD camera is removed carefully and replaced by a spectrometer (Ocean Optics, model: USB2000). Finally, the power of the laser beam is increased to 200 mW. Our substrate sits on a sub-micron precision stage (Newport ULTRAlign, model:

561D) and coupling into all waveguides is achieved by translating the stage one-dimensionally. The surfaces of the waveguides and metallic coatings appeared essentially uniform, with occasional obvious surface flaws (scratches, etc.). In our measurements, these flaws are treated as anomalous, and avoided.

Figure 7-4: Instrumental setup for observing the fluorescence of RhB dye excited via evanescent fields of SPs

7.4 Results and discussions

7.4.1 Fading and emission characterization of RhB

For fluorescence measurements, a spectrometer is able to give us good spectral information of the fluorescence emission, with an option of increasing the integration time for weak signals. However, this integration time cannot be increased infinitely as the fluorescence of RhB suffers from fading, just like any other dyes. Additionally, RhB dissolved in different solvents possesses slightly different fluorescence properties. Hence, we attempt to measure the emission and photobleaching characteristics of RhB (dissolved in water) under green laser excitation prior to our experiments. As shown in Figure 7-5, a 30 mW green laser beam shines through a cuvette containing RhB of concentration $0.104 \mu\text{M}$. The fluorescence emission, collected at right angle to the excitation beam, passes through a 532 nm notch filter before reaching the spectrometer.

Figure 7-5: Experimental setup to measure the fluorescence decay of RhB dyes

A relatively diluted RhB solution is used in order to avoid the inner-filter effect [147] and ensure that the long fading time of RhB is not due to the high concentration of RhB used.

The integration time for this measurement is set to 3 ms. From the spectral data collected (Figure 7-6), we noticed that RhB in water possesses a fluorescence emission peak at ~ 579 nm, and there is no observable fading of RhB even after 120 s of laser excitation. As a reference, we measured the fluorescence signal using an empty cuvette. The collected spectrum shows no signal for all wavelengths (except a small peak at 532 nm), indicating that the cuvette material does not contribute to the background noise at all. The small peak at 532 nm arises due to the absence of RhB to absorb the laser's irradiation, and the multiple reflections of the laser beam within the cuvette. In our later experiments to observe the fluorescence emission of RhB excited with waveguide induced SP evanescent field, the intensity of this SP field is clearly much weaker than the 30 mW laser we used in this experiment. Hence, we can comfortably set longer integration times for weak signals and assume that the fading effect of RhB is negligible.

Figure 7-6: Fluorescence spectrum of RhB dyes after 0 s of laser irradiation, RhB dyes after 120 s of laser irradiation and an empty cuvette

7.4.2 Fluorescence behavior of polymers

As our experiment involves measuring the fluorescence emission from RhB, a very important consideration prior to performing our experiment will be the strength of the background noise, i.e. auto-fluorescence of polymer materials. It is well known that Mylar material fluoresces over a broad spectrum upon green laser excitation [148]. However, as Mylar material is not present in the focus of the 40× objective lens use for imaging and measurement of the fluorescence information, its auto-fluorescence will not be a source of noise. Instead, the fluorescence of our waveguide, i.e. SU-8 material, contributes considerably to the level of noise. Though SU-8 material has popularly been used in the fabrication of microstructures and microfluidic devices, there has been little previous works reporting on the fluorescence characteristics of SU-8 photoresist. With the same configuration as depicted in Figure 7-4, using water sample instead of RhB, the fluorescence image of a waveguide captured by a CCD camera is shown Figure 7-7. On the left side of Figure 7-7, the waveguide is bare while on the right side of the figure, the waveguide is coated with a 30 nm silver – 20 nm gold metallic film. It is quite evident that SU-8 material has a high level of auto-fluorescence upon green laser excitation. On the other hand, the fluorescence of SU-8 is severely quenched when a thin metal film is deposited on the waveguides. We believe that the reason for this is because the broad fluorescence spectrum from the SU-8 waveguide, being weak and incoherent, is unable to penetrate through the highly absorbing metallic film, at least in our case where the launched beam has a power of 200 mW.

