MULTI-MODE SELF-REFERENCING SURFACE PLASMON RESONANCE SENSORS

Jing Guo
University of Kentucky, woshijeanne@hotmail.com

Click here to let us know how access to this document benefits you.

Recommended Citation
https://uknowledge.uky.edu/ece_etds/13

This Doctoral Dissertation is brought to you for free and open access by the Electrical and Computer Engineering at UKnowledge. It has been accepted for inclusion in Theses and Dissertations--Electrical and Computer Engineering by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.
STUDENT AGREEMENT:

I represent that my thesis or dissertation and abstract are my original work. Proper attribution has been given to all outside sources. I understand that I am solely responsible for obtaining any needed copyright permissions. I have obtained and attached hereto needed written permission statements(s) from the owner(s) of each third-party copyrighted matter to be included in my work, allowing electronic distribution (if such use is not permitted by the fair use doctrine).

I hereby grant to The University of Kentucky and its agents the non-exclusive license to archive and make accessible my work in whole or in part in all forms of media, now or hereafter known. I agree that the document mentioned above may be made available immediately for worldwide access unless a preapproved embargo applies.

I retain all other ownership rights to the copyright of my work. I also retain the right to use in future works (such as articles or books) all or part of my work. I understand that I am free to register the copyright to my work.

REVIEW, APPROVAL AND ACCEPTANCE

The document mentioned above has been reviewed and accepted by the student’s advisor, on behalf of the advisory committee, and by the Director of Graduate Studies (DGS), on behalf of the program; we verify that this is the final, approved version of the student’s dissertation including all changes required by the advisory committee. The undersigned agree to abide by the statements above.

Jing Guo, Student

Dr. Jeffrey T. Hastings, Major Professor

Dr. Zhi Chen, Director of Graduate Studies
MULTI-MODE SELF-REFERENCING
SURFACE PLASMON RESONANCE SENSORS

DISsertation

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Engineering at the University of Kentucky

By
Jing Guo
Lexington, Kentucky

Director: Dr. Jeffrey T. Hastings, Professor of Electrical and Computer Engineering Lexington, Kentucky

2012

Copyright © Jing Guo 2012
ABSTRACT OF DISSERTATION

MULTI-MODE SELF-REFERENCING SURFACE PLASMON RESONANCE SENSORS

Surface-plasmon-resonance (SPR) sensors are widely used in biological, chemical, medical, and environmental sensing. This dissertation describes the design and development of dual-mode, self-referencing SPR sensors supporting two surface-plasmon modes (long- and short-range) which can differentiate surface binding interactions from bulk index changes at a single sensing location. Dual-mode SPR sensors have been optimized for surface limit of detection (LOD). In a wavelength interrogated optical setup, both surface plasmons are simultaneously excited at the same location and incident angle but at different wavelengths. To improve the sensor performance, a new approach to dual-mode SPR sensing is presented that offers improved differentiation between surface and bulk effects. By using an angular interrogation, both surface plasmons are simultaneously excited at the same location and wavelength but at different angles. Angular interrogation offers at least a factor of 3.6 improvement in surface and bulk cross-sensitivity compared to wavelength-interrogated dual-mode SPR sensors.

Multi-mode SPR sensors supporting at least three surface-plasmon modes can differentiate a target surface effect from interfering surface effects and bulk index changes. This dissertation describes a tri-mode SPR sensor which supports three surface plasmon resonance modes at one single sensing position, where each mode is excited at a different wavelength. The tri-mode SPR sensor can successfully differentiate specific binding from the non-specific binding and bulk index changes.

KEYWORDS: Surface Plasmon Resonance, SPR Sensor, Biosensor, Cross-sensitivity, Limit of Detection (LOD)
MULTI-MODE SELF-REFERENCING
SURFACE PLASMON RESONANCE SENSORS

By

Jing Guo

Dr. Jeffrey T. Hastings
Director of Dissertation

Dr. Zhi Chen
Director of Graduate Studies
This dissertation is dedicated to my family

Hongbing Guo, Hong Liang, Lianpu Sun, Shuqin Liu, Tao and Roy.
Acknowledgments

First of all, I would like to express my greatest respect and deepest appreciation to my major advisor, Dr. Todd Hastings. I have greatly benefited from his enthusiasm, knowledge, guidance and sharp thoughts on broad scientific topics. Moreover, his inspiration, encouragement, patience and friendship provided me invaluable support during the past six years. I am also grateful to each member of my graduate advisory committee: Dr. Yinan Wei, Dr. Vijay Singh, Dr. Janet Lumpp and Dr. Michael Seigler, whose knowledge are invaluable resources to me. Dr. Wei helped me understanding the bimolecular interactions in my project. Dr. Chuck May and Brian Wadjyk with the CeNSE were a major help during the fabrication process. George Spiggle (1972-2007) gave me the original training on the fabrication processes required for my project.

I would like to thank my former lab mates, Prasanth Bathae Kumaresh and Donnie Keathley. My first study of SPR sensor began with the discussion with Prasanth. Collaboration with Donnie helped me to learn more about design and fabrication of sensors and other projects. I also would like to thank everyone else in our group. Every question or discussion from them gave me deeper understanding and thoughts for my research.

Lastly I would like to thank Tao, my husband for being with me all the time. It would not be possible to start this project without the supporting and encouragement from my loved family.
# Table of Contents

Acknowledgments ........................................................................................................... iii

Table of Contents .......................................................................................................... iv

List of Figures .................................................................................................................. vi

Chapter 1: Introduction .................................................................................................... 1

1.1 Fundamental of Surface Plasmon ........................................................................... 1

   1.1.1 Physical Fundamentals of Surface Plasmons ............................................. 1

   1.1.2 Excitation of Surface Plasmons ............................................................... 4

1.2 Surface Plasmon Resonance (SPR) Sensors ............................................................ 14

Chapter 2 Dual-mode SPR sensor design ......................................................................... 21

2.1 Current research in reference-compensated SPR sensors ........................................ 21

2.2 Current research in self-referencing SPR sensors ................................................... 22

2.3 Design of self-referencing dual-mode SPR sensors ................................................. 22

   2.3.1 Selection of gold and Teflon AF materials ............................................. 23

   2.3.2 Simulation reflected spectrum ............................................................... 25

   2.3.3 The sensing response of dual-mode self-referencing SPR sensors .......... 36

2.4 Optimization of self-referencing dual-mode SPR sensor ......................................... 38

   2.4.1 Optimization of wavelength interrogated dual-mode SPR sensor .......... 38

   2.4.2 Optimization of angular interrogated dual-mode SPR sensor ............... 46

Chapter 3 Dual-mode SPR Sensor Fabrication ................................................................. 53

3.1 Sample Preparation ................................................................................................... 53

3.2 Deposition of Teflon Layer ..................................................................................... 53

3.3 Deposition of Gold film .......................................................................................... 57

   3.3.1 Deposition of Gold by e-beam evaporation ......................................... 57

   3.3.2 Deposition of Gold by sputtering ......................................................... 58

3.4 Thickness measurements ......................................................................................... 60

   3.4.1 Measurement of the Teflon and gold thickness by contact profilometer . 60

   3.4.2 Measurement of the Teflon thickness by ellipsometer .......................... 60

Chapter 4 Dual-mode self-referencing SPR sensor testing and results ............................. 62

4.1 Wavelength interrogated sensors testing and results ................................................. 62

4.2 Angular interrogated sensors testing and results ..................................................... 70

Chapter 5 Tri-mode SPR sensor design .......................................................................... 79

5.1 Selection of materials .............................................................................................. 79

5.2 Simulation of reflected spectrum ............................................................................ 82

Chapter 6 Tri-mode SPR sensor fabrication .................................................................. 86

6.1 Fabrication of a dual-mode SPR sensor .................................................................. 86

6.2 Fabrication of SiO₂ stripes on gold surface ............................................................... 87

   6.2.1 Sputtering of SiO₂ layer ........................................................................ 87

   6.2.2 Photo lithography of SiO₂ layer .............................................................. 89
6.2.3 Wet etch for SiO$_2$ stripes ................................................................. 90
6.2.4 Remove the photo resist ......................................................................... 90
6.3 Thickness measurement ........................................................................... 90
Chapter 7 Tri-mode SPR sensor Testing and Results ......................................... 93
  7.1 Sensing model for tri-mode SPR sensor ....................................................... 93
  7.2 Sensing experiments of a tri-mode SPR sensor .......................................... 94
    7.2.1 Optical system and sensor fabrication ............................................... 94
    7.2.2 Sensing experiment procedure and results ......................................... 96
    7.2.3 Discussion and Conclusion .................................................................. 101
Chapter 8 Conclusions and future work .......................................................... 102
  8.1 Conclusions .............................................................................................. 102
  8.2 Future work .............................................................................................. 103
Reference ......................................................................................................... 105
VITA ............................................................................................................. 109
List of Figures

Figure 1.1 Schematic of surface-plasmon wave propagating along the interface between metal and dielectric layers................................................................. 3
Figure 1.2 Surface plasmon excitation conditions. (a) Surface plasmon waves are exited by incident light using a prism in Kretschmann’s configuration, (b) Illustration of phase matching between the incident light and surface plasmon waves........................................... 5
Figure 1.3 Reflected spectra for both angular (a) and wavelength (b) interrogated systems.................................................................................................................... 7
Figure 1.4 Otto’s scheme for excitation of surface plasmons........................................ 9
Figure 1.5 Excitation of surface plasmon waves by the diffraction grating method. ....... 11
Figure 1.6 Excitation of surface plasmons by waveguide coupling.......................... 13
Figure 1.7 The calculated reflected intensity shifts resulting from surface refractive index changes............................................................ 15
Figure 1.8 Concepts of a SPR biosensor ................................................................ 16
Figure 1.9 Reflected intensity shift as the result of surface binding. ....................... 18
Figure 2.1 Schematic diagram of a self-referencing dual-mode surface-plasmon resonance sensor ........................................................................................................ 24
Figure 2.2 Light reflects at the interface between two materials with different refractive index........................................................................................................... 26
Figure 2.3 Oblique incidence on multilayer dielectric structure. ............................... 27
Figure 2.4 Illustration of a single mode SPR sensor................................................ 30
Figure 2.5 Reflectance as a function of the incident wavelength and polarization for three different thicknesses of the gold film with water on surface....................... 31
Figure 2.6 Reflectance as a function of the incident angle for three different thicknesses of the gold film with water on surface.................................................... 32
Figure 2.7 Reflectance as a function of the incident wavelength of a dual-mode SPR sensor with water on surface................................................................. 34
Figure 2.8 Reflectance as a function of the incident angle of a dual-mode SPR sensor for different thicknesses of the Teflon and the gold film with water on surface........... 33
Figure 2.9. Calculated surface and bulk sensitivities for the long- and short- range surface-plasmon modes of a BK7-Teflon AF-Au sensor illuminated with white light at 65.5° incident angle (inside BK7)......................................................... 42
Figure 2.10 Bulk (a) and surface (b) limits of detection for the dual mode SPR sensor as a function of buffer (Teflon AF) thickness and gold thickness for a source/detector combination with constant power spectral density. (c,d) LODs for the dual mode sensor when interrogated with a tungsten-halogen lamp and Si CCD detector. The angle of incidence is 65.5° inside the BK7 prism......................................................... 44
Figure 2.11 Calculated surface and bulk resonance wavelengths, $\lambda_0$, and minimum reflectivities, $R_{min}$, for the long-range (LRSP) and short-range (SRSP) surface-plasmon modes of a BK7-Teflon AF-Au sensor illuminated at a 65.5° incident angle (inside BK7). 45
Figure 2.12 Calculated resonance angles for long-range and short-range surface-plasmon modes of a BK-7-Teflon AF-Au sensor illuminated with 800 nm light. ......................................................... 47
Figure 2.13 Calculated bulk sensitivities for the long-range and short-range surface-plasmon modes of a BK-7-Teflon AF-Au sensor illuminated with 800 nm light. Bulk sensitivity is in degree/RIU................................................................. 48
Figure 2.14 Calculated bulk sensitivities for the long-range and short-range surface-plasmon modes of a BK-7-Teflon AF-Au sensor illuminated with 800 nm light. Bulk sensitivity is in degree/nm ........................................................................... 49
Figure 2.15 (a) Surface limits of detection for the dual-mode SPR sensor as a function of buffer (Teflon AF) thickness and gold thickness. The wavelength of incident light is 800 nm. (b) Cross-sensitivity for the dual-mode SPR sensor as a function of buffer thickness and gold thickness. ........................................................................................................ 51
Figure 3.1 Refractive index of Teflon AF 1600 polymer........................................................................................................... 56
Figure 3.2 Sputtering process of deposition of gold layer. ........................................................................................................ 59
Figure 3.3 Profilometer measurement of gold layer thickness................................................................................................... 61
Figure 4.1 Schematic diagram of a self-referencing surface-plasmon resonance sensor. ........................................... 64
Figure 4.2 Experimental reflection spectra from a BK7, Teflon AF (430 nm), Au (56 nm) SPR sensor in deionized water with an angle of incidence of approximately 65.5° (inside BK7 prism). Also shown are theoretical fits to the spectrum using two different data sets for the dielectric constant of gold. ......................................................................................................... 66
Figure 4.3 Self-referenced measurement of streptavidin binding to a biotin functionalized gold surface. This experiment illustrates sensor performance for large changes in surface concentration. ........................................................................................................ 68
Figure 4.4 Self-referenced measurement of streptavidin binding to a biotin functionalized gold surface with a large surface concentration of streptavidin already present. This experiment illustrates sensor performance for small changes in surface concentration analyzed using the linear model. ........................................................................................................ 69
Figure 4.5 Comparison of field profiles dual-mode SPR sensors. ........................................................................................................ 72
Figure 4.6 Optical system for angular interrogation of dual mode SPR sensors. The optical system must be designed to measure a broader range of angles (15°) than typical single-mode SPR systems. ........................................................................................................ 74
Figure 4.7 Experimental and theoretical (fit) angular reflection spectra of a dual-mode SPR sensor using 800 nm wavelength light........................................................................................................ 75
Figure 4.8 Sensor response to streptavidin binding on gold surface. (a,b) Resonance angle vs. time for long- and short-range surface plasmon modes. (c,d) Bulk refractive index and relative surface binding thickness change calculated from (a,b). ........................................................................................................ 77
Figure 5.1 Illustration of tri-mode SPR sensor with SiO2 stripes on a gold surface with a low refractive index buffer layer below. ........................................................................................................ 81
Figure 5.2 Schematic diagram of a Tri-mode SPR sensor. The sensor consists of a gold thin film on a low refractive index Teflon layer and SiO2 stripes on top fabricated on a BK7 substrate. ........................................................................................................ 83
Figure 5.3 An example reflection spectrum of a tri-mode sensor. ........................................ 85
Figure 6.1 Measurement of thickness and optical properties for SiO$_2$ strips from ellipsometer.......................................................... 92
Figure 7.1 Experimental and theoretical reflection spectra of a tri-mode SPR sensor..... 95
Figure 7.2 Sensing model for a tri-mode SPR sensor....................................................... 98
Figure 7.3 Sensor response to streptavidin binding on gold surface. Resonance wavelength vs. time for three surface plasmon modes................................. 99
Figure 7.4 The calibrated bulk index, surface specific and non-specific binding change as functions of time based on the linear model. ......................................................... 100
Chapter 1: Introduction

Surface plasmon waves are electromagnetic waves propagating along the interface between a metal and a dielectric media. [1, 2] Under certain conditions, light can resonantly couple from a free space wave to a surface-plasmon wave giving rise to the phenomenon of surface-plasmon resonance. This phenomenon was initially observed in the early 1900s by Wood. Anomalous narrow dark bands were observed in the diffracted spectrum when a metallic diffraction grating was illuminated by polychromatic light [3]. Surface plasmon phenomenon also create a minimum in the reflected spectrum when illuminating thin metal films on a substrate, as observed by Turbadar [4]. In the late 1960s, Otto, Kretschmann and Raether gave the theoretical explanation to Wood’s and Turbadar’s anomalies and found various ways to excite surface plasmons. [5-7] The first usage of SPR for gas detection and biosensing were demonstrated in early 1980s. [8, 9] Interest in optical biosensing keeps increasing, and impressive progress has been made in development of SPR sensors in the last three decades.

