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**INVESTIGATIONS INTO OPTICAL BIOSENSORS IN
VISIBLE-NIR WAVELENGTH BAND FOR
DISEASE DETECTION APPLICATIONS**

**Pae Jian Yi
School of Mechanical and Aerospace Engineering
2021**

**INVESTIGATIONS INTO OPTICAL BIOSENSORS IN
VISIBLE-NIR WAVELENGTH BAND FOR
DISEASE DETECTION APPLICATIONS**

Pae Jian Yi

School of Mechanical and Aerospace Engineering

A thesis submitted to the Nanyang Technological University
in partial fulfilment of the requirement for the degree of
Doctor of Philosophy

2021

STATEMENT OF ORIGINALITY

I hereby certify that the work embodied in this thesis is the result of original research, is free of plagiarised materials, and has not been submitted for a higher degree to any other University or Institution.

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PAE Jian Yi

SUPERVISOR DECLARATION STATEMENT

I have reviewed the content and presentation style of this thesis and declare it is free of plagiarism and of sufficient grammatical clarity to be examined. To the best of my knowledge, the research and writing are those of the candidate except as acknowledged in the Author Attribution Statement. I confirm that the investigations were conducted in accord with the ethics policies and integrity standards of Nanyang Technological University and that the research data are presented honestly and without prejudice.

11 March 2021

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Date

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Murukeshan Vadakke Matham

AUTHORSHIP ATTRIBUTION STATEMENT

This thesis contains material from **9** paper(s) published in the following peer-reviewed journals / from papers accepted at conferences in which I am listed as an author.

Part of Chapter 2 is published as **Jian Yi Pae** and Murukeshan Vadakke Matham, "A review of interferometric techniques with possible improvement in pattern resolution using near-field patterning," *Proc. SPIE 10449, Fifth International Conference on Optical and Photonics Engineering (icOPEN2017)*, 104491B (2017). DOI: 10.1117/12.2270638

The contributions of the co-authors are as follows:

- A/P Murukeshan provided the initial research direction and edited the poster drafts.
- I conducted the study, prepared the figures and poster drafts in the Centre for Optical and Laser Engineering (COLE).
- I also presented the poster presentation during the conference.

Part of Chapter 3 is published as **Jian Yi Pae**, Pankaj Kumar Sahoo and Murukeshan Vadakke Matham, "Grating-Coupled Plasmonic Sensor for Sucrose Sensing Fabricated Using Optical Fiber-Based Interference Lithography (OFIL) System," *IEEE Sensors Journal* **19**(22), 10477 (2019). DOI: 10.1109/JSEN.2019.2933668

The contributions of the co-authors are as follows:

- A/P Murukeshan was the principal investigator, gave research directions and edited the manuscript drafts.
- I prepared the manuscript drafts and all the figures used. All the other authors revised and corrected the manuscript before submission.

-
- I along with Dr. Pankaj and A/P Murukeshan conceived the initial idea.
 - I co-designed the experiment together with Dr. Pankaj. We set up the optical fibre-based interference lithography (OFIL) system in the Micromachines Laboratory (MML).
 - I further optimized the system to improve the fabricated structures and characterized them using the scanning electron microscope (SEM). I also developed the fabricated structures into a sucrose sensor for experimental validation in COLE and analysed the experimental data.

Part of Chapter 3 and Chapter 6 is published as Pankaj Kumar Sahoo, **Jian Yi Pae** and Murukeshan Vadakke Matham, "Enhanced absorption in a graphene embedded 1D guided-mode-resonance structure without back-reflector and interferometrically written gratings," *Optics Letters* **44**(15), 3661 (2019). DOI: 10.1364/OL.44.003661

The contributions of the co-authors are as follows:

- A/P Murukeshan was the principal investigator, gave research directions and corrected the manuscript drafts.
- I, along with Dr. Pankaj and A/P Murukeshan developed the research idea presented in this paper.
- Dr. Pankaj and I jointly prepared the manuscript drafts. I also provided the high-resolution schematic of the experiment setup.
- Dr. Pankaj did the theoretical simulations and I contribute to the design optimisation of the structure.
- I co-designed the experiment together with Dr. Pankaj and we developed the experimental setup in COLE. We also analysed the data.

-
- I fabricated the grating structure in MML and coordinated with the vendors to complete part of the structure necessary for the experiments. I also conducted the characterization of the sample, such as Raman spectroscopy, confocal microscopy and atomic force microscopy (AFM).

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The contributions of the co-authors are as follows:

- Prof. Rajdeep and A/P Murukeshan gave research directions and corrected the poster drafts.
- I prepared all the figures for the poster. All the other authors revised and edited the poster.
- I, along with Dr. Pankaj and Dr. Rohit, co-designed the experiment and performed all the experimental work at COLE.

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The contributions of the co-authors are as follows:

- Prof Balázs and A/P Murukeshan gave research directions and corrected the manuscript drafts.