Figure 7-7: CCD image of a waveguide with water on its surface, where the waveguide is bare on the left and coated with a 30 nm silver – 20 nm gold metallic film on the right

As the spectrometer measures the spectral information within the focus of the objective lens, it is inevitable that weak signals leaking from flawed surface regions within the vicinity of the waveguide will be picked up as well. Hence, to account for this noise, we first measure the fluorescence signal when light is coupled into the waveguide, translate the waveguide $\sim 20\text{ }\mu\text{m}$ sideward to intentionally cause misalignment and measure the spectrum of the noise. Subtracting the noise from the measured signal, we obtain the actual fluorescence signal from the desired regions. The spectral data of the fluorescence emission from the regions where the waveguide is bare and coated with a 30 nm silver – 20 nm gold metallic film is shown in Figure 7-8. As seen from the figure, it is vivid that the metallic film absorbs $> 95\%$ of SU8's autofluorescence. The degree of absorption by the various metallic film combinations will certainly not be the same, since the amount of absorption is dependant on the dielectric functions of gold and silver. At the wavelength of 633 nm, the skin depths of optical penetration in gold and silver are calculated to be $\sim 30\text{ nm}$ and $\sim 23\text{ nm}$ respectively, based on the formula from reference [149]. Since the combined metallic film thickness is much thicker at 50 nm, and that the autofluorescence of SU-8 is weak and incoherent, we

can safely conclude that the penetration of SU-8 autofluorescence through the highly absorbing metallic film will be confined to a narrow range within 5% for all the silver-gold metallic combinations. With further reference to Figure 7-8, it is noted that the autofluorescence of SU-8, peak at ~ 595 nm, overlaps the emission of RhB fluorophores. Therefore, it will be difficult to quantify accurately the fluorescence of RhB dyes that are on top of bare SU-8 waveguides. Nonetheless, as the waveguide auto-fluorescence is highly attenuated by the deposited metallic film, we will be able to obtain accurately the fluorescence spectrum of RhB dyes excited via SPs on top of a metallic layer.

Figure 7-8: Fluorescence spectrum from two separate regions of a waveguide with water on its surface, the first where the waveguide is bare and the second where a 30 nm silver – 20 nm gold metallic film is deposited

7.4.3 Fluorescence of RhB dyes excited with SP evanescent field

When a particular waveguide mode fulfills the SPs resonance condition, energy from this mode is absorbed by the metallic film. Prolong SPs excitation induces a thermal gradient

vertically upwards, normal to the waveguides. It is well known that the intensity of the fluorescence of RhB shows an inverse relationship with temperature. To minimize the temperature dependent effects of RhB, the fluorescence of RhB is measured immediately after the laser beam's power is increased to 200 mW. As soon as the readings are collected, the laser power is attenuated to 0.5 mW and the substrate is translated to couple the laser beam into the next waveguide. Using the same setup as shown in Figure 7-4, we measured the fluorescence emissions of RhB fluorophores on five different waveguide substrates. The fluorescence intensities for all the metallic film combinations are averaged and plotted in Figure 7-9.

Figure 7-9: Averaged fluorescence intensity of RhB dyes measured from regions with different silver-gold combinations

It is evident that RhB dyes on a thicker silver – thinner gold metallic layer emit stronger fluorescence signal. The peak fluorescence intensity at 579 nm for silver – gold combinations of 0 nm – 50 nm, 10 nm – 40 nm, 20 nm – 30 nm and 30 nm – 20 nm are 145, 287, 443 and 584 (arbitrary units) respectively. The fluorescence emission from RhB dyes on

a 30 nm silver – 20nm gold layer is ~ 4.0 times stronger than the emission from dyes on a single 50 nm gold film. This is attributed to the better evanescent field enhancement of silver film which results in more RhB molecules being excited within the SP field. Stronger excitation of RhB might occur due to increased waveguide leakages on rough metallic surfaces. However, we rule out this possibility since the roughnesses of the metallic surfaces are approximately the same, as measured earlier in sub-section 7.3.3.