In this chapter we will start our study with the physical fundamentals and excitation configurations of surface plasmons. Then we will explain the basic concept of the SPR biosensor and give a brief review of SPR biosensor development.

1.1 Fundamental of Surface Plasmon

1.1.1 Physical Fundamentals of Surface Plasmons

At the interface of two materials with real dielectric constants of opposite signs, surface plasmons can be excited by photons when a component of the incident wave vector can match to the wave vector of plasmon oscillations. The electron density will oscillate at the boundary. Maxwell’s equations for planar wave in the material with relative permittivity $\varepsilon$ can be written as
where we consider the media as non-magnetic and source-free. We can reduce Maxwell’s equations to the wave equations:

\begin{align*}
\nabla \times E + \mu_0 \frac{\partial H}{\partial t} &= 0 \\
\mu_0 \nabla \cdot H &= 0 \\
\nabla \times H - \varepsilon_0 \varepsilon \frac{\partial E}{\partial t} &= 0 \\
\varepsilon_0 \varepsilon \nabla \cdot E &= 0
\end{align*}

The oscillating wave associated with electromagnetic field propagating along the interface as shown in figure 1.1 are the solutions of Maxwell’s equations with proper boundary conditions. It can be expressed as below

\[ E = E_0 \exp \left[ i(\mathbf{k} \cdot \mathbf{r} - \omega t) \right] \]  

where \( \mathbf{k} \) is the plasmon wave vector and \( \omega \) is the angular frequency. Since the electromagnetic wave only exist at the surface, the wave equation will simplify as following

\[ E = E_0 \exp \left[ i(\mathbf{k}_d x + \mathbf{k}_z z - \omega t) \right] \]  

The tangential components of the electric-field and the normal components of the magnetic-field should be continuous at the boundary. Applying these boundary conditions to the wave equation, the dispersion relation can be derived as

\begin{align*}
\frac{k_{zd}}{\varepsilon_d} + \frac{k_{zm}}{\varepsilon_m} &= 0 \\
k_x^2 + k_{zd}^2 &= \varepsilon_d \left( \frac{\omega}{c} \right)^2 \\
k_x^2 + k_{zm}^2 &= \varepsilon_m \left( \frac{\omega}{c} \right)^2
\end{align*}

where \( c \) is the speed of light in vacuum. The \( d \) denotes the component in dielectric media and the \( m \) denotes the component in metal layer.
Figure 1.1 Schematic of surface-plasmon wave propagating along the interface between metal and dielectric layers. The x-direction is surface plasmons propagation direction along the interface and the z-direction is the normal direction of the dielectric media. The lines emerging from the interface represent the exponentially decaying electric field associated with the surface-plasmon wave.
Then we can solve the surface wave number (x-direction) from equations (1-9) to (1-11).

\[ k_x = \frac{\omega}{c} \sqrt{\frac{\varepsilon_m \varepsilon_d}{\varepsilon_m + \varepsilon_d}} \quad \text{(1-12)} \]

As the surface plasmon propagates along the interface, it loses energy because of the absorption of the metal. The electromagnetic field of a surface plasmon reaches the maximum at the interface. The evanescent field decays exponentially with penetration depth in the dielectric medium.

1.1.2 Excitation of Surface Plasmons

Optical coupling to surface plasmons requires energy transfer from photons to electrons. The wavevector of propagating surface-plasmon waves is larger than any wavevector associated with the incident light propagating in free space. So, there is no way to excite surface-plasmon waves by directly shining light on a metal. However, there are several effective methods to excite surface-plasmon waves. The most common excitation method uses a prism to provide phase matching between the incident wave and the plasmon wave. Otto[5] and Kretschmann [6] established prism coupling excitation of surface plasmons by two different configurations based on the attenuated total reflection (ATR). One can also use diffraction gratings or optical waveguides to excite surface plasmons[10, 11].

In Kretschmann’s configuration of ATR, a microscope slide was coated with a thin metal film and the slide was coupled to a high refractive index prism as shown in figure 1.2 (a). When light travels from a higher index medium to a lower index medium, it will be totally reflected at the interface if the incident angle is above a critical angle. We call this phenomenon total internal reflection (TIR). At the same time TIR creates an evanescent electrical field along the interface, whose amplitude decreases exponentially with distance from the interface. The distance corresponding to the amplitude reducing to 1/e is the penetration depth, which is about one wavelength.
Figure 1.2  Surface plasmon excitation conditions. (a) Surface plasmon waves are excited by incident light using a prism in Kretschmann’s configuration, (b) Illustration of phase matching between the incident light and surface plasmon waves.
If the interface is coated with a conducting layer with a suitable thickness as shown in figure 1.2, the TM-polarized component of incident light may penetrate the metal layer and excite the a surface-plasmon wave. The plasmon wave will propagate along the metal surface (the interface of metal and the dielectric layer) as shown in figure 1.2.

Surface-plasmon waves only can be excited at a certain combination of incident angle and wavelength when the equation \( k_{sp} = \frac{\omega}{c} = k_0 n_p \sin \theta \) has been satisfied as shown in figure 1.2, where \( k_{sp} \) is the wave number of surface-plasmon wave and \( n_p \) is the prism refractive index. Energy and momentum have been transferred from photons to electrons during the generation process of SP waves. Because surface-plasmon waves are transverse magnetic (TM) waves, the essentially 100% reflection of the transverse electric (TE) wave provides a convenient spectral reference. Consider the reflection spectra as functions of angle and wavelength as shown in figure 1.3; the minimum reflectivity occurs when incident wave and surface plasmon wave are phase matched.

We derived the dispersion relation of the surface plasmon as

\[
k_{sp} = k_x = \frac{\omega}{c} \sqrt{\frac{\varepsilon_m \varepsilon_d}{\varepsilon_m + \varepsilon_d}} = k_0 n_{eff} \text{ in the section above. Combining the dispersion relation with the excitation condition, the angle of maximum coupling will be } \theta = a \sin(n_{eff} / n_p)
\]

where \( n_{eff} \approx \sqrt{\frac{\varepsilon_m \varepsilon_d}{\varepsilon_m + \varepsilon_d}} \). If the incident wavelength is fixed, the relative permittivity of metal and dielectric medium will be known and the coupling angle can be determined. A reflection spectrum using wavelength interrogation is shown in figure 1.3 (b).

We define this coupling as angular interrogation. On the other hand, wavelength interrogation uses a fixed incident angle and then determines the wavelength of maximum coupling. A reflection spectrum using wavelength interrogation is shown in figure 1.3 (b).
Figure 1.3 Reflected spectra for both angular (a) and wavelength (b) interrogated systems.
In Otto’s configuration of ATR, there is a dielectric layer between the prism and the metal layer. When the light reflects totally on the prism at the critical angle, the TM-polarized component of incident light may penetrate the dielectric layer and couple to the surface plasmon waves along the interface of the metal and the dielectric as shown in figure 1.4.

The reflected spectrum can be studied by the Fresnel multilayer reflection theory. We will investigate it thoroughly in Chapter 2.

An optical grating is another method to couple light from free-space to a surface plasmon wave as shown in figure 1.5. As mentioned before, Wood observed related anomalies in the intensity of the light diffracted from a grating in 1902.[3] In the following years many scientists did continuous theoretical and experimental work about the anomalies such as Hessel and Oliner[12], Hagglund and Sellberg[13], Fano etc[14] and Ritchie[10].
Figure 1.4 Otto’s scheme for excitation of surface plasmons.
When a light with a wavevector $k_x$ is incident on a diffraction grating, the diffracted light will be the sum of the light diffracted by each element. And the wavevector of the diffracted light will be:

$$k_m = k_x + mG = k_x + m\frac{2\pi}{\Lambda},$$

where $m$ is the diffraction order (it could be negative or positive), $G$ is the wave number of the grating and the $\Lambda$ is the grating period. When $k_m$ is equal to the wave vector of surface plasmon, the diffracted light can couple with a surface plasmon propagating along the grating surface. The coupling condition is express as the following equation,

$$k_m = k_x + mG = k_x + m\frac{2\pi}{\Lambda} = Re\{k_{sp}\}$$  \hspace{1cm} (1-13)

where the wave vector of surface plasmon is equal to that described in the ATR excitation method. So we can derive the coupling conditions as

$$k_m = k_x + m\frac{2\pi}{\Lambda} = \frac{2\pi}{\lambda_0} n_d \sin\theta + m\frac{2\pi}{\Lambda} = Re\{k_{sp}\} = Re\left\{\frac{2\pi}{\lambda_0} \sqrt{\varepsilon_d\varepsilon_m} \right\}$$  \hspace{1cm} (1-14)

And if we simplify the above equation, we can finally get the condition as

$$n_d \sin\theta + m\frac{\lambda_0}{\Lambda} = Re\left\{\frac{\varepsilon_d\varepsilon_m}{\varepsilon_d + \varepsilon_m} \right\}$$  \hspace{1cm} (1-15)

Various combinations of the incident angle, grating order, and the grating period can satisfy the coupling condition equation. We can design a diffraction grating by fixing the incident wavelength and angle that we wish to excite the surface plasmon wave. We also can detect the coupling angle in the angular interrogation or the coupling wavelength in the wavelength interrogation from the reflected intensity from a grating. A minimum in the reflectivity as a function of incident angle is exactly the coupling angle to excite the plasmons when monochromatic light is incident on a grating. On the other hand the minimum in the reflectivity as a function of wavelength is the coupling wavelength where the energy of the light has been translated into the plasmon wave.

Another excitation method of surface plasmons is waveguide coupling. When the guided modes propagating along a dielectric waveguide pass the region covered by a thin metal film, the evanescent electromagnetic energy can penetrate the metal film and
Figure 1.5 Excitation of surface plasmon waves by the diffraction grating method.
may couple to the surface plasmon wave propagating along the metal film surface (figure 1.6). Here \( k_g \) is the wave vector of the guided mode, and the coupling condition is 

\[ k_g = Re\{k_{sp}\} \]  . Only a narrow wavelength range can satisfy this phase-matching condition to excite the surface plasmon at the outer metal-dielectric boundary.

Prism couplers, diffraction gratings and dielectric waveguides are the general approaches to achieve the phase-matching between the incident light and the surface plasmon wave. ATR using the Kretschmann configuration is the most common method to excite surface plasmons. During the past few years, new excitation schemes have been developed to enhance the resonance intensity or to excite additional resonance modes. The excitation light has also been expanded from visible light to invisible wavelength region. Hibbins et al investigated the grating-excitation of surface plasmons at microwave frequencies in 1999.[15] Telezhnikova and Homola also reported the simultaneous excitation of surface plasmons by a polychromatic light on a grating coupler. [16] As the applications of surface plasmon waves keep expanding, the new excitation schemes will keep improving as required.
Figure 1.6 Excitation of surface plasmons by waveguide coupling.
1.2 Surface Plasmon Resonance (SPR) Sensors

Since surface-plasmon resonance (SPR) was used for biosensing the first time about 30 years ago [8, 9], interest in optical biosensing keeps increasing continuously. SPR sensors are built up based on the very high sensitivity to the refractive index change near the sensor’s surface. Impressive progress has been made in development of SPR sensors in the last three decades. Today SPR sensors are a widely used tool to study biological interactions, to detect of biological and chemical analytes or to monitor the environment. They also have been commercially sold by several companies (GE Healthcare/Biacore, Horiba, Reichart, and many others).

The dispersion relation and the excitation methods of surface plasmon waves have been introduced in the first part. The propagation constant of a surface plasmon will be determined by the dielectric constants of the dielectric and the metal of the structure supporting the surface plasmon. A change in the refractive index of the dielectric media will cause a change of the propagation constant which results a shift of the coupling wavelength or angle in the reflectivity spectrum. The shift of coupling angle and wavelength caused by 0.001 RIU (refractive index unit) is demonstrated in figure 1.7. The blue line is the shifted spectrum caused by the refractive index change on the metal surface.

We define the sensitivity of the refractive index as \( S_n = \frac{\Delta \theta}{\Delta n} \) or \( S_n = \frac{\Delta \lambda}{\Delta n} \) whose unit is degree/RIU or nm/RIU. By tracking the coupling wavelength/angle corresponding to the dip in the reflected intensity, we can figure out the sensitivity of the SPR sensor. The sensitivity of a SPR sensor based on a prism coupler can reach 191 degree/RIU for angular interrogation and 13800 nm/RIU for wavelength interrogation.[17] Including wavelength and angular, the reflected intensity and phase shift can also be measured to characterize a SPR sensor.
Figure 1.7 The calculated reflected intensity shifts resulting from surface refractive index changes. (a) Coupling-wavelength shifts when the surface refractive index changes in wavelength interrogation, (b) Coupling-angle shifts when the surface refractive index changes in angular interrogation.
Figure 1.8 Concepts of a SPR biosensor. (a) A SPR biosensor placed on a prism with surface functionalized with biorecognition elements (blue y-shaped structures); (b) A solution containing analyte of interest (red spots) is introduced onto the sensor; (c) The analyte interacts with the recognition element and locally modify the refractive index near the sensor surface.
If the surface of a SPR sensor is functionalized by suitable biorecognition elements such as antibodies, it will be able to recognize and interact with a specific analyte. We define these SPR sensors as SPR biosensors. When a solution containing analyte is introduced into a SPR biosensor, the refractive index near the metal surface will increase because of the interaction/binding between the biorecognition and the analyte molecules. The refractive index change can be detected by the output measurement of the SPR biosensor. The interaction process is illustrated in figure 1.8. The calculated reflected intensity shift caused by the surface binding is shown in figure 1.9 in a wavelength interrogation.

The main performance characteristics of an unfunctionalized SPR sensor include coupling wavelength, angle, intensity and phase shifts. For those surface-functionalized SPR biosensors, the main performance characteristics are to be sensitivity, linearity, resolution, accuracy and limit of detection (LOD). Sensor sensitivity $S$ is defined as the ratio of the change in sensor output $dY$ to the change in what is measuring $dX$:

$$S = \frac{dY}{dX} \quad (1-16)$$

The sensor output can be the coupling angle or wavelength of the plasmon resonance, or intensity. The sensitivity to an analyte concentration can be written as $S_c = \frac{dY}{dc}$. The sensitivity to the refractive index can be written as $S_n = \frac{dY}{dn}$ which depends on bulk refractive index change and surface refractive index change caused by surface interaction/binding.
Figure 1.9 Reflected intensity shift as the result of surface binding. The example spectra illustrate the formation of a 5 nm thick film of refractive index 1.45.
The sensor linearity means the relationship between the sensor output and the actual measurand may be considered as linear where the sensitivity is slope of the linear curve. To simplify the analysis we sometimes only solve the sensor response based on the linear model when the nonlinear effect is negligible. But there are often nonlinear phenomena in the SPR biosensor response, and for more accurate calibration we should carefully consider the nonlinear effect.