-
- I, along with Dr. Radhika and A/P Murukeshan developed the initial idea with inputs from Dr. Parasuraman.
 - Dr. Radhika and I also prepared the manuscript drafts and conducted the theoretical simulations to support the work.
 - I prepared the high-resolution schematic of the experiment setup including the image for the back cover of the journal.
 - I co-designed the experiments together with Dr. Radhika and we developed the experimental setup in COLE. We also analysed the data.
 - Together with Dr. Radhika, I did characterisation of the nanoparticles using transmission electron microscopy (TEM) and imaged the fibrillation of beta amyloid using field emission scanning electron microscope (FESEM) at the Facility for Analysis Characterisation Testing and Simulation (FACTS).

Part of Chapter 5 is published as **Jian Yi Pae**, Rohit Medwal, Radhika Nair, Avinash Chaurasiya, Marco Battiato, Rajdeep Singh Rawat and Murukeshan Vadakke Matham, "Electro-ionic control of surface plasmons in graphene-layered heterostructures," *Nano Letters* **20**(11), 08305 (2020). DOI: 10.1021/acs.nanolett.0c03471

The contributions of the co-authors are as follows:

- I, along with Dr. Rohit, developed the research along with inputs from the project leads, A/P Murukeshan and Prof. Rajdeep.
- I prepared the drafts of the manuscript including all the figures. The manuscript was first edited by Dr. Rohit and Dr. Radhika.
- The manuscript draft was corrected and edited by Asst/P Marco, A/P Murukeshan and Prof. Rajdeep.

-
- Dr. Rohit and I co-designed the experiment, and we conducted the measurement in COLE.
 - Mr. Avinash synthesised the ion-gel at the Plasma Engineering and Applied Research Lab (PEARL).
 - Dr. Radhika provided the theoretical formulation and Asst/P Marco provided the theoretical model to describe the proposed device.

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The contributions of the co-authors are as follows:

- I, along with Dr. Rohit, developed the research along with inputs from the project leads, A/P Murukeshan and Prof. Rajdeep.
- I prepared the drafts of the manuscript including all the figures. The manuscript was first edited by Dr. Rohit and Dr. Joseph.
- The manuscript draft was corrected and edited by A/P Murukeshan and Prof. Rajdeep.
- Dr. Rohit and I co-designed the experiment and synthesised graphene at the Plasma Engineering and Applied Research Lab (PEARL).
- I conducted the sensing study in PEARL and also analysed the substrate using Raman spectroscopy at the School of Mechanical and Aerospace Engineering (MAE).
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The contributions of the co-authors are as follows:

- All authors corrected the poster manuscript.
- I developed the initial idea for the work under the direction of A/P Murukeshan.
- I designed the study with assistance from Dr. Rohit and performed all the experimental work at PEARL. I also characterized the substrate using SEM and analysed all the experimental data.
- I prepared the poster drafts including all the figures. The poster was also corrected by Dr. Rohit.

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- I, along with Dr. Rohit, developed the research along with inputs from Dr Radhika.
- The project leads, A/P Murukeshan and Prof. Rajdeep provided the direction of the research.
- Avinash assisted in preparing the ion-gel substrate used in the experiment.
- All authors corrected the paper.
- I prepared the presentation slides and presented the paper orally at the conference.

Apart from the ones listed above, the thesis also contains materials from 1 paper that is under review.

11 March 2021

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Date

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PAE Jian Yi

ABSTRACT

In the latest published results from Global Burden of Disease (GBD) in 2017, the top two contributors to the disease burden which requires the most urgent attention are Alzheimer's Disease (AD) and female breast cancer. As AD is a neurological disorder with unknown causes, it is almost impossible to adopt any preventive measures. Additionally, current medical tools are unable to reverse the effects of AD. Hence, the best approach is to detect the early onset of AD so that measures can be implemented early to halt or slow down its progress. However, existing techniques for the early detection of AD primarily focus on using neuroimaging which may be too expensive to conduct regularly. Therefore, there is an urgent need for a low-cost method for the early detection of AD. In the case of female breast cancer, despite being the most diagnosed form of cancer, the data also have shown that it has very promising survival rates when the cancer is detected and treated in the early stages. However, the effectiveness of existing breast cancer detection techniques is hugely limited by the low uptake of the screening program. This could be due to the lack of awareness or misconception regarding breast cancer and due to the perceived hassle of having to go for regular screening at periodic intervals. Hence, there is a need to explore alternative breast cancer screening methods that are more hassle-free.