Though waveguide SPs has popularly been employed for sensing applications, to our knowledge, no work has been done so far on waveguide SPs for localized fluorescence excitation. Waveguide SPs for fluorescence spectroscopy offers good signal-to-noise-ratio due to the highly localized excitation of fluorophores. From a theoretical perspective, prism or grating excited SPs possess short propagation lengths, ranging from tens to hundreds of micrometers. However, our results have indicated waveguide SPs is capable of inducing fluorescence emissions substantially along 2 mm metallic films. We attribute our observation to several reasons. When a particular waveguide mode satisfies the SPs resonant condition, upon first contact with the metallic film, it evanescently penetrates through the metal layer exciting a surface plasmon wave at its outer boundary. The optical energy coupled to SPs is a continuously process and spreads over a distance. In addition, since our multi-modal waveguide has a rectangular cross-section, mode conversion and cross polarization effects will likely surface. Hence, powers from adjacent modes will “replenish” the optical power in the weakened resonant mode. We believe that these are the primary reasons for significant SPs evanescent field excitation of RhB particles along the metallic deposited regions.

7.4.4 Transmittance study of BSA solutions

In addition to SPR-fluorescence applications, it will be worthwhile to demonstrate the merits of the proposed configuration in sensing, where the bulk of SPR research works is

concentrated in. A popular and straight forward scheme for monitoring analytes binding and disassociation using the prism SPR configuration involves illuminating the metal transduction surface at a fixed angle of incidence, while measuring the intensity profile of the reflected beam as different biological samples are flowed across the metal surface [100]. In the waveguide SPR configuration, similar study for monitoring analytes of different concentrations will be possible through measuring the transmittance profile.

The experimental setup and considerations for sensing analytes are slightly different in comparison to that of fluorescence excitation. Earlier, waveguides with multi-modal characteristics are intentionally fabricated so as to ensure that one of these modes satisfy the SP resonance requirement, hence creating a platform for SP field – fluorescence excitation. However, in transmittance studies of different analyte concentrations, single mode waveguides are preferred for better signal-to-noise-ratio. Besides, these analytes will have to be injected and flowed through the sensing surface using microfluidic channels.

The waveguide structure employed earlier, which comprises of a SiO₂ lower cladding and SU-8 core, permits multimode propagation due to the large index difference. To realize single mode propagation, we introduce an additional layer of lower-index SU-8 as the bottom cladding. Technically, this bottom cladding is processed in similar fashion as the side claddings of the waveguide, yielding a refractive index difference of $\sim 8 \times 10^{-4}$ with the core. To begin, a layer of SU-8 2005 is spun onto the substrate at a spin speed of 3000 rpm, soft-baked at 65 °C for 2 mins and 95 °C for 2 mins, left to cool for 5 mins, subjected to flood UV exposure, and finally post-baked at 65 °C for 2 mins and 95 °C for 5 mins. Next, another layer of SU-8 2005 is spun onto the cross-linked SU-8 at a spin speed of 3000 rpm, soft-baked at 65 °C for 2 mins and 95 °C for 2 mins, left to cool for 5 mins, subjected to UV exposure under a patterned mask, post-baked at 65 °C for 2 mins and 95 °C for 20 mins, left to cool for 5 mins, subjected to flood UV exposure, and finally post-baked at 65 °C for 2

mins and 95 °C for 5 mins [137]. The aforementioned procedures yield a final single mode waveguide structure of dimensions $5\ \mu\text{m} \times 5\ \mu\text{m}$. Next, the waveguides are deposited with 2 mm wide of metallic film with the e-beam evaporator, under similar conditions as described earlier in sub-section 7.3.3

The procedures of constructing microfluidic channels with Mylar material are well documented in literature [150]. Briefly, a double-sided adhesive Mylar (100 μm thick), engraved with a 2 mm wide linear channel for fluidic flow, is pasted onto the waveguides. Next, a layer of non-adhesive Mylar (30 μm thick), engraved with two little circular openings permitting fluidic in-flow and out-flow, is placed on top to cover the channel. The cross-section of the integrated microfluidic channel – waveguide microchip is shown in Figure 7-10.