The sensor resolution is the smallest change which can be predictably detected in the measurand. For SPR biosensor it is usually the smallest change of refractive index which can result a measurable shift in the output. The noise in the sensing output is the main factor determining the sensor resolution. The sensor accuracy is the maximum difference between the actual value and the theoretical value in the sensor output. High resolution does not imply high accuracy. A high resolution sensor may have poor measurement accuracy in some experiments.

Another important performance characteristic of a SPR biosensor is the limit of detection (LOD). LOD is defined as the smallest concentration of an analyte that can be measured under defined conditions by the International Union of Pure and Applied Chemistry. [18, 19] In SPR biosensors, LOD is the equivalent of the resolution. In generally accepted rule the LOD is defined as the background (blank signal) plus three times the standard deviation of the blank which is given by the equation as follow:

\[ Y_{\text{LOD}} = Y_{\text{blank}} + 3SD_{\text{blank}} \]  

where \( Y_{\text{blank}} \) is the measurement of the sample without analyte. If we set the background signal as zero, the LOD will be simplified to \( Y_{\text{LOD}} = 3SD_{\text{blank}} \).  

To optimize the sensing the LOD should be as low as possible. There are different methods to calculate LOD. Appropriate calculations should be decided based on different situations. We will discuss the LOD of our SPR biosensor in details in the next chapter.

Compared to the traditional biosensing process, SPR sensors have several
distinguishing advantages. Firstly SPR is a label free detection method that uses refractive index transduction to determine changes on the sensor surface. Secondly the large electric field associated with surface plasmon waves can provide high sensitivity to surface effects such as antibody-antigen binding. Finally SPR provides real-time detection with high speed (<ms) measurements. The most commonly employed configuration of the best commercial SPR sensor is Kretschmann’s configuration with angular interrogation. The refractive index resolution of such a prism-based SPR sensor can reach as low as 1×10^{-7} RIU.[20] In order to achieve higher sensitivity and lower resolution, novel structures of SPR sensors have been investigated. In the research of dual-mode SPR sensors based on the Kretschmann configuration, a additional dielectric layer is placed under the metal film to create a nearly symmetric refractive index profile that supports both symmetric [long-range (LR)] and antisymmetric [short-range (SR)] modes at a single observing spot.[21-25]The multilayer structure with symmetric refractive index profile can support long-range surface plasmons (LRSP).[26]The performance of a SPR sensor supporting LRSP has been intensively studied and improved while the resolution can reach as low as 5×10^{-8} RIU and the sensitivity of the refractive index can reach as high as 59000 nm/RIU[27, 28].

In summary SPR sensors are optical sensing devices using the high electromagnetic field associated with surface-plasmons on metal surfaces to detect surface refractive index changes. With appropriate biorecognition elements on sensor surface, it can be used to detect the specific bio-molecules by surface binding reaction. Sensitivity, resolution, accuracy and limit of detection are performance characteristics of a SPR biosensor to describe the sensor ability. To improve the performance of a SPR sensor, research continues worldwide focusing on novel structures and materials.
Chapter 2 Dual-mode SPR sensor design

We briefly introduced the electromagnetic theory and excitation of surface plasmon resonance (SPR) and SPR biosensor in chapter 1. However, one fundamental problem of SPR sensors remains unsolved. Standard SPR sensors cannot differentiate a bulk index change from a specific biological or chemical interaction on the sensor surface. Our solution to this problem is to develop a self-referencing dual-mode SPR sensing system that can differentiate surface interaction from interfering bulk refractive index changes.

2.1 Current research in reference-compensated SPR sensors

Multichannel SPR sensors have been developed to compensate for background index changes[29, 30] and non-specific binding[31]. In a dual-channel system, the SPR sensor configuration has been divided into two sensor surfaces with separate flow cell volumes. A high refractive index dielectric layer is coated on the second channel to shift the coupling wavelength to longer wavelength. The second channel is used for reference compensation of the primary channel during the biosensing experiments. A novel multichannel SPR sensor was established based on parallel-channel structure [31]. The innovation of the multichannel sensor was a combination of two parallel dual-channel sensing surfaces coated to minimize non-specific adsorption. One of two channels was functionalized with biomolecular recognition elements to interact with specific molecule. Such a SPR sensor allows distinguishing specific response from non-specific surface and bulk effects. In order to achieve the multichannel SPR sensor, spectral interrogation has to be conducted on two or more region and separate flow cell channels are required. Consequently, the spatially separated measurement and referencing regions make accurate calibration difficult. At the same time Homola et al. recognized that multichannel system cannot totally compensate the non-specific binding effects in complex realistic analyte.
2.2 Current research in self-referencing SPR sensors

Typical SPR sensors support only one surface plasmon mode; however, dual-mode SPR sensors have been developed to support two modes: long-range surface plasmons (LRSPs) and short-range surface plasmons (SRSPs) \([24, 25, 32, 33]\). Such sensors use an additional layer with a refractive index closely matched to the solution of interest. This layer is placed under the metal film to create a nearly symmetric refractive index profile that supports both symmetric [long-range (LR)] and antisymmetric [short-range (SR)] modes \([21, 22]\). Because their fields penetrate to different depths in the solution, these modes can be used to differentiate between surface interactions of interest and interfering bulk refractive index changes.

Homola et al. demonstrated SPR biosensor based on angular spectroscopy on an array of diffraction gratings \([34, 35]\). Large number of sensing channels (over 100) has been fabricated on one sensor chip. This approach enables the integration of thousands of sensing spots in conjunction with the preparation of dense arrays of different molecular probes on the sensor surface. Bulk refractive index changes can be compensated by designing the gratings to couple the incident light to more than one surface-plasmon wave. The sensor presents a tool for label-free, high-resolution and parallelized observation of biomolecular interactions.

2.3 Design of self-referencing dual-mode SPR sensors

We designed self-referencing dual-mode SPR sensors based on a transmission matrix. The sensing experiments proved that the dual-mode SPR sensors can successfully compensate the background index change of the surface interactions.
2. 3.1 Selection of gold and Teflon AF materials

Surface-plasmons are bound electromagnetic waves that propagate along the interface of two materials with real dielectric constants of opposite signs. Because the dielectric media has positive real dielectric constants, we should chose special metal with negative real relative permittivity such as gold or silver. Gold is general used for the metal layer in SPR sensors because of its stability.

For very thin metal layers, the SP modes associated with each metal-dielectric interface can be coupled. If the metal film is surrounded by media with a symmetric structure (similar dielectric constants), this coupling leads to the long- and short- range (or symmetric and antisymmetric) surface plasmon modes. The innovation of dual-mode SPR sensor is to add a Teflon AF layer under the gold layer to create a symmetric profile. Teflon AF has a dielectric constant (1.31 at 632 nm) that is very close to the dielectric constant of water (1.33 at 632 nm). However, dual-mode structures will not be useful as SPR sensors if we cannot excite the LRSP and SRSP simultaneously. Fortunately the LRSP and SRSP can successfully be generated by controlling and adjusting the thickness of gold and Teflon layers. The velocity and attenuation of the SP modes depends on the optical properties and thickness of the Teflon and gold layer.

Figure 2.1 illustrates the basic optical system for a self-referencing dual-mode SPR sensor with wavelength interrogation. Two surface-plasmon (SP) modes are generated at the same observation location at the same incident angle but at different wavelengths.

The proper thickness of gold and Teflon layer will be determined by the excitation conditions. Theoretical simulation is calculated by a transmission matrix.
Figure 2.1 Schematic diagram of a self-referencing dual-mode surface-plasmon resonance sensor. The structure supports two surface plasmon modes excited at different wavelengths: a symmetric (long-range, LRSP) mode whose fields extend deeper into the solution and an anti-symmetric mode (short-range, SRSP) whose fields are concentrated near the surface of the metal.
2.3.2 Simulation reflected spectrum

When a white light is incident on a SPR sensor, the reflected spectrum can be calculated using the Fresnel multilayer reflection theory [36-38].

First, we will introduce the simple layer reflection. When a light reaches the interface of two different medium at an oblique angle, reflection and transmission will happen at the same time as shown in figure 2.2. The reflection and transmission coefficient are

\[
\begin{align*}
    r_{TE} &= \frac{n_1 \cos \theta_1 - n_2 \cos \theta_2}{n_1 \cos \theta_1 + n_2 \cos \theta_2} \\
    t_{TE} &= \frac{2n_1 \cos \theta_1}{n_1 \cos \theta_1 + n_2 \cos \theta_2} \\
    r_{TM} &= \frac{n_2 \cos \theta_1 - n_1 \cos \theta_2}{n_1 \cos \theta_1 + n_2 \cos \theta_2} \\
    t_{TM} &= \frac{2n_1 \cos \theta_1}{n_1 \cos \theta_1 + n_2 \cos \theta_2}
\end{align*}
\]

Where TE denotes the transverse electric field and TM denotes the transverse magnetic field. The refractive indexes of two materials are \( n_1 \) and \( n_2 \). Incident angle is \( \theta_1 \) and the refracted angle is \( \theta_2 \). The reflectance \( R \) is calculated by

\[
R = |r|^2
\]

and the transmittance \( T \) is calculated by

\[
T = \frac{n_2 \cos \theta_2}{n_1 \cos \theta_1} |t|^2
\]

Considering the multilayer structure of SPR sensors, we will use the transmission matrix method to calculate the reflection coefficient. For a multilayer structure illustrated in figure 2.3, the light (the electric field of the light wave) will reflect and refract at each interface.
Figure 2.2 Light reflects at the interface between two materials with different refractive index.
Figure 2.3 Oblique incidence on multilayer dielectric structure.
Each reflection/refraction angle is determined by Snell’s law:

\[
n_0 \sin \theta_0 = n_i \sin \theta_i = n_t \sin \theta_t \quad i = 1,2,...M
\]  

(2-7)

The phase shift $\beta$ in each layer is calculated by $\beta_i = k_i l_i \quad (i = 1,2,...M)$ where $k_i = k_0 n_i \cos \theta_i$ and $k_0 = \omega / c_0 = 2\pi / \lambda$ is the free-space wave number. Combined with the Snell’s law the phase shift can be expressed as follow,

\[
\beta_i = \frac{2\pi}{\lambda} l_i n_i \sqrt{1 - \frac{n_0^2 \sin^2 \theta_0}{n_i^2}} \quad i = 1,2,...M
\]  

(2-8)

The transverse field reflection and transmission coefficients at the M+1 interface is

\[
r_{T_i} = \frac{n_{T,i-1} - n_{T,i}}{n_{T,i-1} + n_{T,i}} \quad i = 1,2,...M + 1
\]  

(2-9)

\[
t_{T_i} = \frac{2n_{T,i-1}}{n_{T,i-1} + n_{T,i}} \quad i = 1,2,...M + 1
\]  

(2-10)

Where the refractive index for each layer is

\[
n_{T_i} = \begin{cases} 
\frac{n_i}{\cos \theta_i} & \text{TM} \\
n_i \cos \theta_i & \text{TE}
\end{cases} \quad i = 0,1,2,...M,t
\]  

(2-11)

The reflection and transmission of light from the up- and under-side of a multilayer may be described by a matrix equation.

\[
\begin{bmatrix} E_{T,i+} \\ E_{T,i-} \end{bmatrix} = \frac{1}{t_{T,i}} \begin{bmatrix} r_{T,i} & 0 \\ 0 & \exp(-i\beta_i) \end{bmatrix} \begin{bmatrix} r_{T,i+1} & 0 \\ \exp(i\beta_i) & 1 \end{bmatrix} \begin{bmatrix} E_{T,i+1} \\ E_{T,i+1-} \end{bmatrix}
\]  

(2-12)

The complex reflection coefficient for transverse electric field is given by the ratio of the amplitudes.

\[
r_{TE} = \left| \frac{E_{T,i-}}{E_{T,i+}} \right|
\]  

(2-13)

The total reflectance is given by $R_{TE} = |r_{TE}|^2$. We can also calculate the total reflectance of the TM mode $R_{TM}$. The reflectivity, $R$, of an SPR sensor is given by the ratio of the reflectance $R = R_{TM} / R_{TE}$.

For single mode SPR sensors there is only one gold layer on the substrate as shown in figure 2.4. In a wavelength interrogation system, the reflectivity can be calculated versus wavelength by fixing the thickness of gold layer. Figure 2.5 shows a simulated reflected spectrum of a single mode SPR sensor for three different gold film thicknesses.
The wavelength corresponding to the dips of the spectrum is the coupling wavelength which will shift as the incident angle changes. The thickness of the gold film has strong influences on the magnitude of the dip in the reflectivity spectrum. The magnitude of the dip increases as the gold film thickness decreases in the range from 45 nm to 65 nm.

If the incident wavelength is fixed, the reflectance can be measured as a function of the incident angle in angular interrogation system. Figure 2.6 shows the reflectance of a single mode SPR sensor for different thicknesses of the gold film. The dip occurs in the reflectance of angular interrogation when the surface plasmon is excited at a certain incident angle. Similarly, the magnitude of the dip is influenced by the thickness of the gold layer.
Figure 2.4 illustration of a single mode SPR sensor
Figure 2.5 Reflectance as a function of the incident wavelength and polarization for three different thicknesses of the gold film with water on surface.
Figure 2.6 Reflectance as a function of the incident angle for three different thicknesses of the gold film with water on surface. The parameters in simulation are listed as follow: BK7 glass prism and substrate ($n_p=1.51$), gold film ($\varepsilon_m=-25+1.44i$) and water ($n_{\text{water}}=1.329$).
To simulate the reflectivity of a dual-mode SPR sensor shown in figure 2.1, we need to apply the transmission matrix one more time for the additional Teflon layer. Two surface plasmon resonances can be excited simultaneously with proper thicknesses of the Teflon layer and gold film. Figure 2.7 illustrates the calculated reflectance of a dual-mode sensor with different thicknesses of the gold film and Teflon layer in a wavelength interrogation system. The incident angle is fixed of 65° for the entire measurement. Long-range (LR) and short-range (SR) surface plasmon (SP) modes are generated from different wavelengths simultaneously. The thickness of the Teflon layer is fixed at 600 nm, figure 2.7(a), and the LRSP and SRSP (coupling wavelengths) get closer while the gold thickness increases. In figure 2.7(b), the magnitude of the dips in spectrum increases while the Teflon thickness decreases when the gold film is 50 nm thick.

The simulated reflectance as the function of the incident angle of a dual-mode SPR sensor in an angular interrogation optical system with different parameters is shown in figure 2.8. Different combinations of the Teflon layer and gold film thicknesses significantly change the coupling angles, the width and the magnitude of the dips.