A biosensor is a promising candidate in this context, which could provide a low-cost and hassle-free approach for the screening of diseases such as AD and female breast cancer. Electrochemical biosensors are well-established and have already been commercialised such as blood glucose monitors. However, such biosensors typically have low sensitivity which could not be used to accurately diagnose diseases. With the recent improvement

in the optics field, optical biosensors are also gaining popularity as they have high sensitivity and a low detection limit. Novel optical biosensors based on the surface plasmon resonance (SPR) effect are even able to achieve label-free sensing by using specially bioengineering bioreceptors with high specificity towards the targeted analytes. One critical limitation is that current fabrication methods are unable to mass produce the nanoscale features which are necessary for the development of compact optical biosensors. Therefore, part of the objectives of this thesis would be the investigations into potential fabrication techniques for the low-cost fabrication of the nanoscale features necessary for forming the optical biosensors. Another research objective is to improve the sensitivity of these optical biosensors so that they will be comparable to conventional laboratory-based detection techniques for high accuracy. Lastly, this thesis would also be investigating the potential of using graphene, which is a 2D material, to develop a novel optical biosensor that can be interrogated electrically and optically.

Based on the extensive literature review, interference lithography (IL) was selected as a cost-effective method for producing the nanoscale grating features necessary to form the grating coupled SPR biosensor. IL also has the flexibility of producing different periodic features to suit different biosensor designs. The interference of light waves was first validated using theoretical formulation and further confirmed using theoretical simulations based on the finite-difference time-domain method. Next, two different IL configurations were experimentally validated; the first one was based on a specially fabricated phase grating and the other one was based on a custom fabricated single-input multiple-output (SIMO) optical fibre splitter. The major advantage of optical fibre interference lithography (OFIL) is that there is no need to realign the beams when the angle of incidence was varied. This was successfully demonstrated as nanoscale grating

with a range of periodicity from 514 nm to 1,646 nm was easily fabricated by simply adjusting the angle of incidence. The nanoscale grating features were then further developed into a grating coupled SPR biosensor for the sensing of glucose concentration in a sample fluid. The operating range of the biosensor is in the spectral range from around 600 nm to approximately 800 nm which is in the visible – near-infrared (NIR) range. The benefit of this is that the optical sensors in the wavelength are more economical and easier to integrate with conventional optical components.

Additionally, nanoparticles were demonstrated as a potential approach to amplify the signal of SPR biosensors. Theoretical simulations showed that the amplification or field enhancement factors of spiky shaped nano-urchins are several orders of magnitude better than in conventional nano-spheres shaped nanoparticles. This was experimentally validated in a gold (Au) nano-urchins based localised surface plasmon resonance (LSPR) biosensors for the sensing of beta-amyloid fibrillation which is an essential biomarker for the early detection of AD. The Au nano-urchins were then added to the surface of a prism coupled SPR biosensor for the sensing of estrogen receptor alpha ($ER\alpha$) which is a useful biomarker for $ER\alpha$ -positive breast cancer.

Lastly, different methods were also investigated for the synthesis of high-quality monolayer graphene. Raman spectroscopy was used to characterise the synthesised graphene which confirms the quality of the synthesised graphene. The transfer of graphene from the growth substrate to the desired substrates was also successfully demonstrated. This allows the development of a graphene-layered heterostructure which was further developed into an electrically controlled SPR biosensor.

It is envisioned that the output of this research can contribute to the efforts to improve the current process for disease diagnostics, especially for AD and female breast cancer.

The primary advantage is that this method can allow patients to conduct the test at their convenience. This may greatly increase the uptake and screening frequency to ensure the diseases are detected early which would greatly reduce their risks. Additionally, the low-cost fabrication methods for producing the biosensors could also alleviate some of the financial burdens of existing disease screening techniques and improve their effectiveness for the detection of diseases. Lastly, additive manufacturing (AM) methods could also be integrated with the IL system to develop a hybrid manufacturing method where the benefits of both techniques can be exploited. This will form part of the future research work to extend this thesis.

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LIST OF SYMBOLS

Symbol	Description
α	Acceptance angle
θ_i	Incidence angle of interfering beams
θ_{mi}	Incidence angle (with respect to normal axis of mirror surface)
θ_{mr}	Reflectance angle (with respect to normal axis of mirror surface)
θ_{tilt}	Angle of mirror rotation (tilt)
m	Diffraction order
$\mathbf{E}$	Electric field (vector form)
$\mathbf{H}$	Magnetic field (vector form)
I	Intensity of light
I_{max}	Maximum intensity value
I_{min}	Minimum intensity value
c	Speed of light in vacuum
A	Absorbance
R	Reflectance
T	Transmittance
Γ	Amplitude reflection coefficient
T	Amplitude transmission coefficient
R	Off-resonance (Fresnel) amplitude reflection coefficient
t	Off-resonance (Fresnel) amplitude transmission coefficient
γ	External leakage rate
δ	Intrinsic loss rate
ϵ	Dielectric constant
Λ	Periodicity
n	Refractive index
$\mathbf{k}$	Wave vector
λ	Wavelength
λ_0	Centre wavelength of light source
$\Delta\lambda$	Spectral bandwidth of light source
x	x-spatial coordinate
y	y-spatial coordinate
z	z-spatial coordinate
Δz	Height difference in the z-spatial coordinate
J	Surface charge density
σ	Conductivity
U	Electric field generated due to an oscillating electric field
h	Width of the positive layer