Figure 7-10: Cross-section of integrated microfluidic channel – waveguide microchip

In our proof-of-principle experiments to demonstrate the potential of the bimetallic configuration for sensing analytes of lower concentration, we employ bovine serum albumin (BSA, Sigma) of different concentrations as the model analyte. BSA absorbs readily onto gold surfaces forming a thin layer, and prolonged flowing leads to gradual thickening of the

BSA layer due to aggregation. Shown in Figure 7-10 is the modified setup for these sensing experiments. The output from the waveguide is directly coupled into a fibre (9/125) which connects to a spectrometer. A motorized pump, loaded with 1 mL of phosphate buffered saline (PBS, pH 7.4) solution, acts as the driver for fluidic flow along the micro-channels. BSA solution is loaded into a 500 μL sample loop (Upchurch Scientific) and the flow rate for fluidic injection is programmed to be 1 $\mu\text{L sec}^{-1}$. In a single pump cycle, 500 μL of BSA solution first flows across the sensing surface, followed by 500 μL of PBS which acts as a flushing mechanism. Thereupon, the next higher concentration BSA solution is loaded into the sample loop and the cycle is repeated. To ensure that there is minimal contamination, BSA solutions are used in ascending concentration, in the order of 5 $\mu\text{g mL}^{-1}$, 50 $\mu\text{g mL}^{-1}$ and 500 $\mu\text{g mL}^{-1}$. To highlight the contrast between the single gold and bimetallic silver-gold configurations, results for the 50 nm gold and 30 nm – 20 nm silver-gold metallic layers are presented. Depicted in Figure 7-11 are the transmittance profiles for the two metallic film waveguide SPR configurations. The transmittance profile is computed by normalizing with respect to the measured waveguide output power with PBS as analyte.

Figure 7-11: Instrumental setup for transmittance measurement, as BSA solutions of different concentrations are flowed across the metal sensing surface

From Figure 7-11, we observe that the transmittance profile for the single gold SPR configuration rises steeply when BSA solution is flowed across the metal surface. However, the change in transmittance reaches a standstill before the flow of BSA solution ends. This implies that BSA has absorbed onto most or all of the metal surface, and that the increase in the BSA layer thickness due to aggregation cannot be visibly detected. Additionally, the single gold configuration shows no clear trend for further analysis when higher concentration BSA solutions are flowed across the metal surface.

Figure 7-12: Transmittance profile as BSA solutions of different concentrations are flowed across the metal sensing surface

On the contrary, the transmittance profile for the bimetallic SPR configuration rises steadily as $5 \mu\text{g mL}^{-1}$ of BSA solution is flowed across the metal surface. There are only marginal fluctuations in the transmittance profile when PBS solution is flushed through the channel, indicating that BSA has absorbed firmly onto the metal surface. When $50 \mu\text{g mL}^{-1}$ and $500 \mu\text{g mL}^{-1}$ of BSA solutions are injected into the microfluidic channels, the transmittance profile demonstrates smaller extent increases. Further thickening of the BSA layer can probably be monitored too with the bimetallic film configuration.

The smaller profile change as BSA solutions of increased concentrations flows through the channel is probably attributed to the following reason. When $5 \mu\text{g mL}^{-1}$ of BSA solution first flows through the channels, BSA absorbs readily onto most of the metal surface, leaving little vacant sites. Subsequently, when increased concentrations are introduced into the channels, BSA will aggregate gradually on top of a BSA layer. This process, which occurs at

increasing distances from the metal surface, can be easily monitored with an extended SP field.

In the previous sub-section, we have excited RhB dyes on metal-coated regions along a waveguide in order to demonstrate the extended SP evanescent field achievable with the two-layered metallic structure. In this sub-section, we have shown that the enhanced SP field is capable of monitoring biological events that occur at increasing distances from the metal surface. The enhanced electric field is especially useful for biosensing as biological bindings that occur further into the analyte can be probed, bigger cells can be imaged and longer ligands can be immobilized on the sensing surface. When the proteins of interest get larger (and usually the ligands as well), the enhanced sensitivity due to field extension will be more pronounced, as a weak evanescent field might have decayed too severely to sense the biological binding. This complements the narrower SPR resonance width advantage of the proposed configuration discussed earlier.