From the simulations, we conclude that two surface plasmon resonance modes can be excited simultaneously at the same observing location by different wavelength in wavelength interrogation. There are wide ranges of thickness for both Teflon layer and gold layer which can satisfy the excitation conditions. Different combinations result in different width of the resonance, different magnitude of the reflectivity dip.
Figure 2.7 Reflectance as a function of the incident wavelength of a dual-mode SPR sensor with water on surface. The incident angle is 65°. (a) thickness of Teflon=600 nm (b) thickness of Gold=50 nm.
Figure 2.8 Reflectance as a function of the incident angle of a dual-mode SPR sensor for different thicknesses of the Teflon and the gold film with water on surface. The parameters in simulation are listed as follow: incident wavelength=800 nm, BK7 glass prism and substrate ($n_p=1.51$), gold film ($\varepsilon_m=-25+1.44i$) and water ($n_{water}=1.329$). (a) The thickness of Teflon layer is 600 nm. (b) The thickness of gold film is 50 nm.
2.3.3 The sensing response of dual-mode self-referencing SPR sensors

Dual-mode self-referencing wavelength interrogated SPR sensors

For a dual-mode SPR sensor, the symmetric surface plasmon mode exhibits lower absorption loss, and is termed the long-range surface plasmon (LRSP). Conversely, the anti-symmetric mode exhibits higher absorption loss and is referred to as the short-range surface plasmon (SRSP). The fields of the symmetric surface-plasmon mode penetrate more deeply into the dielectric while the fields of the anti-symmetric mode are concentrated at the metal surfaces. As a result, surface binding influences the propagation constant of the short-range mode more strongly than the propagation constant of the long-range mode. This key difference forms the foundation for the self-referencing SPR sensor.

For dual-mode wavelength interrogated SPR sensors, both background index changes and surface binding will result in different shifts for the excitation wavelength of the long-range (LR) and short-range (SR) surface-plasmon resonances. Thus, we can use the two resonances to differentiate between the two effects. If the sensor’s response to both bulk index change and surface binding is approximately linear, the LR and SR surface-plasmon resonance shifts, $\Delta \lambda_{LR}$ and $\Delta \lambda_{SR}$ respectively, are given by

\[
\Delta \lambda_{LR} = S_{S-LR} \Delta d + S_{B-LR} \Delta n_B, \quad (2-14)
\]

\[
\Delta \lambda_{SR} = S_{S-SR} \Delta d + S_{B-SR} \Delta n_B \quad (2-15)
\]

where $S_{S-LR}$ and $S_{S-SR}$ are the surface sensitivities in nm-wavelength/nm-thickness for the long- and short-range modes, and $S_{B-LR}$ and $S_{B-SR}$ are the bulk refractive index sensitivities in nm-wavelength/refractive index unit (RIU). The values $\Delta d$ and $\Delta n_B$ are the binding layer thickness change and the bulk refractive index change respectively. In many cases, the analyte partially cover the surface and it is more appropriate to write
the surface sensitivities in terms of a change in surface concentration of the analyte. If we know the sensitivities and resonance wavelength shifts, the surface layer thickness and bulk index changes can be calculated directly as following:

\[
\Delta d = \frac{\Delta \lambda_{LR}}{S_{B-LR}} - \frac{\Delta \lambda_{SR}}{S_{B-SR}} \\
\Delta n_B = \frac{\Delta \lambda_{LR}}{S_{B-LR}} - \frac{\Delta \lambda_{SR}}{S_{B-SR}}
\]

(2-16)

Thus, by measuring two different resonances we can separately determine the two unknowns of interest: surface layer thickness change, or equivalently fractional coverage, and bulk index change.

In some experiments involving large changes in bulk index or surface concentrations, nonlinear responses cannot be ignored. In this case, the dependence of the resonance wavelengths on the product of the bound layer and the background index changes can be significant and we must consider the more complicated relationships

\[
\Delta \lambda_{LR} = S_{B-LR} \Delta d + S_{B-1R} \Delta n_B + S_{SB-LR} \Delta d \Delta n_B
\]

(2-18)

\[
\Delta \lambda_{SR} = S_{B-SR} \Delta d + S_{B-1R} \Delta n_B + S_{SB-SR} \Delta d \Delta n_B
\]

(2-19)

We have introduced \( S_{SB-LR} \) and \( S_{SB-SR} \) to describe the product dependence for the long- and short-range resonances respectively. One must numerically solve a set of nonlinear equations to determine \( \Delta d \) and \( \Delta n_B \) from the measurements of \( \Delta \lambda_{LR} \) and \( \Delta \lambda_{SR} \); however, this is a small price to pay for the improved accuracy provided by the product term. It should also be noted that the surface and bulk sensitivities in the last equation will not be exactly the same as those found for the linear model because the basis functions (\( \Delta d, \Delta n_B \)) are not orthogonal.

Dual-mode self-referencing angular interrogated SPR sensors

In angular interrogation system, we write the shift in coupling angle as a function of
the sensitivities. $\Delta \theta_{LR}$ and $\Delta \theta_{SR}$ respectively, are given by

$$\Delta \theta_{LR} = S_{S-LR} \Delta d + S_{B-LR} \Delta n_B$$

$$\Delta \theta_{SR} = S_{S-SR} \Delta d + S_{B-SR} \Delta n_B$$

The binding layer thickness and bulk index changes are

$$\Delta d = \frac{\Delta \theta_{LR} / S_{B-LR} - \Delta \theta_{SR} / S_{B-SR}}{S_{S-LR} / S_{B-LR} - S_{S-SR} / S_{B-SR}}$$

$$\Delta n_B = \frac{\Delta \theta_{LR} / S_{S-LR} - \Delta \theta_{SR} / S_{S-SR}}{S_{B-LR} / S_{S-LR} - S_{B-SR} / S_{S-SR}}$$

The surface layer thickness change and bulk index change can be determined by measuring the shifts of two different coupling angles of two surface plasmon resonances.

2.4 Optimization of self-referencing dual-mode SPR sensor

2.4.1 Optimization of wavelength interrogated dual-mode SPR sensor

For dual-mode wavelength interrogated SPR sensors, both the background index changes and the surface binding will result in different shifts in the excitation wavelength of the long-range (LR) and short-range (SR) surface-plasmon resonances. Thus, Dual-mode SPR sensors use two surface plasmon waves to distinguish background refractive index change from surface binding. We have discussed that the sensing characteristics of a sensor include sensitivity, accuracy, resolution and limit of detection (LOD). An optimal design must minimize limit-of-detection for surface interactions in the presence of bulk index changes. Determining limit-of-detection requires knowledge of both the sensor’s sensitivity and the accuracy with which one can estimate a change in resonance wavelength. Estimating accuracy depends on the depth and width of the spectral feature of interest.[32, 39] In addition, we cannot simply optimize one
surface-plasmon mode based on these characteristics, but must consider the trade-off between the sensitivities and estimation accuracies for both surface-plasmon modes.

Homola et al. addressed this design problem by minimizing cross-sensitivity between the two channels. As they noted, this approach is especially effective when calibration errors dominate errors in sensor response.[23] However, this approach does not consider the impact of noise nor does it fully optimize the design using limit-of-detection as the figure of merit. We optimize our dual-mode sensors based on the Cramer-Rao bound (CRB) for spectral shift estimation in Poisson noise as derived by Karl and Pien.[40] The CRB provides a theoretical lower limit on the uncertainty at which a given parameter, e.g. surface concentration of an analyte, can be estimated in the presence of noise. The existence of an estimation algorithm that reaches the CRB, a so-called efficient estimator, is not guaranteed. However, Karl and Pien showed that efficient algorithms do exist for spectral shift estimation. Here we apply this technique to dual mode sensors, and we experimentally demonstrate this approach by monitoring biotin-streptavidin binding in solutions of varying refractive index.

In chapter 1 we introduced the definition of LOD which is generally defined as 3 times of the standard deviation of the blank. If we assume the measurement noise for $\Delta \lambda_{LR}$ and $\Delta \lambda_{SR}$ is not correlated, then we can add the variances of the terms in the numerators of $\Delta d$ and $\Delta n_B$ equations of the linear mode. The resulting LOD of $\Delta d$ and $\Delta n_B$ are given by

$$LOD(\Delta d) = 3 \sqrt{\frac{\text{var}(\Delta \lambda_{LR})/S_{B-LR}^2 + \text{var}(\Delta \lambda_{SR})/S_{B-SR}^2}{(S_{S-LR}/S_{B-LR} - S_{S-SR}/S_{B-SR})^2}}$$  \hspace{1cm} (2-24)$$

$$LOD(\Delta n_B) = 3 \sqrt{\frac{\text{var}(\Delta \lambda_{LR})/S_{LR}^2 + \text{var}(\Delta \lambda_{SR})/S_{SR}^2}{(S_{B-LR}/S_{S-LR} - S_{B-SR}/S_{S-SR})^2}}$$  \hspace{1cm} (2-25)$$

We optimized the sensor configuration used for previous dual-mode SPR sensors[32, 33] and shown in Figure 2. This sensor consists of a BK7 glass prism, a low-refractive index amorphous Teflon AF (DuPont, Inc.) buffer layer, and a thin gold
film. The variable parameters in the optimization are the angle of illumination, the Teflon AF thickness, and the gold thickness. Water, described by the optical constants determined by Hale and Querry[41], was used for the solution. Optical constants for gold were obtained from Johnson and Christy[42]. Optical constants for BK7 were established by Sellmeier dispersion coefficients supplied by Schott North America Inc.[43], and optical constants for Teflon AF were taken from a Cauchy dispersion formula fit to the measurements of Lowry et al.[44]

To optimize for surface limit-of-detection, we need to determine all four sensitivity parameters and the variance of each resonance wavelength shift estimate. To determine sensitivities we calculated reflection spectra for candidate sensor designs using the transmission matrix approach and Fresnel formulae described the simulation section. The reflection minima were identified using an iterative search as implemented in MATLAB’s (Mathworks Inc.) fminbnd function. To determine the surface sensitivities, a uniform layer with refractive index of 1.45 was added to the surface of the gold and the shift in wavelength for minimum reflectivity was calculated. The bulk sensitivity was determined by increasing the solution index by 0.001 refractive index units (RIU) and determining the resulting resonance wavelength shifts.

Figure 2.9 maps the sensitivities of the short range and long range surface-plasmons to bulk and surface changes. Figure 2.10 shows the resonance wavelengths and minimum reflectivity associated with each mode for each design. All maps were generated with an incident angle of 65.5° (inside the BK7 prism). With regard to bulk sensitivities, there is a clear tradeoff between the sensitivity of the short- and long-range modes. Thin buffer layers provide high short-range sensitivity and thicker buffer layers provide high long-range sensitivity. However, the short range sensitivity varies by only 20% over the entire design space while the long range sensitivity becomes very small for thin buffer layers. Likewise surface sensitivities are relatively constant for the short range mode (again varying by approximately 20%) whiles the long range sensitivity
falls to very small values where the short range sensitivity is highest. This suggests that operating at a point of high long-range sensitivity is likely to offer better performance, despite the slight sacrifice in short-range sensitivity.

To determine the variance with which we can estimate the resonance wavelength shift we turn to the Cramer-Rao bound for the shift of a known spectrum. This is equivalent to assuming that a well established baseline exists before any sensing event occurs. Karl and Pien recently derived the Cramer-Rao bound for the shift of discretely sampled spectrum[40]. Here we reformulate Karl and Pien’s result to take into account the wavelength dependence of the light source and detector. We find that the variance of the spectrum shift estimate is given by

\[
\text{var}(\Delta \lambda_0) \geq \left( \sum_{i=1}^{N} P(\lambda_i) \left( \frac{dR(\lambda)}{d\lambda} \bigg|_{\lambda = \lambda_i - \Delta \lambda_0} \right)^2 \right)^{-1}
\]

where \( R(\lambda_i) \) is the noise free reflectivity of the sensor and \( P(\lambda_i) \) is the number of detected photons at a given wavelength. To separately quantify the variance for the short and long range modes, we divide the summation at the inflection point between the resonance dips.

Let us first consider optimization for an illumination and detection system with a constant spectral power density. Figure 2.10 (a,b) maps the surface and bulk limits of detection based on calculated sensitivity and variance using Eqns. \( LOD(\Delta d) \) and \( \text{var}(\Delta \lambda_0) \) as a function of Teflon AF thickness and gold thickness at 65.5°. Moving to smaller angles and longer wavelengths holds the potential for even better performance, but places the short-range resonance beyond the detection limit of a Si detector array.
Figure 2.9 Calculated surface and bulk sensitivities for the long- and short- range surface-plasmon modes of a BK7-Teflon AF-Au sensor illuminated with white light at 65.5° incident angle (inside BK7). Bulk sensitivity is in nm/RIU and surface sensitivity is in nm (wavelength)/ nm (thickness).
Figure 2.10(c,d) shows the limits of detection with experimentally determined spectra from a tungsten-halogen fiber coupled light source (Ocean Optics DH-2000 with the deuterium source disabled) and a silicon CCD based spectrometer (Ocean Optics HR-4000). In this case, the characteristics of the interrogation system establish an optimal angle between 65.5° and 66.0°. As expected, this angle places the short-range resonance well within the efficient detection region for the silicon detector. For a 65.5° incident angle, the optimum sensor design consisted of a 400 nm thick Teflon layer with a 52 nm thick gold layer. As shown in Figure 2.11 the long- and short-range resonances fall at 625 nm and 897 nm respectively for the lowest limit-of-detection.

Importantly, the optimal design based on the limit of detection is reasonably close, though not exactly the same as, Homola’s optimal design (45 nm gold layer and 355 nm thick Teflon AF layer) based on minimizing cross-sensitivity.[32] The analysis here is appropriate when the sensor is accurately calibrated and the noise is the dominant source of error. If errors in the calibration coefficients are the dominant source of measurement error, then minimization of cross-sensitivity may be more appropriate.
Figure 2.10 Bulk (a) and surface (b) limits of detection for the dual mode SPR sensor as a function of buffer (Teflon AF) thickness and gold thickness for a source/detector combination with constant power spectral density. (c,d) LODs for the dual mode sensor when interrogated with a tungsten-halogen lamp and Si CCD detector. The angle of incidence is 65.5° inside the BK7 prism. LODs are normalized to the square root of the number of detected photons. Note that the optimal design clearly depends on the illumination and detection system and that the design for optimal surface LOD is almost identical to design for optimal bulk LOD.
Figure 2.11 Calculated surface and bulk resonance wavelengths, $\lambda_0$, and minimum reflectivities, $R_{min}$, for the long-range (LRSP) and short-range (SRSP) surface-plasmon modes of a BK7-Teflon AF-Au sensor illuminated at a 65.5° incident angle (inside BK7).
2.4.2 Optimization of angular interrogated dual-mode SPR sensor

We also optimized the angular interrogated dual-mode SPR sensor by the same method. Considering the shifts in coupling angles as the function of the sensitivities, we derived the binding layer thickness and bulk index changes expressed in sensitivities.