LIST OF ABBREVIATIONS

Abbreviation	Explanation
1D	One dimensional
2D	Two dimensional
3D	Three dimensional
$A\beta$	Beta-amyloid
AD	Alzheimer's disease
AM	Additive manufacturing
ASR	Age-standardised incidence rates
ASRS	Age-standardised relative survival
ATR	Attenuated total reflection
Bio-MEMS	Bio micro-electro-mechanical system
CCD	Charge-coupled device
CVD	Chemical vapour deposition
DALYs	Disability-adjusted life years
DI water	Deionised water
DLW	Direct laser writing
EIU	Economist Intelligence Unit
$ER\alpha$	Estrogen receptor alpha
EW	Evanescent waves
FDTD	Finite-difference time-domain
FLG	Few-layer-graphene
FWHM	Full width at half-maximum
γ -ray	Gamma ray
GBD	Global Burden of Disease
GMR	Guided-mode resonance
HBMS	Health and Biomedical Sciences
HER-2	Human epidermal growth factor receptor 2
HMDS	Hexamethyldisilane
IC	Integrated circuit
IHD	Ischemic heart diseases
IL	Interference lithography
IMHE	Institute for Health Metrics and Evaluation
IPA	Isopropanol alcohol
IR	Infrared
LSPR	Localized surface plasmon resonance
MPA	Multi-photon absorption
MRI	Magnetic resonance imaging
MUC1	Mucin1

NIR	Near-infrared
NIRF	Near-infrared fluorescence
OFIL	Optical-fibre based interference lithography
OPD	Optical path difference
PDMS	Polydimethylsiloxane
PECVD	Plasma-enhanced chemical vapour deposition
PET	Polyethene terephthalate
PM fibre	Polarization-maintaining fibre
RET	Resolution enhancement techniques
RF	Radio-frequency
RI	Refractive index
RIE2020	Research, Innovation and Enterprise 2020 Plan
RIU	Refractive index unit
SCCM	Standard Cubic Centimetres per Minute
SEM	Scanning electron microscopy
FESEM	Field emission scanning electron microscopy
SLA	Stereolithography apparatus
SP	Surface plasmons
SPR	Surface plasmon resonance
SPECT	Single-photon emission computed tomography
TAA	Tumour-associated antigens
TE	Transverse electric
TM	Transverse magnetic
ThT	Thioflavin T
TPA	Two-photon absorption
UV	Ultraviolet
WHO	World Health Organization
YLDs	Years lived with disability
YLLs	Years of life lost

CHAPTER 1

INTRODUCTION

The first chapter begins with the background motivation of the thesis. The existing health strategies are reviewed to identify the limitations in the current state of the art followed by highlighting the main objectives and scope of the thesis. This chapter concludes with the research roadmap conceived for meeting the thesis objectives and a summary on the organization of the whole thesis is given at the end.

1.1 Motivation

The global life expectancy at birth has increased by 20 years over the past 50 years as shown in Figure 1.1. This could be attributed primarily to the improvement in general health conditions all over the world [1]. As countries developed, sanitary conditions are upgraded, and medical care becomes better and more easily accessible [2].

Figure 1.1: Global life expectancy at birth from 1960-2017 (data source [3])

However, even with improved life expectancy, many people still die or fall into ill health before reaching their full life expectancy [4]. Therefore, this loss of human potential is a

huge global problem and an international consortium of researchers have come together on the Global Burden of Disease (GBD) project to collect data on the different types of diseases, injuries, and risk factors that could potentially lead to premature death or disability [5]. These data are analysed by the Institute for Health Metrics and Evaluation (IHME) which is an independent global health research centre at the University of Washington and their results are published annually.

This trend is also consistent in Singapore where the life expectancy has increased by 20.3 years from 62.9 years in 1960 to 83.2 years in 2018 [6]. Due to Singapore's rapidly ageing population, the disease burden would inevitably have a much more significant impact as compared to other countries [7]. In this context, Singapore's government has developed a science and technology plan for five years (2016-2020); the Research, Innovation and Enterprise 2020 Plan (RIE2020), where a total of S\$19 billion will be invested to address Singapore's future national challenges [8]. Out of the four strategic technology domains, the largest proportion (S\$4 billion) of this budget has been allocated for research and development in Health and Biomedical Sciences (HBMS). This is targeted to help to improve Singapore's disease burden.

According to the latest findings from the GBD 2017 report, the top three most fatal diseases are – (1) mental & neurological disorders, (2) cardiovascular diseases, and (3) cancers [9]. These three diseases have also been identified in part of the five therapeutic areas recognised in the HBMS domain of RIE2020. This emphasises the urgent need to target these three diseases for Singapore to overcome the challenge of its disease burden.