7.5 Conclusion

In this chapter, the merit of the bimetallic film configuration in providing a higher sensitivity in terms of sensing region is presented. The use of a two-layer silver-gold film allows us to obtain enhanced fluorescence emission of up to 4× improvement due to SP wave extension. The stronger fluorescence intensity is ascribed to the enhanced evanescent field induced using the two-layered metallic film configuration. Rhodamine B (RhB) solution, placed on top of four metallic films with various silver and gold thickness combinations, are excited via SP evanescent fields and the respective fluorescence intensities are measured. Extending the bimetallic silver – gold SPR configuration for sensing, it was demonstrated that BSA aggregations occurring at increased distances from the metallic surface can be probed as well. The better electric field enhancement of the two-layered metallic film configuration

facilitates several applications. In SPR – fluorescence spectroscopy, less concentrated fluorophore-tagged analyte solution can be monitored. In sensing, biological bindings that occur further into the analyte can be probed and longer ligands can be immobilized on the sensing surface. An enhanced SP evanescent field can be further exploited in applications such as imaging, cells manipulation and sorting, nano-photolithography and numerous other domains of research where an extended probing electric field is desirable.

CHAPTER 8

CONCLUSION & RECOMMENDATIONS

8.1 Conclusion

In this thesis, investigation of the advantages possible with the two-layer metallic film configuration for the SPR enhancement was reported. Briefly, the enhancements were evaluated comprehensively from three approaches: the signal-to-noise ratio; the sensitivities in terms of $d\theta/dn$ and $d\lambda/dn$; and the sensitivity in terms of wider sensing region.

A multi-layer model based on magnetic field continuity was first developed to theoretically analyze the respective enhancements. Theoretical comparison between the popular single gold film SPR configuration and the bimetallic silver-gold structure with prism in-coupling concludes that the latter yields better SNR, poorer sensitivity in terms of $d\theta/dn$, no distinctive differences for sensitivity in terms of $d\lambda/dn$ and better sensitivity in terms of sensing region. Experimental data with prism geometry reinforced the theoretical deduction that the bimetallic silver-gold configuration improves SNR of the SPR reflectivity curves.

The bimetallic configuration was extended to the waveguide geometry to examine the

sensitivity differences ($d\lambda/dn$) achievable as compared to single film structures. The waveguide scheme was employed as it permits easy realization of wavelength interrogation, and its compact nature facilitates numerous lab-on-a-chip applications and potential commercial exploitation. Advanced lithography may reduce the overall cost as well. Wavelength interrogated sensing of glucose solution concludes that the bimetallic configuration achieves much better sensitivity ($d\lambda/dn$) than the single gold film waveguide SPR scheme.

Furthermore, two new lithographic procedures were proposed and explored for the fabrication of embedded waveguides, easing the integration process of fluidic chambers and channels with waveguides for SPR sensing. The first, known as photothermal lithography, involves photo-induced polymerization of the UV-exposed regions under a patterned mask, and subsequently cross-linking of the unexposed regions via high thermal treatment, achieving an index contrast of $\sim 7.2 \times 10^{-3}$. The second technique, known as dual-UV exposure lithography, involves a UV expose – bake – UV expose procedure to marginally modulate the refractive index between the initially exposed and later exposed regions, resulting in a maximum index contrast of $\sim 8 \times 10^{-4}$. Both processes can be implemented on commercial SU-8 photosensitive material without needing any development. Characterization and measurement data reveal that the waveguides, especially the ones fabricated with dual-UV lithography, possess good surface quality and demonstrate high optical transparency.