The LOD of $\Delta d$ and $\Delta n_B$ are determined by

$$LOD(\Delta d) = 3 \sqrt{\frac{\text{var}(\Delta \theta_{LR})/s_{LR}^2 + \text{var}(\Delta \theta_{SR})/s_{SR}^2}{(S_{LR}/S_{SR})^2}}$$

(2.27)

$$LOD(\Delta n_B) = 3 \sqrt{\frac{\text{var}(\Delta \theta_{LR})/s_{LR}^2 + \text{var}(\Delta \theta_{SR})/s_{SR}^2}{(S_{LR}/S_{SR})^2}}$$

(2.28)

We optimized the sensor configuration used for previous dual-mode SPR sensors[25]. The sensors used for angular interrogation is identical to the sensor fabricated for the wavelength interrogation system. We still need to determine all four sensitivity parameters and the variance of each resonance angle shift estimate.

We still used the transmission matrix to calculate reflection spectra for candidate sensor designs. Four sensitivities were determined from the calculated reflectance. The solution index change by 0.0001 refractive index units was applied to determine the bulk sensitivity by resonance angle shifts. A uniform 1 nm layer binding was added to the surface of the gold to calculate the surface sensitivity. The resonance angles and minimum reflectivity associated with long range and short range surface-plasmons are illustrated in Figure 2.12. Figure 2.13 and Figure 2.14 map the sensitivities of each mode to bulk and surface changes. All simulations were generated with an incident light of 800 nm. Compared to the sensitivities of wavelength interrogated dual-mode SPR sensor, there is still obvious tradeoff between the sensitivity of the long- and short- range modes. With regard to bulk sensitivities thinner buffer layers (above 550 nm) provide high sensitivity for long-range mode and thicker buffer layers (above 550 nm) provide high sensitivity for short-range mode. There is an overlap around buffer layers with 550 nm thick and gold layers with 55 nm thick where sensitivities can reach relatively high for both surface-plasmon modes. We choose sensor design around this point to reach
relatively high sensitivities.

Figure 2.12 Calculated resonance angles for long-range and short-range surface-plasmon modes of a BK-7-Teflon AF-Au sensor illuminated with 800 nm light.
Figure 2.13 Calculated bulk sensitivities for the long-range and short-range surface-plasmon modes of a BK-7-Teflon AF-Au sensor illuminated with 800 nm light. Bulk sensitivity is in degree/RIU.
Figure 2.14 Calculated bulk sensitivities for the long-range and short-range surface-plasmon modes of a BK-7-Teflon AF-Au sensor illuminated with 800 nm light. Bulk sensitivity is in degree/nm.
To determine the variance with which we can estimate the resonance angle shift we turn to the Cramer-Rao bound for the shift of a known spectrum [40]. The angle shift is reformulated to be dependence of the light source and detector which is given by

\[ \text{var}(\Delta \theta_0) \geq \left( \sum_{i=1}^{N} P \frac{\left| \frac{dR(\theta)}{d\theta} \right|_{\theta = \theta_i - \Delta \theta_0}}{R(\theta_i - \theta_0)} \right)^{-1} \]  

(2.29)

where \( R(\theta_i) \) is the noise free reflectivity of the sensor and \( P \) is the number of detected photons at a given wavelength. We consider optimization for an ideal illumination and detection system with a constant spectral power density. Figure 2.15 (a) shows the surface limit of detection based on calculated sensitivity and variance. During the design range of the buffer layer and gold thickness, the surface LOD increases as the gold thickness increasing for a fixed buffer thickness. Figure 2.15 (b) shows the merit of cross-sensitivity introduced by Homola. His optimal design based on minimizing cross-sensitivity. [23] For a fixed gold thickness of a sensor, the cross-sensitivity decreases as the buffer thickness decreasing. Combining with the maps of surface and bulk sensitivities the optimal angular sensor design consisted of a 500 nm thick Teflon layer with a 60 nm thick gold layer. As shown in Figure 2.12 the long- and short-range surface plasmon resonances fell at 63.2° and 67.4° respectively for the optimal design.
Figure 2.15 (a) Surface limits of detection for the dual-mode SPR sensor as a function of buffer (Teflon AF) thickness and gold thickness. The wavelength of incident light is 800 nm. (b) Cross-sensitivity for the dual-mode SPR sensor as a function of buffer thickness and gold thickness.
For wavelength interrogation the optimal dual-mode sensor design consisted of a 400 nm thick Teflon layer with a 52 nm thick gold layer with a 65.5° incident angle inside the BK7 prism. For angular interrogation the optimal design consisted of a 500 nm thick Teflon layer with a 60 nm thick gold layer with an 800 nm incident light.
Chapter 3 Dual-mode SPR Sensor Fabrication

According to the simulation and optimization of dual-mode SPR sensors in chapter 2, we successfully fabricated the sensors with desirable properties. The experimental dual-mode SPR sensor consists of a BK7 glass substrate, a low refractive index Teflon AF layer (~450 nm thick), and a thin gold layer (~55 nm thick). The fabrication process includes basic cleaning, spin coating, thin film deposition and property measurement.

3.1 Sample Preparation

A 20×20 mm² BK7 glass slide (Fisher Scientific) was chosen as the substrate of the sensor. Initially, thin slides were used as substrates, but they were too fragile for fabrication. As a result, thicker slides (~1mm) were adopted as substrates for the sensors. General cleaning steps are critical before the deposition of Teflon. The substrate was rinsed with acetone and then ethanol to remove the organic compounds and impurities, then rinsed by deionized water and blown to dry.

3.2 Deposition of Teflon Layer

Teflon is a well know brand of synthetic fluoropolymers from Dupont Co. Teflon AF is a summary of amorphous fluoropolymers with strong carbon-fluorine bonds. Compared to other amorphous polymers, Teflon AF has similar mechanical properties, excellent chemical resistance, high optical clarity and good heat resistance. Teflon AF is stable at temperatures up to 360°C /680°F, permitting more flexibility in processing options. The significant advantage of Teflon AF polymers is that they can be dissolved in certain solvents. Teflon AF polymers have the lowest refractive index among all solid polymers which is from 1.29 to 1.31. Due to its distinct optical, electronic, chemical and mechanical properties, Teflon AF polymers are used in a wide range of applications in many different areas [45].
Because of the high clarity and low refractive index, Teflon AF polymer is a great choice for optical materials. They can be a clear coating on optical device with a low refractive index which is exactly required for the buffer layer of the dual-mode SPR sensor. We coated Teflon AF-1600 from Dupont Co. on the substrate with comparable refractive index with water (1.32 to 1.34) to fabricate a symmetric profile so that two SPR modes can be excited at the same observing position. Figure 3.1 shows the refractive index of Teflon AF during the wavelength range of the visible light.

Because Teflon AF polymers are soluble in certain solvents, it is possible to deposit a thin film by spin coating method. The optimization of dual-mode SPR sensor requires the thickness of Teflon AF buffer layer to be around 500 nm. In order to achieve such a thin layer, we prepare the Teflon AF solution with proper concentration. Teflon AF 1600 is a liquid solution of Teflon diluted in FC-40 fluorinert solvent by 18% in weight. Teflon AF 1600 is too viscous, so it was further diluted with FC-40 (3 M Inc.) at a ratio of 1:2.5 in weight. The dilute solution is spin coated to a thickness of 500 nm at a spin speed of 3000 RPM.

Teflon AF has good adhesion to titanium, aluminum and electrolytic copper, but it does not adhere to the glass substrate. Surface treatment is necessary to improve the adhesion if we want to produce stable sensors which can be reused. A fluorosilane, 1H, 1H, 2H, 2H perfluorodecyltriethoxy silane (Lancaster Synthesis, Inc.) was used as adhesion promoter to increase the adhesion of Teflon AF to BK7 glass substrate. The adhesion promoter is prepared by mixing fluorosilane, ethanol and water with a weight ratio of 1:95:5. Clean BK7 glass substrate was mounted in vacuum chamber. The adhesion promoter was applied on the substrate and spun at 2000 RPM (round per minute) for 30 seconds to form a super thin layer. Then the sample was baked at 110°C for 10 minutes to remove the solvent. Then the diluted Teflon AF 1600 was dispensed on the substrate surface and spun at 500 RPM for 10 seconds so that Teflon AF solution can be spread.
uniformly on the sample. Then the sample was spin coated at 3500 RPM for 30 seconds to reach the expected thickness. Finally the sample was baked at 165°C for 20 minutes.

After the spin coating process, we measure the thickness of Teflon AF layer before deposition of gold film. A profilometer or an Ellipsometer is used to measure the Teflon film thickness. We will discuss the thickness measurement in the last part of this chapter.
Figure 3.1 Refractive index of Teflon AF 1600 polymer.
3.3 Deposition of Gold film

There are different methods to deposit gold on the sample. After many experiments, sputtering was determined to be more compatible with our process than e-beam evaporation.

3.3.1 Deposition of Gold by e-beam evaporation

During the e-beam evaporation process, an electron beam is used to heat the gold target (99.9% pure from Kurt J. Lesker, Inc) until it boils and evaporates. In a vacuum chamber, the evaporated gold particles condense freely on any surface inside the chamber including the sample. E-beam evaporation is performed at a base pressure of approximately $5 \times 10^{-6}$ torr. The evaporation was maintained by a constant acceleration voltage of 8KV. The deposition process was controlled at deposition rate of 2 Å/sec and the vacuum in the chamber increased to approximately $4.7 \times 10^{-5}$ torr. A quartz crystal is used to monitor the deposition rate. After calibrating the monitor reading, we can deposit 50 nm gold film as required by e-beam evaporation.

We got the gold film with proper thickness, but we ignored the process temperature during the evaporation. The high energy electron beam that heats the gold to evaporation also heats the Teflon beyond its stability temperature of 360°C. Teflon AF decomposes above 360°C resulting in a change in the refractive index. Concurrently, the thickness of Teflon AF layer also decreases. This resulted the failure to excite surface plasmon resonances as predicted by the simulation. After changing the distance between the target and the sample and adjusting the acceleration voltage of the evaporation, we still can not maintain the Teflon properties.

We experienced a serious concern of evaporator systems, which is radiation damage. The X-rays generated by the process damages the substrate and the dielectric, so evaporators can not be used in MOS or other technologies that are sensitive to this type
damage unless later thermal annealing steps are adequate to remove the damage. Compared to evaporator systems, sputtering systems have several advantages. It has better step coverage than evaporation and produce far less radiation damage. Sputtering is also much better at producing layers of compound materials and alloys. So the sputtering was chosen as the alternative method to deposit the gold. The sample during sputtering process will not be heated as high as e-beam evaporation. Teflon can keep low refractive index during the sputtering process.

3. 3.2 Deposition of Gold by sputtering

Sputtering is a process to eject the material from the target, and deposit it on the substrate. A high energy Ar plasma bombards the target to eject the source particles. Then the source particles will travel in the vacuum chamber and condense on the substrate. The sputter deposition process is illustrated in figure 3.2. Multi-targets sputtering machine can set several different targets in one chamber, which enable in-situ multi-layer deposition. We use a single-target sputtering machine for our gold deposition.

Gold (99.9%) target used in sputtering is from Kurt J. Lesker, Inc. When the chamber vacuum reached 0.16 mTorr, Argon gas was injected into the chamber at the speed of 15 scc/m. Sputtering is performed at a pressure of 3.6 mTorr and a DC power of 75 W. A crystal monitor was used to monitor the deposition thickness. The power supply was shut down automatically when the desired thickness is satisfied. After calibration of the monitor, we can deposit a 50 nm thick gold film as desired.
Figure 3.2 Sputtering process of deposition of gold layer.
The sputtering power and deposition pressure has two important influences on the properties of the deposited film. We will discuss them in the chapter 6 of tri-mode SPR sensor fabrication.

3.4 Thickness measurements

We used two different methods to measure the thickness of Teflon AF layer. We also placed a plain glass substrate in the chamber with the sample during sputtering so that we can measure the thickness of gold film.

3.4.1 Measurement of the Teflon and gold thickness by contact profilometer

The contact profilometer is an instrument that measures the roughness of a sample surface by detecting the vertical position of a stylus moving horizontally across the sample surface. A tiny line scratch was usually made at the edge of the sample. When the stylus scans across the scratch, the profilometer will show the vertical difference between the Teflon surface and the substrate surface, which is the thickness of Teflon layer. By the same way we can measure the gold film thickness on the plain BK7 glass substrate. We use Dektak 6M profilometer (Veeco, Inc) to measure the thickness of Teflon layer and gold layer. One measurement window is shown in figure 3.3.

3.4.2 Measurement of the Teflon thickness and refractive index by ellipsometer

The ellipsometer is an instrument used to study the properties of thin films by measuring the change of polarization of reflection or transmission. Ellipsometer M-2000 (J.A. Woollam Co. Inc) was used to measure the thickness of Teflon layer while our Teflon sample was attached on a 1cm thick BK7 glass substrate using BK7 refractive index matching fluid (Cargille, Inc.). At the same time we can also measure the refractive index of Teflon AF to make sure that Teflon layer can be used as a buffer layer for the dual-mode SPR sensor.
Figure 3.3 Profilometer measurement of gold layer thickness.
Chapter 4 Dual-mode self-referencing SPR sensor testing and results

We designed the dual-mode self-referencing SPR sensors based in chapter 2 and then successfully fabricated them. In order to validate the effectiveness of our sensors, it was tested using the well-established biotin-streptavidin binding interaction.

4.1 Wavelength interrogated sensors testing and results

For dual-mode wavelength interrogated SPR sensors, both background index changes and surface binding will result in different shifts for wavelength of excitation of the long-range (LR) and short-range (SR) surface-plasmon resonances. Thus, we can use the two resonances to differentiate between the two effects. If the sensor’s response to both bulk index change and surface binding is approximately linear, the LR and SR surface-plasmon resonance shifts, $\Delta \lambda_{LR}$ and $\Delta \lambda_{SR}$ respectively, are given by

$$\Delta \lambda_{LR} = S_{S-LR}\Delta d + S_{B-LR}\Delta n_B$$

$$\Delta \lambda_{SR} = S_{S-SR}\Delta d + S_{B-SR}\Delta n_B$$

(4-1)

(4-2)

Where $S_{S-LR}$ and $S_{S-SR}$ are the surface sensitivities in nm-wavelength/nm-thickness for the long- and short-range modes, and $S_{B-LR}$ and $S_{B-SR}$ are the bulk refractive index sensitivities in nm-wavelength/refractive index unit (RIU) also for both modes. The values $\Delta d$ and $\Delta n_B$ are the binding layer thickness change and the bulk refractive index change respectively. In many cases, the analyte partially covers the surface and it is more appropriate to write the surface sensitivities in terms of a change in surface concentration of the analyte. If we know the sensitivities and resonance wavelength shifts, the surface layer thickness and bulk index changes can be calculated directly as follows.
\[ \Delta d = \frac{\Delta \lambda_{LR}}{S_{B\text{-LR}}} / S_{B\text{-LR}} - \frac{\Delta \lambda_{SR}}{S_{B\text{-SR}}} / S_{B\text{-SR}} \]

\[ \Delta n_B = \frac{\Delta \lambda_{LR}}{S_{B\text{-LR}}} / S_{B\text{-LR}} - \frac{\Delta \lambda_{SR}}{S_{B\text{-SR}}} / S_{B\text{-SR}} \]

(4-3)  

(4-4)

In some experiments involving large changes in bulk index or surface concentrations, nonlinear responses cannot be ignored. In this case, the dependence of the resonance wavelengths on the product of the bound layer and the background index changes can be significant and we must consider the more complicated relationships

\[ \Delta \lambda_{LR} = S_{S\text{-LR}} \Delta d + S_{B\text{-LR}} \Delta n_B + S_{SB\text{-LR}} \Delta d \Delta n_B \]

(4-5)

\[ \Delta \lambda_{SR} = S_{S\text{-SR}} \Delta d + S_{B\text{-SR}} \Delta n_B + S_{SB\text{-SR}} \Delta d \Delta n_B \]

(4-6)

We have introduced \( S_{SB\text{-LR}} \) and \( S_{SB\text{-SR}} \) to describe the product dependence for the long- and short-range resonances respectively. One must numerically solve a set of nonlinear equations to determine \( \Delta d \) and \( \Delta n_B \) from measurements of \( \Delta \lambda_{LR} \) and \( \Delta \lambda_{SR} \); however, this is a small price to pay for the improved accuracy provided by including the product term. It should also be noted that the surface and bulk sensitivities in the last equation will not be exactly the same as those found for the linear model because the basic functions \( \Delta d, \Delta n_B \) and \( \Delta d \Delta n_B \) are not orthogonal.