Among the three top diseases, it is observed that the cardiovascular disease's burden has been declining over the years. On the other hand, the burden of cancer and mental & neurological disorders have experienced rapid growth. Out the so many different types

of cancer, female breast cancer is the most commonly diagnosed type of cancer and is the deadliest among women making it a key contributor to the disease burden [10]. Additionally, the greatest contributor to the mental and neurological disorder's burden is dementia which has been declared by the World Health Organization (WHO) as a public health priority and most dementia cases are caused by Alzheimer's disease (AD). Therefore, there is an urgent need to improve existing medical and health technologies to reduce the risk of these diseases. In this context, this thesis will be targeting female breast cancer and AD which are two of the deadliest diseases.

1.2 Limitations of Current Health Strategies to Manage Diseases

There are many health strategies that governments or policymakers can adopt to reduce the fatality of the risks associated with diseases. They can be broadly classified into three main categories as detailed in Table 1.1. Each strategy has its strengths and shortcomings; certain strategies are more effective against some types of diseases. Therefore, each strategy will be assessed based on its effectiveness to reduce the risk of female breast cancer and AD.

Table 1.1: Different health strategies for managing diseases

Stage of Disease	Strategies	Examples
Pre-growth	Prevention	Health promotion, active lifestyle, personal hygiene, immunisations
Early – middle	Early detection	Health screening, vital signs monitoring, medical diagnosis
Early – end-of-life	Medical intervention	Therapy, treatment, medication

Among all these, preventive strategies are the most ideal as prevention is better than cure [11]. This can be achieved by educating the general public on the risk factors for the different diseases so they can adopt the necessary precautions such as immunisation to lower their risks [12]. A recent study has shown that the risk of stroke and coronary heart

disease can be reduced by almost 70% simply by adopting a more active lifestyle and changing to a healthier diet [13]. However, preventive strategies are only effective in people who are highly motivated and proactive in maintaining their health. It may also not provide a hundred percent protection against diseases where the risk factors are not fully understood such as breast cancers and AD.

Since it is impossible to completely prevent all forms of diseases, the next better strategy is to detect and treat them early. This could be done through self-diagnosis where patients themselves monitor their vital signs and symptoms or a clinical diagnosis where medically trained experts employ advanced medical tools to reveal any diseases. However, it is often challenging to pinpoint the actual disease due to the overlapping signs and symptoms. For example, in diagnosing AD, doctors employ neuroimaging to measure the cognitive profile of the patient. However, it is difficult to differentiate between the early onset of AD from a normal patient undergoing healthy ageing which results in an inaccurate diagnosis [14]. Therefore, the effectiveness of early detection techniques is limited by their sensitivity towards early-stage diseases.

In the unfortunate case where the disease is diagnosed, the patients can then opt for medical intervention to cure it. With today's modern medical facilities, almost all diseases can be treated, however, the success rates are typically linked to the progression stage of the disease. Late-stage breast cancer or invasive breast cancer has very high chances of relapse making it almost impossible to completely eradicate the cancer [15]. As for AD, there is currently no known cure to effectively stop or reverse the damage, however, therapy can be employed to slow down its progress [16]. Hence, the effectiveness of existing medical intervention techniques is limited by the effectiveness of existing early detection strategies.

For diseases to be detected early, the patients must periodically go for medical screening to ensure any early signs or symptoms are captured. However, its effectiveness is limited by the patient's motivation to keep up with the routine and the hassle of having to make frequent visits to the clinic. This would lead to low uptake and delay the process of disease detection [17]. By the time the disease is detected, it would have already progressed to an advanced stage which would result in a lower chance of survival. Additionally, going for periodic testing would inevitably generate an extra financial burden which may also deter some people from persisting with the recommended screening schedule [18]. There is also the fear of overdiagnosis where patients with non-life threatening diseases are sent for unnecessary tests [19]. Hence, there is an urgent need to improve current disease detection techniques to ensure diseases are diagnosed at an early stage.

A potential method is to use biosensors to complement conventional clinical diagnostic techniques. Biosensors are analytical equipment that could be used to detect specific biomolecules or analytes from a sample fluid. This is achieved by using a specially bioengineered bioreceptor that binds specifically to the analyte. In recent years, biosensors have found commercial success, such as the development of blood glucose monitors by using glucose oxidase to measure blood glucose levels [20]. Biosensors could also be used to detect disease biomarkers which can be used to diagnose certain diseases or provide an indication of their progression [21]. Hence, there is an increasing interest in biosensors and it is projected that the global biosensors market will break US\$ 33.76 billion by 2026 [22].

With the improvement in bio micro-electro-mechanical system (bio-MEMS) research, the size of biosensors has shrunk significantly over the years [23]. In recent literature, modern optical biosensors can integrate full-scale conventional laboratory processes or

tests into a single compact chip with even better sensitivity and detection limits than electrochemical biosensors. However, a critical limitation is that the fabrication techniques for producing the biosensors are too slow and not economical [24]. Furthermore, as compared to the existing well-established laboratory-based techniques, the accuracy of biosensors is still lacking for disease diagnosis. Hence, these form the untapped domains to explore for achieving the desired research targets. Another research objective would be to develop an electrically controlled biosensor by exploiting the unique electrical property of 2D materials such as graphene. The electrically controlled biosensor would combine the high sensitivity of optical biosensors with the relatively low cost and ease of use of electrical biosensors.