Subsequently, the fabricated embedded waveguides were integrated with a fluorescence chamber for the experimental observation of enhanced fluorescence emission due to bimetallic silver-gold layer induced SP wave extension. RhB solution, placed on top of metallic films with various silver and gold thickness combinations, are excited via SP evanescent fields and the intensity of the collected fluorescence presents a direct analysis of

the SP fields' penetration depth. Furthermore, the proposed SPR configuration has also been employed for monitoring BSA aggregations occurring at increased distances from the metallic surface, based on transmission measurement. Both fluorescence intensities and waveguide transmittance measurements conclude that the bimetallic configuration achieves much better sensitivity (sensing region) than the single gold film waveguide SPR scheme. The waveguide structure used highlighted the viability of SPR for applications where compactness and portability is appreciated.

To-date, most research works on SPR centers on SP excitation on a single gold film to achieve stable test results as well as ride on the more matured ligand immobilization techniques on gold surfaces. Only in some cases, silver film is used for enhanced results in preliminary studies. With the proposed bimetallic configuration, it is expected that colleagues in the scientific community working on SPR sensors can benefit from its better SNR and improved sensitivity in terms of sensing region. The beauty of the proposed configuration is that an overlying gold material, popularly used for many applications due to its inertness and good bio-adhesion, protects the underlying silver, which provides huge enhancement of the evanescent field. Moreover, this configuration can be easily realized as both metallic layers can be deposited consecutively in an electron beam evaporator without breaking vacuum.

Concisely, the proposed bimetallic configuration is potentially viable to be exploited for many applications. In this thesis, the merits of the bimetallic configuration for 4 times improvement in fluorescence emission, as well as for continuous monitoring of BSA aggregations at extended penetration depths had been presented. Additionally, in a collaborative work, the proposed configuration has also been employed for particle propulsion with improved efficiency [151]. A $2\times$ increase in particle velocities is achieved in comparison to a pure gold configuration, enforcing the prospect of an on-chip particle

sorting device. In essence, the enhanced SP evanescent field can be extended to applications such as imaging, cells manipulation and sorting, nano-photolithography and numerous other domains of research where an extended probing electric field is desirable.

From a board perspective, bulk of the works presented in this thesis lies in the study, design and experimental verification of the merits and consequences of the two-layer silver-gold SPR configuration, while only a moderate level of sophistication is ascribed to the theoretical element of the bimetallic configuration. Therefore, certain areas in the theoretical modeling sections of this thesis require further considerations in subsequent works. Predominantly, the frequency dependent characteristic of the respective materials, i.e. silver, gold, BK7 prism, SU-8 waveguide etc., have not been discussed in detail. Employing an appropriate software (finite-difference-time-domain), further deliberation to verify the merits of proposed configuration from a theoretical perspective would be invaluable and fruitful.

8.2 Recommendations for future research

We have demonstrated the advantages possible with the two-layer metallic film configuration for SPR enhancement, as compared to single film structures used currently. In addition to the improved SNR, the extended SP evanescent field enables the monitoring of biomolecular interactions that occur further into the analyte. Potentially, there are still much further works that can be done to further verify our results, as well as levitating the use of SPR to greater heights. The following are some specific challenges and improvement to be made for the future research works.

1. To perform a study, both theoretical and experimental, on the influence of wavelength dispersion of all components and elements used, via single- and multi-mode waveguides – surface plasmon resonance geometry. In this thesis, in-depth theoretical analyses and

experimental works on the frequency dependency of materials on the plasmons resonance condition have not been performed. It will be extremely useful to further investigate the phenomenon of single- and multi-mode waveguides induced surface plasmons, both theoretically and experimentally.

2. To take into consideration a more sophisticated treatment on the influence of autofluorescence from extraneous sources on the interpretation of the measured fluorescence values. In this thesis, the degree of autofluorescence from raw materials such as Mylar and SU-8 has not been precisely quantified. Moving forward, if the proposed setup is employed in single molecule detection with fluorescence spectroscopy, elimination of the background noise is of paramount importance, since the concentration of the labeling fluorophores is low and photobleaching is significant.
3. To perform further considerations and experimental study on waveguide – SPR transmission measurements. In this thesis, we had shown that the aggregation of BSA on the metal film led to a change in the transmission values of the waveguide. However, the SPR phenomenon was investigated only at a single wavelength, with three concentrations of BSA solutions. Further works may involve employing lasers of different wavelengths, as well as working with more concentrations of BSA solutions. Additionally, more comprehensive works can be investigated, primarily by extending the transmission type waveguide SPR sensor for antigen – antibody sensing.
4. To continue all aspects of the work, especially in micro-fabrication, which bear upon integrating microfluidics with the waveguide microchip. Though a compact microfluidics-integrated-waveguide-device had been constructed in this thesis, the presented device can be improved further by (1) shrinking the dimensions of the waveguide and microfluidics chamber or (2) packing many microfluidics-integrated-waveguide-devices on a single chip for high throughput diagnostics applications. The