The sensors were fabricated using a series of thin-film deposition processes as described in Chapter 3. Biotin labeling of the gold surface was performed as described by Kim et al. [46]. Briefly, the sensor was immersed in a solution containing 200 \( \mu \)M of \( N\text{-}(6\text{-[biotinamido]hexyl})\text{-3'}\text{-}(2'\text{-pyridyl)dithio})\text{propionamide} \) (biotin-HPDP, Pierce Biotechnology) in a 20 mM phosphate buffer, pH 7.4. The reaction was incubated at the room temperature overnight for approximately 16 hours. Next the sensor was removed, rinsed with de-ionized water and air dried. The biotin labeled probe was kept dry in a clean container until use.
Figure 4.1 Schematic diagram of a self-referencing surface-plasmon resonance sensor. The sensor consists of a gold thin film on a buffer layer (Teflon AF) that closely matches the refractive index of the solution of interest. The structure supports two surface plasmon modes excited at different wavelengths: a symmetric (long-range, LRSP) mode whose fields extend deeper into the solution and an anti-symmetric mode (short-range, SRSP) whose fields are concentrated near the surface of the metal.
As shown in Figure 4.1, we placed the sensor substrate in contact with a BK7 equilateral prism (Esco Products, Inc.) using a BK7 specific index matching fluid (Cargille, Inc.). The prism and sensor were clamped in a custom made acrylic flow cell with fluorinated ethylene propylene (FEP) coated channels. Liquids were introduced to the sensor surface through polytetrafluoroethylene (PTFE) tubing using low-pulsation peristaltic pump (Isamatec). The sensor was illuminated with light from a halogen lamp (Model DH-2000, Ocean Optics, Inc.) using a 200 μm core multi-mode optical fiber and a collimating lens. The polarization of the incident light was controlled by a calcite Glan-Taylor polarizer (ThorLabs, Inc.). The reflected light was collected by another lens and coupled to a multimode fiber which routed it to a CCD based spectrometer (Ocean Optics model HR-4000). Spectrum analysis was performed by custom software developed in LabView (National Instruments). A single measurement of the TE reflection spectrum was used as a reference. Figure 4.2 shows the experimental reflection spectrum from a dual-mode sensor with each resonance labeled. Two fits are also shown in figure 2 with two different data sets for the dielectric constant of gold. One is obtained from Johnson and Christy[42]. Another uses values provided by J.A. Woollam Co[47]. Both data sets provide a good fit for the reflectance. The measured excitation wavelength for each resonance is very close to the expected value.

Sensing experiments were carried out in 50 mM Tris buffer (Sigma-Aldrich) with pH 8.0. We prepared a second buffer solution with 1% glycerol (Sigma-Aldrich) by weight to provide a known bulk refractive index change of 0.00113. Streptavidin binding was carried out in a solution of 0.05 mg/mL streptavidin (Invitrogen) in 50 mM Tris buffer. As can be seen in Figure 4.3(a,b) both the LRSP and SRSP resonance wavelengths shift as the bulk solution changes and as the streptavidin binds with the biotin. After calibrating based on the change in bulk index and the steady state surface concentration, one can separately determine the bulk index change and the surface concentration.
Figure 4.2 Experimental reflection spectra from a BK7, Teflon AF (430 nm), Au (56 nm) SPR sensor in deionized water with an angle of incidence of approximately 65.5° (inside BK7 prism). The two reflection minima correspond to coupling to the long-range (LRSP) and the short-range (SRSP) surface plasmons. Also shown are theoretical fits to the spectrum using two different data sets for the dielectric constant of gold. Fit 1 uses Johnson and Christy’s values and fit 2 uses values provided by J.A. Woollam Co.
Figure 4.3(c, d) shows the calculated surface and bulk changes based on the nonlinear model. The non-linear model is necessary in this case because of the large change in surface index resulting from streptavidin binding. The dual mode sensor compensates for the background index change and allows the surface concentration change to be measured separately.

A 10 mM HCl solution was introduced to remove some of the bound streptavidin, and the experiment was repeated on the same sensor surface. In this case, most of the streptavidin remained and the change in surface concentration was considerably smaller. Figure 4.4(a,b) shows the change in resonance wavelength for this experiment while Figure 4.4 (c,d) shows the calculated surface and bulk changes based on the linear model. In this case we find the linear model effectively distinguishes surface and bulk changes; however, some residual cross talk can be observed that likely arises from small non-linearity or errors in the calibration coefficients.

The linear sensitivities for the short and long range modes were found to be 6300 nm/RIU and 1300 nm/RIU respectively. This is in excellent agreement with the theoretical predictions shown in Figure 2.9, and the short range bulk sensitivity is competitive with traditional SPR sensors. Although it is difficult to estimate absolute surface sensitivity based on a streptavidin-biotin experiment, we can compare the surface and bulk sensitivity ratios for each mode. These ratios were measured to be $S_{B-SR}/S_{B-LR} = 4.8$ and $S_{S-SR}/S_{S-LR} = 3.4$ which are also in good agreement with the theoretical predictions from Figure 2.9 ($S_{B-SR}/S_{B-LR} = 5.1$ and $S_{S-SR}/S_{S-LR} = 3.7$). The minor discrepancies between the predicted and measured sensitivities are likely the result of uncertainty in the optical properties of gold as noted in Figure 4.2.
Figure 4.3 Self-referenced measurement of streptavidin binding to a biotin functionalized gold surface. This experiment illustrates sensor performance for large changes in surface concentration. (a,b) SRSP and LRSP wavelengths vs. time. (c) bulk index change calculated from non-linear model. (d) change in surface concentration calculated from non-linear model. Experiments were conducted in 50mM Tris buffer (1) with 1% glycerol (2) or streptavidin (3) added. (c) and (d) clearly indicate that the dual-mode sensor compensates for changes in bulk index.
Figure 4.4 Self-referenced measurement of streptavidin binding to a biotin functionalized gold surface with a large surface concentration of streptavidin already present. This experiment illustrates sensor performance for small changes in surface concentration analyzed using the linear model. (a,b) Resonance wavelength vs. time for the long- and short-range surface plasmon modes. (c,d) Bulk refractive index and relative surface concentration change calculated from (a). Solutions are based on 50mM Tris buffer (1) with 1% glycerol (2) or streptavidin (3) added.
In order to determine the bulk LOD we recognize that Eq. 2-25 depends only on the ratio $S_{B-SR}/S_{S-LR}$ and not the absolute values of the surface sensitivity. Based on the variance of the resonance wavelengths in flowing Tris buffer we find the bulk LOD is $2.3 \times 10^{-5}$ RIU for 1 second sampling. The surface limit of detection, in terms of relative surface concentration change, was determined to be 0.012. If we assume a surface density of 1 protein/100 nm$^2$ for streptavidin[48], we estimate a mass LOD of 11 pg/mm$^2$. It should be noted that the variance used to calculate this LOD includes the effects of pump pulsation and represents the performance of a high-noise, CCD-based spectrometer. Even with these considerations, and despite the inherent tradeoffs in optimizing self-referencing sensors, these LODS remain competitive with typical SPR sensors while allowing operation in solutions with varying refractive index.

We conclude that spectrally interrogated SPR sensors supporting both long- and short-range surface-plasmon modes can differentiate between background refractive index changes and surface-binding of a target analyte. The sensitivities of dual mode sensors can be adequately predicted based on bulk-optical properties and Fresnel reflection calculations. For a given interrogation system a clear optimum sensor design exists. When properly optimized, dual-mode SPR sensors can be competitive with traditional SPR sensors in terms of sensitivity and LOD for surface effects while compensating for bulk index changes.

4.2 Angular interrogated sensors testing and results

Sensor performance in self-referencing wavelength interrogation systems is limited by the fact that the long range mode, with fields penetrating more deeply into the solution, is excited at shorter wavelengths, while the short range mode, with fields more localized on the metal surface, is excited at longer wavelengths. This phenomenon results in less differentiation between bulk and surface effects than anticipated and thus higher cross-sensitivity. In this case, cross sensitivity is defined as the measurement
error of a surface layer thickness or concentration change due to a change in bulk index or vice-versa.[23]

In order to improve sensor performance, and especially to achieve lower cross-sensitivity between surface and bulk responses, we have developed an optical system for angular interrogation of dual-mode sensors. Such a system can excite both long- and short-range surface-plasmon modes simultaneously at the same location and the same wavelength using different incident angles. As a result, the two modes are more strongly differentiated with respect to the field strength at the metal surface and field penetration into the solution. The calculated fields for both wavelength and angular interrogation are shown in Figure 4.5 and show that angular interrogation exhibits superior differentiation between the two modes.

Both background index changes and surface binding will result in different shifts for angle of excitation of the long-range (LR) and short-range (SR) surface-plasmon resonances. Thus, we can use the two resonances to differentiate between the two effects. If the sensor’s response to both bulk index change and surface binding is approximately linear, the LR and SR surface-plasmon resonance shifts, $\Delta \theta_{LR}$ and $\Delta \theta_{SR}$ respectively, are given by

$$\Delta \theta_{LR} = S_{S-LR} \Delta d + S_{B-LR} \Delta n_B$$

$$(4-7)$$

$$\Delta \theta_{SR} = S_{S-SR} \Delta d + S_{B-SR} \Delta n_B$$

$$(4-8)$$

Where $S_{S-LR}$ and $S_{S-SR}$ are the surface sensitivities in degree-angle/nm-thickness for long and short-range modes, and $S_{B-LR}$ and $S_{B-SR}$ are the bulk refractive index sensitivities in degree/refractive index unit (RIU) also for both modes. The values $\Delta d$ and $\Delta n_B$ are the binding layer thickness change and the bulk refractive index change respectively. If we know the sensitivities and resonance angle shifts, the surface layer thickness and bulk index changes can be calculated directly as follows
Figure 4.5 Comparison of field profiles dual-mode SPR sensors. (a) Electrical field (component normal to the metal surface) profiles for the long-range (LRSP) and short-range (SRSP) surface plasmons excited at 800-nm in an angular interrogation configuration. Note the enhanced field at the surface for the SRSP and the deeper penetration of the field into the solution for the LRSP. (b) Electric field profiles for the LRSP and SRSP modes in a wavelength interrogation configuration. The resonance wavelengths are characteristic of a typical dual-mode sensor. The angular interrogation configuration produces greater differentiation in the field profiles and thus reduced cross sensitivity between surface and bulk effects.
\[ \Delta d = \frac{\Delta \theta_{LR} / S_{B-LR} - \Delta \theta_{SR} / S_{B-SR}}{S_{S-LR} / S_{B-LR} - S_{S-SR} / S_{B-SR}} \]  
\[ \Delta n_B = \frac{\Delta \theta_{LR} / S_{S-LR} - \Delta \theta_{SR} / S_{S-SR}}{S_{B-LR} / S_{S-LR} - S_{B-SR} / S_{S-SR}} \]  

(4-9)  
(4-10)

Figure 4.6 illustrates the optical system used for dual-mode angular interrogation. A combination of two cylindrical lenses (Thorlabs Inc.) with a line camera detector (Thorlabs, Inc.) allows for reflection measurement over approximately 15°. This represents a broader range of incident angles than for a typical single mode, angularly interrogated SPR sensor.[49] Resonance angles were calibrated from output pixel positions on the line camera. An 800 nm filter was placed before the linear camera while a halogen white light source was used (Ocean Optics, Inc.) for illumination. A Glan-Taylor polarizer (Thorlabs, Inc.) was used to select TM illumination for sensing and TE illumination for recording a reference reflection spectrum. A custom made flow cell (thickness \( \approx 250 \mu m \)) was mounted to the sensor attached on a quasi-hemi cylindrical prism (Thorlabs Inc.) such that different solutions could be introduced to the sensor surface. All analytes were introduced to the sensors using a peristaltic pump (Ismatec SA).

The sensor was fabricated as the same method and was attached on the prism by BK7 index matching fluid (Cargille, Inc.). The flow cell was mounted on the sensor to allow the different solutions switch on the sensor surface. Figure 4.7 illustrates calculated and experimental reflectance spectra for the dual-mode sensor with Teflon AF thickness of 520 nm and gold layer thickness of 62 nm using deionized water as the solution. Transmission matrix calculations (WVASE32 Software, J.A. Woollam Co., Inc.) were used to fit the thickness to experimental data. The measured angle for each resonance is very close to the expected value with long-range excitation at 63 degrees and short-range excitation at 66 degrees. The difference in reflection amplitude is most likely attributable to the uncertainty in the optical constants of gold and Teflon AF.
Figure 4.6 Optical system for angular interrogation of dual mode SPR sensors. The optical system must be designed to measure a broader range of angles (15°) than typical single-mode SPR systems.
Figure 4.7 Experimental and theoretical (fit) angular reflection spectra of a dual-mode SPR sensor using 800 nm wavelength light. The theoretical fit indicates that the Teflon AF layer is 520 nm thick and the Au layer is 62 nm thick.
In order to demonstrate the effectiveness of our sensor we allowed streptavidin (Invitrogen Inc.) to non-specifically bind to the gold surface in the presence of different flowing buffer solutions. The experiment was carried out using a 50 mM Tris (Sigma-Aldrich) buffer. The binding solution contained 0.5 mg Streptavidin dissolved in 10 ml 50 mM Tris Buffer. A reference buffer was prepared by combining 50 mM Tris buffer with 1% glycerol by weight to provide a bulk refractive index change of 0.001.

The solutions were introduced to the sensor in the following order: (1) 50 mM Tris buffer, (2) 50 mM Tris buffer with 1% glycerol, (3) 50 mM Tris buffer, (4) streptavidin in 50 mM Tris buffer, (5) 50 mM Tris buffer, (6) 50 mM Tris buffer with 1% glycerol, (7) 50 mM Tris buffer. During the experimental process, a water bath was used to control all solution temperatures at 24.0±0.1ºC.

As shown in Figure 4.8 (a,b), both the LRSP and SRSP resonance angles shifted as the buffer solution is changed and as streptavidin binds to the gold surface. The small shift before introducing the streptavidin solution was caused by residual DI water in the tubing. Figure 4.8 (c,d) shows the calculated surface binding and bulk index change as functions of time based on the linear model. The bulk refractive index measurements are largely unaffected by the thickness increase. Similarly, the surface binding layer thickness measurements show only a small influence from the bulk index change between the two buffer solutions.