With the development of integrated optical devices, it is envisioned that such biosensors would allow point-of-care diagnosis of diseases where people can monitor themselves regularly without having to frequently visit the clinic. The routine screening of disease would reduce the delay for seeking treatment and greatly improve the chances of recovery.

1.3 Main Objectives

The main objectives of this research are derived based on the outcome of the literature review and the identified research gaps. They are summarized as follows:

- a) Conceptualization and development of a flexible nanofabrication technique for fabricating features ranging from 500 nm – 1.5 μ m for biosensing applications (theoretical and experimental). The targeted spectral response of the optical biosensor is expected to be in the wavelength band from 400 nm – 1000 nm (visible – near-infrared).

-
- b) Research into the design and development of an optical biosensor for disease detection with possible enhancement to its sensitivity using gold nano-urchins. The targeted applications are for the detection of Estrogen Receptor-Alpha ($ER\alpha$) positive female breast cancer and early-stage AD detection.
 - c) Investigations into electro-ionic control of surface plasmons in a graphene-layered heterostructure for the development of a sensitive plasmonic device (theoretical analysis and experimental).

1.4 Scope

The different research tasks to be carried out to meet the above-mentioned research objectives are given below based on the identified research gaps:

- a) Flexible nano-fabrication technique
 - i. Theoretical formulation and simulation to confirm the formation of the interference pattern from counter-propagating light waves
 - ii. Investigations into grating-based interference lithography (IL) system
 - iii. Conceptualization and development of a highly reconfigurable optical-fibre based IL (OFIL) system
 - iv. Experimental validation and characterisation of the gratings feature fabricated using the OFIL system
 - v. Realisation of a grating coupled SPR biosensor using the grating features fabricated by the OFIL system
- b) Optical biosensor for disease detection
 - i. Design and development of a fully automated system for the surface plasmon resonance (SPR) sensor

-
- ii. Theoretical simulation and experimental validation of a prism coupled SPR for comparison with a grating coupled SPR sensor
 - iii. Investigation into the SPR signal enhancement using gold nano-urchins as a localized SPR (LSPR) sensor for AD detection
 - iv. Investigation into a gold nano-urchins enhanced SPR sensor for female breast cancer detection
- c) Graphene layered heterostructure
- i. Development and parametric studies of a thermal chemical vapour deposition (CVD) system for graphene synthesis
 - ii. Development and parametric studies of a plasma-enhanced CVD (PECVD) system for reduced temperature graphene synthesis
 - iii. Realization of a graphene-layered heterostructure by transferring the graphene from the growth substrate to the desired substrate
 - iv. Theoretical formulation of a graphene layered heterostructure model to explain the electrical control of the surface plasmons
 - v. Development of an electro-ionic controlled SPR device as a multi-level optical switch

Figure 1.2 summaries the above-mentioned main objectives and various research tasks into a research roadmap.

Figure 1.2: Research roadmap

1.5 Organization of the Thesis

This thesis is divided into six chapters. Each chapter begins with a short description of the primary contents of that chapter.

This chapter introduces the motivation for this thesis and the limitations of current health strategies for managing female breast cancer and AD was also discussed. Based on this, the research gaps were identified which forms the main objectives and scope of this thesis. A research roadmap was also presented to summarise the research tasks and the main objectives of this thesis.

Chapter 2 gives in an in-depth review of the state-of-the-art literature in the related research fields and is divided into three sections. The first section is a detailed introduction of the global disease and highlights the few most critical diseases which pose

the greatest challenge for Singapore's future. Additionally, existing techniques for the early detection of these diseases will also be reviewed to identify the main research gaps of this thesis. The second section introduces the potential of using biosensors as a promising method for the early detection of diseases. Various reported methods for improving the sensitivity of biosensors will also be discussed. The last section reviews the limitations of existing techniques for the fabrication of compact and low-cost biosensors. On top of that, the significance of graphene as a potential substrate to realise a novel electrically controlled biosensor is also discussed. The chapter then concludes with a summary of the outcome of the literature review which highlights the important research gaps that are identified which led to the main objectives of this thesis.

In **Chapter 3**, the potential of using optical interference lithography (IL) for the fabrication of nanoscale grating features which could be used in a compact grating coupled surface plasmon resonance (SPR) biosensor. In this chapter, the phenomenon of light wave interference is first studied by theoretical formulation and simulation to better understand the critical parameters and their effects on the quality of the features fabricated by the IL. Next, two different types of IL systems based on a diffraction grating and single-input multiple-output (SIMO) optical fibre splitter are experimentally validated. The nanoscale grating features were then developed into a grating coupled SPR biosensor to demonstrate its potential as an economical method for the fabrication of compact biosensors.