forementioned improvements surely require thorough planning and consideration in the micro-fabrication aspect.

5. To employ the bimetallic silver-gold film configuration for imaging. It is meaningful to employ SPR for imaging and monitoring of translational activities occurring on the cellular membrane, as this membrane is the place where cellular communications with its surroundings take place. Furthermore, investigation of binding affinity between antigens and antibodies is primarily achieved with SPRi as well. With the bimetallic configuration, the enhanced SP evanescent field is capable of deeper penetration into the analyte medium, probing more information. In cell microscopy, cellular activities occurring further away can be imaged; in antigen – antibody array microscopy, longer assembly can be pictured as well.
6. To extend the waveguide SPR for lab-on-a-chip sensing or fluorescence spectroscopy of low concentration fluorophore-labeled analytes. The extended SP field encompasses more analyte molecules within its effective probing range, amounting to higher sensitivity of low concentration analytes at the tail-end of the SP field during SPR sensing, as well as exciting more fluorescence particles in fluorescence spectroscopy. A compact waveguide structure has been proposed in this thesis for SPR sensing. It will be significant if further development works can be done to incorporate light sources and detectors on a chip, realizing a true lab-on-a-chip micro-device. Besides, biological analytes, bigger/longer ones especially, can be monitored with the bimetallic film waveguide SPR configuration.
7. To employ the bimetallic silver-gold film configuration in enhanced optical transmission through subwavelength periodic hole arrays. In this thesis, it was shown that the bimetallic film is capable achieving SP electromagnetic field enhancement close to silver's, while a overlying gold film ensures that the stability of the metallic structure is

not comprise. Currently, extraordinary transmissions are realized with subwavelength holes patterned on silver films due to better enhancement of silver, hence leading to stronger SP interference and higher transmission. However, in terms of practicality and commercial exploitation, this structure will not be stable after time or be valuable for biological applications as silver oxidizes very easily. Therefore, the employment of the bimetallic film structure for EOT will surely extend its use for more potential applications.

AUTHOR'S PUBLICATIONS

Journal Papers:

1. B. H. Ong, X.-C. Yuan, Y. Y. Tan, R. Irawan, X. Q. Fang, L. S. Zhang and S. C. Tjin, *Two-layered metallic film induced surface plasmon polariton for fluorescence emission enhancement in on-chip waveguide*, Lab on a Chip, 7, 506-512, (2007).
2. Y. Y. Tan, X.-C. Yuan, B. H. Ong, J. Bu and Q. Y. Lin, *Two-layered metallic film induced surface plasmons for enhanced optical propulsion of microparticles*, Applied Physics Letters, 91, 141108 (2007).
3. B. H. Ong, X.-C. Yuan and S. C. Tjin, *Bimetallic silver-gold film waveguide surface plasmon resonance sensor*, Fiber and Integrated Optics, 26, 229-240, (2007).
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1. B. H. Ong, X.-C. Yuan and S. C. Tjin, *Bimetallic silver-gold film waveguide surface plasmon resonance sensor*, Proceedings of SPIE Vol. 6123, Integrated Optics: Devices, Materials, and Technologies X, San Jose, CA, USA, 61230B, (21-26 January 2006).
2. B. H. Ong and X.-C. Yuan, *Surface plasmon resonance imaging at nm resolution*, 2nd NTU Nanotech Symposium, Nano Magnetism/Photonics and Nano Materials Synthesis, Nanyang Technological University, Singapore, (24 October 2005).

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