Bulk sensitivities for both modes were calculated from the linear model and found to be. \( S_{B\_LR} = 9.92^\circ / RIU \) and \( S_{B\_SR} = 103.4^\circ / RIU \). The short range mode bulk sensitivity remains competitive with the typical single mode SPR sensors (97º/RIU for a single mode sensor operating at 850 nm)[17]. Although it is difficult to estimate the surface binding thickness change in our non-specific binding experiment, we can determine the ratio of surface sensitivities for the two modes. Slavik et al. showed that the cross-sensitivity of a dual-mode sensor is inversely proportional to difference in the sensitivity ratios, \( \chi = \left| \frac{S_{S\_SR}}{S_{S\_LR}} - \frac{S_{B\_SR}}{S_{B\_LR}} \right| \)[32]

76
Figure 4.8 Sensor response to streptavidin binding on gold surface. (a,b) Resonance angle vs. time for long- and short-range surface plasmon modes. (c,d) Bulk refractive index and relative surface binding thickness change calculated from (a,b). Solutions are carried out in 50mM Tris buffer (1) with 1% glycerol (2) or streptavidin (3) added.
The measured ratios for our angularly interrogated sensor were $S_{_SR}/S_{_LR} = 15.4$ and $S_{_{SR}}/S_{_{LR}} = 10.4$. The figure of merit, $\chi = 5.0$, is significantly better than our wavelength interrogated design based on minimizing surface limit-of-detection, $\chi = 1.4$,[39] and Slavik's wavelength interrogated design based on minimizing cross-sensitivity, $\chi = 1.46$.[23]

We conclude that both the long- and short- range surface-plasmon modes can be simultaneously excited using single-wavelength angular interrogation, and that the two modes can differentiate surface-binding from bulk refractive index changes. Angularly interrogated dual-mode SPR sensors reduce cross-sensitivity by at least a factor of 3.6 compared to optimized wavelength-interrogated sensors. As a result, they are preferred when calibration inaccuracy is the dominant error source.[23] However, system noise is often the dominant error source, and minimum reflectivity and the width of the spectral features influence spectral shift estimation.[51]
Self-referencing dual-mode SPR sensors can successfully differentiate surface binding interactions from bulk index changes. However, distinguishing specific binding from non-specific binding effects in a complex analyte presents another challenge. Based on the dual-mode SPR sensors, at least one more SPR mode should be achieved to realize this goal. If we can design a tri-mode SPR sensor and each resonance can respond to specific and non-specific surface interactions, as well as bulk index changes differently, it is possible to distinguish one effect from the other two. Ultimately, this will permit SPR sensors to operate in complicated fluids. These efforts will further bridge the gap between laboratory research and real-world instruments, so that self-referencing SPR sensor could replace the traditional SPR sensors for biosensing, environmental detection, pharmaceutical research, or wherever they are needed.

5.1 Selection of materials

The design of a tri-mode sensor is based on a dual-mode sensor. Firstly we need to designing and fabricate a dual-mode SPR sensor. BK7 glass is still chosen to be the substrate because of the widespread availability of high-quality material. As mentioned because the real relative permittivity of gold is negative for visible to near-infrared light and gold is stable in most chemical environments of interest, it is chosen to be the metal layer to generate surface plasmon resonance. A low refractive index Teflon AF
Layer is placed under the gold film to create two surface plasmon resonances.

If a dual-mode sensor is covered by a thin SiO₂ layer, the two coupling wavelengths/angles will shift. That means it is possible to realize a tri-mode spectrum by fabricating SiO₂ stripes on the gold surface. We chose SiO₂ as the stripe material because of its ease of deposition and chemical modification. In this configuration, the short wavelength modes associated with the bare and coated gold region typically overlap in the reflection spectra. However, the longer wavelength modes separate sufficiently that they can be independently interrogated. For the SiO₂ coated regions the refractive index on either side of the gold film is longer symmetric, and it is no longer meaningful to refer to long- and short-range modes. Figure 5.1 shows an illustration of an ideal tri-mode SPR sensor with SiO₂ stripes on a gold surface with a low refractive index buffer layer below.
Figure 5.1 Illustration of tri-mode SPR sensor with SiO$_2$ stripes on a gold surface with a low refractive index buffer layer below.
5.2 Simulation of reflected spectrum

Figure 5.2 illustrates the optical system used for tri-mode SPR sensor by wavelength interrogation. To calculate the theoretical reflection spectra of a tri-mode SPR sensor we still need to apply the Fresnel equations and transmission matrix introduced in Chapter 2 to tri-mode situation. But now the surface of a tri-mode sensor is covered by SiO$_2$ stripes instead of a uniform layer. Because the space between each two stripes is the width of a stripe and the spacing is large compared to the wavelength, we can consider two structures; one is a dual-mode SPR sensor and another is a dual-mode SPR sensor covered by SiO$_2$ layer. Then the average of the reflection spectra of these two structures can be considered as the reflection spectrum of a dual-mode sensor covered by SiO$_2$ stripes with the same space as stripe width. Firstly we design a tri-mode sensor with satisfactory reflectivity. Specifically, we desire low reflectivity for each mode and that all modes can be detected within a practical wavelength range of 400 to 1000 nm. This range corresponds to the effective range of a silicon detector-based spectrograph.

One example spectrum of a tri-mode design with DI water on the sensor surface is shown in Figure 5.3. Figure 5.3(a) shows two spectra calculated based on transmission matrix for two structures (a dual-mode SPR sensor with/without thin SiO$_2$ layer). R1 is the ratio of TM to TE reflectivity of a normal dual-mode SPR sensor. R2 is the ratio of TM to TE of this sensor covered by a thin SiO$_2$ layer. The final reflected theoretical spectrum is the average of R1 and R2 shown in figure 5.3(b).
Figure 5.2 Schematic diagram of a Tri-mode SPR sensor. The sensor consists of a gold thin film on a low refractive index Teflon layer and SiO$_2$ stripes on top fabricated on a BK7 substrate. This sensor structure supports three SPR modes excited by different wavelengths whose electrical fields penetrate difference distances into the solution.
The incident angle is fixed and the variable parameters are Teflon thickness, gold thickness and SiO$_2$ stripe thickness. All the simulation parameters are listed in the figure. The data sets of optical properties of the materials are from the same sources as in the simulation of a dual-mode sensor. Water, described by the optical constants determined by Hale and Querry[41], was used for the solution. Optical constants for gold were obtained from Johnson and Christy[42]. Optical constants for BK7 were established by Sellmeier dispersion coefficients supplied by Schott North America Inc.[43], and optical constants for Teflon AF were taken from a Cauchy dispersion formula fit to the measurements of Lowry et al[44]. And the optical constants for SiO$_2$ were measured by ellipsometer (J.A. Woollam Co., Inc.).

The calculation proved that we can create a tri-mode SPR sensor if we can fabricate it properly. Three surface plamon resonances can be excited at the same incident angle but at different wavelengths. The optimization of a tri-mode sensor becomes quite complicated as many characteristics must be considered including sensitivities of three modes, accuracy, resolution and LOD.
Figure 5.3 An example reflection spectrum of a tri-mode sensor (a) Simulated reflection spectra of a dual-mode SPR sensor (Teflon: 420 nm Gold: 60 nm) with and without a thin SiO$_2$ layer (45 nm) on the gold surface. The incident angle is 67°. (b) Simulated reflection spectra of a tri-mode SPR sensor. Teflon: 420 nm Gold: 60 nm and SiO$_2$ stripes: 45 nm.
Chapter 6 Tri-mode SPR sensor fabrication

We fabricated a tri-mode sensor based on the design parameters established in Chapter 5. A successful dual-mode sensor is the foundation of a tri-mode sensor. In order to create three surface plasmon modes during the measurement wavelength range (400 nm to 1100 nm), the thickness of gold layer should increase compared to that of an optimized dual-mode sensor. The thicker gold layer compresses the modes of the dual mode sensor into the shorter wavelength region, and provides bandwidth to add the third mode.

6.1 Fabrication of a dual-mode SPR sensor

A 1mm-thick 20×20 mm² BK7 glass slide (Fisher Scientific) was chosen as the substrate. The substrate was prepared for film deposition by rinsing with acetone and then ethanol. An adhesion promoter (1,1,1,3,3,3-Hexamethyldisilazane (HMDS), AZ Electronic Materials) was applied on the substrate and spun at 2000 RPM (revolutions per minute) for 30 seconds to form a thin layer that increases the adhesion of Teflon to the glass surface. Then the sample was baked at 110°C for 10 minutes to remove the solvent. Teflon AF 1600 was applied on the sample with adhesion promoter and firstly spun at 500 RPM for 10 seconds so that Teflon AF solution can be spread uniformly on the sample. Then the sample was spin coated at 3500 RPM for 30 seconds to reach the expected thickness. Finally the sample was baked at 165°C for 20 minutes. Before deposition of a gold film, the film thickness is measured by a stylus profilometer and an ellipsometer to determine the thickness and optical properties of the Teflon layer.

A gold (99.9%) target from Kurt J. Lesker, Inc. was used to sputter a thin film (~60 nm) on the Teflon. When the chamber vacuum reached 0.16mTorr, Argon gas was released into the chamber at the speed of 15 scc/m. A constant power of 75w was supplied while the sputtering deposition was conducted at a pressure of 3.6mTorr. The power supply will be shut down automatically when the desired thickness is reached. A plain BK7
substrate was placed in chamber as a monitor sample for every deposition so that we can directly measure the gold thickness using a profilometer. We also tested the dual-mode sensor by measuring its reflected spectrum to guarantee it can work properly before the next step (deposition of SiO\textsubscript{2} layer).

6.2 Fabrication of SiO\textsubscript{2} stripes on gold surface

In order to create SiO\textsubscript{2} stripes on gold surface we need to deposit SiO\textsubscript{2} layer and then perform a wet etch after photolithography. After removing the photo resist residue only SiO\textsubscript{2} stripes remain on the gold surface.

6.2.1 Sputtering of SiO\textsubscript{2} layer

We briefly introduced sputtering system in Chapter 3. The structure and properties of a sputtered film depend on sputtering power, substrate temperature and deposition pressure. The structure zone model (SZM) was firstly introduced by Movchan and Demchishin and later modified by Thornton[52-54]. Messier and Giri revised the SZM for thin film coating structure in 1984[55]. Mobility of the adatoms at the substrate surface determines the structure of a thin film. The Thornton SZM is the most commonly used model to describe the relationship between the substrate temperature (usually referenced to the ratio of substrate temperature, T, to the coating-material melting point, T\textsubscript{m}), kinetic energy of the ions and the deposition rate. The sputtered films on ZONE III appear milky or hazy with rough surface. The films on ZONE II form with tall narrow columnar grains that grow vertically from the surface. Only ZONE II can produce dense films with highly specular surface and very small grains. [53] Thornton only considered the thermal induced mobility. The revised structure zone model included the induced mobility from bombardment effects.
The SiO$_2$ was also deposited on the gold surface by sputtering. The SiO$_2$ target was from Kurt J. Lesker, Inc. Firstly SiO$_2$ was sputtered on our sample at the same conditions as the deposition of gold. When the chamber vacuum reached 0.16 mTorr, Argon gas was released into the chamber at the flow rate of 15 scc/m. A constant power of 75 W was supplied while the depositing with a pressure of 3.6 mTorr. Under these conditions, the SiO$_2$ would crack and peel during the next fabrication step (photolithography), and the gold surface was also destroyed when SiO$_2$ peeled off. The refractive index of SiO$_2$ measured by Cauchy fitting from an ellipsometer (J. A. Woollam Co. Inc) was 1.35 which was much lower than pure, dense SiO$_2$. Every appearance showed that such a SiO$_2$ layer had a loose porous structure which should fall in ZONE I.

The melting point of SiO$_2$ is 1,600°C (2,912°F), and room temperature is about 24°C (75°F). There is no heating instrument in the sputtering system, so the ratio of T/T$_m$ is a very small value (below 0.1). From Thornton’s SZM we can conclude that the lower the pressure is, the more likely the deposited film structure will be falling into ZONE T.

In order to get a tight structure SiO$_2$ film we should control the sputtering pressure as low as possible. Higher power should be applied to excite Ar gas plasmon at lower pressure. When the chamber vacuum reached 0.16 mTorr, Argon gas was released into the chamber at the speed of 7 scc/m. A constant power of 200 W was supplied while the sputtering deposition at the pressure with 2.1 mTorr. At the pressure with 1.1 mTorr, the plasma will be extinguished. Thus, we must keep the pressure higher than 1.1 mTorr, and the reason that 7 scc/m is the minimum gas flow rate. Ultimately, we obtained a 40 nm thick SiO$_2$ layer with refractive index of 1.48 at 800 nm incident light measured by ellipsometer.

In order to improve the adhesion of SiO$_2$ to gold surface, we also pre-sputtered several nanometer thick Ti layer on the gold film before depositing SiO$_2$. Starting pressure was 3.7 mTorr. Gas flow was 15 scc/m and the DC power was 80 W.
6.2.2 Photo lithography of SiO$_2$ layer

Initially, photo resist (Shipley S1813) stripes were patterned on gold surface before deposition of SiO$_2$ layer. The ideal situation would be removal of the photo resist after sputtering SiO$_2$ layer. Then the sample will be a tri-mode sensor with SiO$_2$ stripes left on gold surface. However, sputtering technology has good step coverage, and it became difficult to completely remove photoresist from the gold surface while keeping SiO$_2$ stripes. In addition, the gold surface was significantly contaminated with photoresist residue after the process.

Alternative plan was to deposit the SiO$_2$ layer before the photo lithography step. Photo lithography creates photoresist stripes on SiO$_2$ layer. After wet etching the SiO$_2$ and removing the photo resist, SiO$_2$ stripes remained on the gold surface. There is no strict criterion to define the stripe width. Sufficient numbers of stripes in the observing light spot (1.5mm diameter circle) were achieved to average the spectra. Four 20mm×20mm square patterns were designed consisting of 30μm width stripes with 30μm space between each two stripes, 50μm width with 50μm space, 60μm width with 40μm space and 70μm width with 30μm space. The patterns without 50:50 duty cycles were chosen to correct for over etching of the SiO$_2$. The 70:30 line to space pattern was finally used for our tri-mode sensor.

Before applying Shipley S1813 positive photo resist onto the sample, the sample should be prebaked at 115°C for 2 minutes. Photo resist was applied on the sample and spun at 5000 RPM for 30 seconds to form a protection layer about 1μm. Then the sample was baked at 115°C for 1 minute to remove the solvent. Contact photolithography was used here. One can estimate the exposure time by $T = E/I$ where $T$ is the exposure time (second), $E$ is the exposure dose in unit (mJ/cm$^2$) and $I$ is the intensity of exposure light in unit (mW/cm$^2$). The exposure dose for 1 μm thick S1813 is 90 mJ/cm$^2$.[56] The light intensity can be measured by a UV light meter. The estimated exposure time was about 10 seconds. Because the mask pattern is as the
same size as our sample, the alignment was not difficult. MF319 developer is used for photo resist S1813. After each 10-second development the sample was checked under an optical microscope to ensure integrality of the SiO$_2$ layer and total development of the photo resist. The optimal development time was approximately 40 seconds. After development the sample was blown dry with nitrogen.