Chapter 4 introduces the potential of using nanomaterial to amplify the signal in an SPR biosensor. First, a conventional prism coupled SPR biosensor was studied using theoretical simulation and experimental validations. A pair of highly precise rotation stage was employed to ensure good repeatability and high accuracy in the angular

interrogation mode. The next section compares different geometries of nanomaterials and the effect on amplifying the SPR signal when coupled to a biosensor. For demonstration, the optimised nanomaterial design was developed into an LSPR biosensor to detect beta-amyloid ($A\beta$) which is an AD biomarker. Furthermore, the nanomaterial was also integrated into a nanomaterial enhanced SPR biosensor to detect estrogen receptor alpha ($ER\alpha$) which is a common female breast cancer marker. These demonstrate the potential of using nanomaterial to enhance the signal in an SPR biosensor and the possibility of using such biosensors to detect disease markers.

In **Chapter 5**, the potential of using a graphene layered heterostructure was investigated for the development of an electro-ionic controlled SPR biosensor. The first section of this chapter discusses the advantages and disadvantages of two different approaches of synthesising high-quality monolayer graphene to improve the sensitivity of biosensors. This is followed by the steps needed to transfer the graphene onto the desired substrate to realise the graphene layered heterointerface structure. The last section demonstrates the potential of using the graphene layered heterointerface structure in an SPR biosensor which is electrically controlled.

The thesis concludes with **Chapter 6** where the major contributions of the research work are highlighted. This is followed by a section, giving recommendations for future work directions.

CHAPTER 2

LITERATURE REVIEW

*This chapter is an in-depth review of the state-of-the-art literature in relation to the main research objectives. It is classified into three distinct sections to better present the different key research gaps to be addressed by the thesis. **Section A** introduces the global disease burden and the few critical diseases which pose the biggest challenge for Singapore. In this section, the significance and limitations of existing techniques for the early detection of diseases will also be highlighted. **Section B** discusses the potential of using biosensors as an alternative method for the early detection of diseases. The advantages and methods to improve the sensitivity of various state-of-the-art biosensors are also discussed. **Section C** reviews the limitations of existing techniques for the fabrication of compact biosensors. Following this, graphene is also introduced as a potential material to realise a novel form of biosensing configuration. The chapter concludes with an outcome of the literature review which highlights the important research gaps to be fulfilled by this research.*

SECTION A

2.1 Global Burden of Disease

Diseases are negative conditions that weaken the health of people. In most cases, if left untreated, the disease may cause irreversible damage to the body which, in certain scenarios, could even result in death. Therefore, the affected people are not able to live until their fullest potential due to the premature deaths and disabilities caused by diseases. The loss of human potential is of great significance as it creates an additional burden on the economy and has negative social impacts such as depression due to the

passing of loved ones or anxiety caused by the fear of contracting diseases [25]. Therefore, researchers all over the world have come together under the Global Burden of Disease (GBD) project to quantify the overall global disease burden and their associated risk factors [26]. The data is also an indication of the gap between the current health standards and the ideal disease-free health standards. Managing the disease burden in Singapore is an even greater challenge due to its ageing population [27]. Figure 2.1 shows the distribution of Singapore residents by age group which shows that the majority are above the age of 40. Therefore, Singapore's disease burden would be expected to worsen as older people are more prone to diseases and disabilities.

Figure 2.1: Total number of Singapore residents by age group in June 2019 (data source [27])

In this context, Singapore's Ministry of Health has been working in collaboration with the Institute for Health Metrics and Evaluation (IHME) to produce a comprehensive and detailed report on Singapore's disease burden. According to the report, the three greatest contributors are – (1) mental and neurological disorders (16.8%), (2) cardiovascular diseases (14.2%), and (3) cancers (13.3%) (refer to Appendix A for the data). These diseases might not necessarily cause the most deaths, but instead, result in the most significant loss of human potential. This is calculated by combining both the number of years of life lost (YLLs) due to premature death and the number of years lived with

disability (YLDs) because of the disease. The resultant metric is the disability-adjusted life years (DALYs) which is a much more accurate estimation of the overall public health standard as it accounts for both fatal diseases and diseases that cause disabilities. The following sections discuss in detail the three top-ranked diseases and the effectiveness of current health strategies in reducing their burdens.

2.1.1 Cancer

According to the latest available data consolidated by the International Agency for Research on Cancer, World Health Organization (WHO), there are more than 18 million new cancer cases and about 9.6 million cancer-related deaths in 2018. In total, it is estimated that 43.8 million people are still being inflicted by cancer that is diagnosed within the last five years [28]. Currently, the three most common types of cancer in the world are – female breast cancer (15.7%), non-melanoma skin cancer (11.9%), and prostate cancer (8.5%) (refer to Appendix B for the data). Also, female breast cancer has the highest estimated age-standardised incidence rates (ASR) of 46.3 per 100,000 as shown in Figure 2.2.