6.2.3 Wet etch for SiO$_2$ stripes

Buffered oxide etch (BOE 6:1) was used to etch off the SiO$_2$. BOE (6:1) means the solution comprises a 6:1 volume ratio of 40% NH$_4$F in water to 49% HF in water. BOE (6:1) was diluted again by DI water with the volume ratio of 1:1 to slow down the etching speed. To totally etch 30 to 40 nm thick SiO$_2$ layers required about 8 sec.

6.2.4 Remove the photo resist

The photoresist was removed by immersing the sample in Shipley 1165 positive resist remover for several seconds, and then checking the pattern under an optical microscope. The removal process was repeated if evidence of the photoresist was visible.

The times for development, etching and removing resist are less than the manufacturers recommended values. For all cleaning steps the sample was immersed in DI water instead of rinsed under it. The reason for these operations is to make sure the SiO$_2$ not peeling off the gold surface.

6. 3 Thickness measurement

The method to measure the thickness of Teflon and gold film is as the same as the dual-mode SPR sensor. The thickness of Teflon layer can be measured both by profilometer and ellipsometer. Gold thickness was measured by profilometer. SiO$_2$ stripe thickness can be measured by Cauchy fitting of ellipsometer which can be confirmed
using profilometer after the stripes are successfully fabricated on the gold surface.

The optical constants of Teflon layer and SiO$_2$ were both measured by Cauchy fitting using an ellipsometer. Figure 6.2 shows the optical properties measured by ellipsometer for the two materials. Both of them have reached the range we expected so that we can generate three surface plasmon resonances.
Figure 6.1 Measurement of optical properties for Teflon AF and SiO$_2$ strips from ellipsometer. (a) Optical properties for Teflon AF, (b) Optical properties for SiO$_2$ strips.
Chapter 7 Tri-mode SPR sensor Testing and Results

7.1 Sensing model for tri-mode SPR sensor

Based on the dual-mode SPR sensors, the addition of one more SPR mode should enable the sensor to distinguish specific binding from non-specific binding. As previously described, SiO₂ stripes with the same spacing as stripe width on the gold surface of a dual-mode sensor create the 3rd surface plasmon resonance. Background index changes, specific binding to a functionalize gold surface, and non-specific binding to both the gold and SiO₂ surfaces will result in different shifts for the wavelength of excitation of the three surface-plasmon resonances. Thus, we can use the three resonances to differentiate these three effects. If the sensor’s response to bulk index change, specific binding and non-specific binding is approximately linear, the three surface-plasmon resonance shifts, \( \Delta \lambda_1 \), \( \Delta \lambda_2 \) and \( \Delta \lambda_3 \) respectively, are given by

\[
\begin{pmatrix}
\Delta \lambda_1 \\
\Delta \lambda_2 \\
\Delta \lambda_3 \\
\end{pmatrix} = S \times \begin{pmatrix}
\Delta n_B \\
\Delta C_n \\
\Delta C_s \\
\end{pmatrix}
\]

(7-1)

where \( S \) is the sensitivity matrix given by

\[
S = \begin{pmatrix}
S_{B-1} & S_{Sn-1} & S_{SS-1} \\
S_{B-2} & S_{Sn-2} & S_{SS-2} \\
S_{B-3} & S_{Sn-3} & S_{SS-3} \\
\end{pmatrix}
\]

where \( S_{Sn-1} \), \( S_{Sn-2} \) and \( S_{Sn-3} \) are the surface non-specific binding sensitivities in nm-wavelength/arb for the three modes, \( S_{SS-1} \), \( S_{SS-2} \) and \( S_{SS-3} \) are the specific binding sensitivities, and \( S_{B-1} \), \( S_{B-2} \) and \( S_{B-3} \) are the bulk refractive index sensitivities in nm/refractive index unit (RIU) also for three modes. The values \( \Delta C_n \), \( \Delta C_s \) and \( \Delta n_B \) are the binding layer surface concentration change and the bulk refractive index change respectively. Typically the surface concentration change is specified as a fraction of full coverage rather than an absolute unit such as molecules/m². If we know the
sensitivities and resonance wavelength shifts, the surface layer concentration and bulk index changes can be calculated directly from the inversion of the sensitivities matrix.

7.2 Sensing experiments of a tri-mode SPR sensor

7.2.1 Optical system and sensor fabrication

The optical system used for a tri-mode sensor wavelength interrogation is illustrated in figure 5.2. A Glan-Taylor polarizer (Model SM05PM5, Thorlabs, Inc.) was used to select TM illumination for sensing and TE illumination for recording a reference reflection spectrum. A custom made flow cell (channel thickness ≈250 μm) was mounted to the sensor and attached to a BK7 prism (ESCO Inc.) such that different solutions could be introduced to the sensor surface. All analytes were introduced to the sensors using a peristaltic pump (Model REGLO Digital, Ismatec SA).

The tri-mode sensor consists of a BK7 glass substrate (Fisher Scientific, Inc.), a spin coated, low refractive index Teflon AF 1600 (Dupont, Inc.) layer, a sputter deposited gold layer and SiO₂ stripes with the same spacing as stripe width. The detail fabrication process is described in Chapter 6.

Figure 7.1 illustrates calculated and experimental reflectance spectra for the tri-mode sensor using deionized (DI) water as the solution. The sensor had a Teflon AF thickness of 460 nm, a gold layer thickness of 67 nm, and a SiO₂ stripe thickness of 35 nm. The thickness of the gold layer and Teflon layer were measured by contact profilometer. Ellipsometry measurements confirmed the thickness of Teflon layer by Cauchy fitting. The thickness of SiO₂ layer was measured by ellipsometer after sputtering and was confirmed by profilometer after photolithography. Three surface plamon resonances are excited by different wavelengths at the same incident angle. The measured incident angle is 66.3°. The difference in reflection amplitude is most likely attributable to the uncertainty in the optical constants or chromatic aberrations in the optical system.
Figure 7.1 Experimental and theoretical (fit) reflection spectra of a tri-mode SPR sensor. The theoretical fit indicates that the Teflon AF layer is 460 nm thick, the Au layer is 62 nm thick and the SiO$_2$ stripes is 35 nm thick. Incident angle is indicated to be approximately 66°.
7.2.2 Sensing experiment procedure and results

In order to demonstrate the effectiveness of our sensor we allowed streptavidin (Invitrogen Inc.) to non-specifically bind to the SiO$_2$ stripe surface and we also monitored the specific binding of biotin-streptavidin on the gold surface. To functionalize the gold surface with biotin, the sensor was immersed overnight (~18 hours) in a biotin-HPDP solution (Pierce Biotechnology) at room temperature. The biotin labeled sensor was rinsed with DI water and kept dry until use. The sensing experiment was carried out using a 50mM Tris (Sigma-Aldrich) buffer. The non-specific binding solution contained 0.5mg streptavidin and 475 $\mu$g biotin dissolved in 10ml 50mM Tris Buffer. The extra biotin bound to streptavidin in solution to saturate the binding sites and prevent binding to the biotin labeled gold surface. The specific binding solution contained 0.5mg streptavidin dissolved in 10ml 50mM Tris buffer. A reference buffer was prepared by combining 50mM Tris buffer with 1% glycerol by weight to provide a bulk refractive index change of 0.001. [50, 57] Figure 7.2 illustrates the sensing model. The solutions were introduced to the sensor in the following order:

1. 50mM Tris buffer,
2. 50mM Tris buffer with 1% glycerol,
3. 50mM Tris buffer,
4. streptavidin and biotin in 50mM Tris buffer,
5. 50mM Tris buffer,
6. 50mM Tris buffer with 1% glycerol,
7. 50mM Tris buffer,
8. streptavidin in 50mM Tris buffer,
9. 50mM Tris buffer.

As shown in Figure 7.3(a, b) the first 2 surface plasmon resonance wavelengths shifted as the buffer solution changed index and streptavidin bound to the SiO$_2$ stripes and the gold surface. The surface-plasmon resonance bound on SiO$_2$ stripes only
responded to bulk and non-specific binding changes as shown Figure 7.3 (c). The specific binding did not cause the wavelength shift for the SPR mode on SiO$_2$ because the streptavidin selectively only connected with biotin-HPDP on gold surface. Fig. 4 (a, b, c) shows the calibrated bulk index, surface specific and non-specific binding changes as functions of time based on the linear model. The bulk refractive index measurements are largely unaffected by the binding change, neither specific nor non-specific interaction. Non-specific binding cannot be separated from the whole sensing procedure clearly. However, the biotin-HPDP-streptavidin binding is differentiated from the index change and saturated streptavidin surface binding. Some residual cross talk can be observed when the solution changes on the sensor. That might be caused by the nonlinear relationship between the surface and bulk changes and the resonance wavelength shifts.
Figure 7.2 Sensing model for a tri-mode SPR sensor. Red spots are streptavidin and the blue y-shapes on the gold surface are biotin-HPDP. The blue y-shapes on the glass surface are normal biotin molecule. (a) Specific binding of Streptavidin-Biotin on Gold surface, (b) non-specific binding of saturated-site Streptavidin binding on SiO₂ strips.
Figure 7.3 Sensor response to streptavidin binding on gold surface. Resonance wavelength vs. time for three surface plasmon modes. Solutions contain 50mM Tris buffer (1) with 1% glycerol (2) or streptavidin and biotin (3) added or streptavidin (4) added.
Figure 7.4 The calibrated bulk index, surface specific and non-specific binding change as functions of time based on the linear model.
7.2.3 Discussion and Conclusion

Bulk sensitivities for all three modes were calculated from the linear model and found to be. 
\[ S_{B,1} = 1000 \text{nm} / \text{RIU} \], 
\[ S_{B,2} = 5400 \text{nm} / \text{RIU} \] and 
\[ S_{B,3} = 13000 \text{nm} / \text{RIU} \]. The bulk sensitivity of the SPR mode on the SiO\(_2\) strips remains competitive with the traditional SPR sensors.[17] The relative specific binding sensitivity of SPR mode bound at gold surface is 2.2 for complete coverage without SiO\(_2\) strips. The surface sensitivity of our dual-mode sensor was 2.7 for wavelength interrogation and 2.6 for angular interrogation. Although it is difficult to estimate the absolute surface binding concentration change in our experiment, we can determine the ratio of surface sensitivities for the three modes.

As we mentioned in the dual-mode SPR sensor sections and in reference [39], Slavik et al. showed that the cross-sensitivity of a dual-mode sensor is inversely proportional to difference in the sensitivity ratios.[23] Similarly we introduce the figure of merit 
\[ \chi_1 = \left| \frac{S_{ss,2}}{S_{ss,1}} - \frac{S_{B,2}}{S_{B,1}} \right| \] denoted for the differences in the sensitivity ratios of bulk index change and specific binding. The measured bulk and specific binding surface sensitivity ratios for our tri-mode sensor were 
\[ \frac{S_{B,2}}{S_{B,1}} = 5.4 \] and \[ \frac{S_{ss,2}}{S_{ss,1}} = 4.9 \].

So the introduced figure of merit \( \chi_1 = 0.48 \) which is lower than that of our dual-mode SPR sensor performance. We got difference in the sensitivity ratio of 1.4 for the wavelength interrogation and 5 for the angular interrogation.[25, 39].

We draw the conclusion that three SPR modes can be excited at one observation point by different wavelength at the same incident angle on our tri-mode SPR sensor. In addition, the three modes respond differently to the bulk index change, non-specific binding and specific binding. Specific binding was differentiated from the other two effects successfully. Noise is always an import factor in data analysis. Nonlinear response of the sensor will increase the difficulty to differentiate one effect from the others.
Chapter 8 Conclusions and future work

8.1 Conclusions

This dissertation presents a study about self-referencing surface-plasmon resonance sensors. In order to solve the basic problem of the traditional single-mode SPR sensor, i.e. its inability to distinguish the surface interaction from the bulk reflective index change, we designed, simulated, optimized, fabricated and tested the multi-mode self-referencing SPR sensors (both dual- and tri-mode versions). The reflection spectra for multi-mode self-referencing SPR sensor designs were generated using the multilayer transmission matrix and Fresnel multilayer reflection theory.

Dual-mode SPR sensors use the long- and short-range surface plasmon modes to differentiate surface binding from bulk effects. Both long- and short-range surface-plasmon modes can be simultaneously excited at the same location and incident angle but different wavelengths in spectral interrogation. Both surface plasmon modes can also be simultaneously excited using single-wavelength angular interrogation. Angularly interrogated dual-mode SPR sensors reduce cross-sensitivity by at least a factor of 3.6 compared to optimized wavelength-interrogated sensors.

The optimum dual-mode sensor design for wavelength interrogation consisted of a 400 nm thick Teflon layer with a 52 nm thick gold layer for lowest surface limit of detection. For angular interrogation the optimal design consisted of a 500 nm thick Teflon layer with 60 nm thick gold layer with incident light of 800 nm wavelength. When
properly optimized, dual-mode SPR sensors can be competitive with traditional SPR sensors in terms of sensitivity and LOD for surface effects while compensating for bulk index changes.

Three surface plasmon resonance modes can be excited at one observation point at a single incident angle on our wavelength interrogated tri-mode SPR sensor. The three modes respond differently to the bulk index change, non-specific binding and specific binding. Tri-mode self-referencing SPR sensor can successfully differentiate specific binding from bulk index changes and non-specific binding effects.

Noise became a significant factor in sensing experiments and data analysis. There are many factors affecting the sensing noise for a tri-mode sensor design. Three-thin-layer deposition process cannot perfectly match the simulation parameters. The roughness, surface energy and chemistry and the physical structure are unpredictable especially for SiO$_2$ strips patterned by photolithography after wet etching. We built up an ideal linear sensing model for our tri-mode sensor; however, there is always nonlinear response for a real sensing test which increases the difficulty of differentiating specific-binding from the other two effects.

8.2 Future work

To reduce the noise in sensing experiments of a tri-mode sensor, we should do further study on both the sensor design and the sensing experiment design. Further measurements of the surface properties of the sensor should be conducted because the
sensor surface is a critical factor in sensing performance. For the original sensing test, we assumed the non-specific binding only occurred on SiO$_2$ strips whereas the specific binding only on gold surface. It could be possible that the non-specific binding happens on both surfaces, but with different binding affinities. Because non-specific binding will differ on gold and SiO$_2$ due to different surface energy and chemistry, we should design more sensing models for our tri-mode sensor to verify the sensing ability and increase the sensing performance.
Reference

19. Nomenclature and definitions for use in NRSCL and other NCCLS documents. NRSCL-8-P2. 13(1).
35. Dostalek, J. and J. Homola, Surface plasmon resonance sensor based on an array of diffraction gratings for highly parallelized observation of biomolecular


VITA

Jing Guo was born in Huangshi city, Hubei Provence, China. She received her Bachelor of Science degree in Physics from Wuhan University in 2003. She was accepted as a doctoral student in Physics and Astronomy in the fall of 2004. She transferred to Electrical and Computer Engineering in the spring of 2006. She is currently completing her doctoral degree in the laboratory of Dr. J T. Hastings.