Figure 2.2: Estimated global ASR for the 5 most common cancer types in 2018 (data source [29])

The reason cancer is especially challenging to manage is due to the mechanism behind its growth. Figure 2.3 is a graphical representation of the life cycle of a cancer cell. The

beginnings of cancer are typically linked to a mutated cell that has damaged deoxyribonucleic acid (DNA) [30]. Under the normal protocol, the mutated cells would automatically self-destruct or be removed by the immune system. However, sometimes, the mutated cells managed to avoid death which allows them to reproduce uncontrollably. The mutated cells then slowly accumulate to form a mass of tissue commonly called a lump, growth, or tumour. If the growth remains aggressive and starts to invade neighbouring tissue, it would be classified as malignant tumours or cancerous [31]. Late-stage invasive cancer is diagnosed when the cancerous cells broke away from the original tumour and are transported to other parts of the body through the blood circulatory system or the lymphatic system [32].

Figure 2.3: Life cycle of a cancer cell (adapted from [33])

The root cause for the DNA of healthy cells to become damaged is still unexplained and cancer can grow from any parts of the body [34]. Hence, preventative strategies are not as effective as it is very challenging to develop a vaccine to prevent cancer from developing [35]. Due to the aggressive nature of cancer, it is best to detect and treat it before it can metastasize. Therefore, early detection would be a better approach than medical intervention. However, current cancer detection techniques are unable to pinpoint the exact location where cancer initiates as it produces no visible symptoms during the early stages [36]. The signs would only show when the mass had grown into a

significant size and affect the normal function of other organs. By then, it would be racing against time to remove the cancerous tumour before it can metastasize. Therefore, there is an urgent need to improve current cancer detection techniques to ensure high survival rates.

2.1.1.1 Female Breast Cancer

The female breast is an intricate organ which undergoes many changes as the body grows and ages. It mainly consists of three major parts, namely – fatty tissue, lobes, and ducts as shown in Figure 2.4. The fatty tissue extends out from the chest wall and supports the lobes. Milk is produced in the lobes and delivered via the ducts to the nipple [37]. Besides, lymph nodes are also embedded within the adipose tissue of the breast which is connected by a network of lymph vessels forming part of the lymph system [38].

Figure 2.4: Anatomy of the female breast (with permission from [39])

Female breast cancer originates from cells in the breast and can be further classified according to the specific site. The most common form of female breast cancer begins from

the cells in the ducts and are called ductal carcinoma [40]. Less common seen is lobular carcinoma which starts from cells in the lobes [41]. Occasionally, inflammatory breast cancer is also diagnosed which is a very aggressive form of breast cancer that blocks the lymph nodes in the skin and causes swelling [42]. Other rare forms of female breast cancers include breast sarcomas which initiate from the fatty tissues and breast lymphomas which starts from the lymph vessels near the breast [43].

The risk factors associated with a higher probability of female breast cancer can be classified into environmental and genetic factors. Environmental factors are closely linked with a person's lifestyle and diet such as eating an unbalanced diet high in saturated fat [44]. Additionally, hormone replacement therapy drugs would also increase the occurrence of breast cancer and may even accelerate the growth of a tumour [45]. Genetic factors are the genetic makeup of a person which are inherited from the parents. Two specific genes have been identified to be closely related to a person's chance of developing breast cancer. They are named BRCA1 and BRCA2 which is an abbreviation for "breast cancer gene" [46]. These two genes do not cause breast cancer but are tumour suppressor genes which play a big role in preventing breast cancer. People with BRCA gene mutations have higher risks of developing breast cancer. Therefore, gene-panel sequencing can as a useful method to predict a person's risk of breast cancer [47].

Besides being the most prevalent form of cancer, inevitably, female breast cancer is also one of the most fatal forms of cancer. In 2018, breast cancer accounts for about 15% of the total cancer-related deaths among females (refer to Appendix B for the data) [48]. An even more worrying trend is that the global ASR for female breast cancer has been steadily increasing. This is especially true in the Asia-Pacific region where the ASR for female breast cancer in some countries is growing at eight times the increase in the global

average ASR [49]. In Singapore, female breast cancer had topped the table as the most frequently diagnosed cancer type among females since 1968 (refer to Appendix C for the data) [50]. Also, the ASR for female breast cancer in Singapore has been creeping up over the years as shown in Figure 2.5. It is now more than 2.5 times that of the second most frequent cancer type in females and is also fast approaching that of other developed countries [51,52]. With such a high incidence rate, it is unsurprising that female breast cancer is also the most fatal form of cancer among Singapore females which accounts for about 17.2% of cancer-related deaths in females in 2018 [53].

Figure 2.5: Age-standardised incidence rate, per 100,000 population, for female breast cancer by five-year period from 1968-2017 in Singapore (data source [51])

Despite such high mortality rates, the survival rates of female breast cancer patients show surprising promising results which are measured in terms of five-year age-standardised relative survival (ASRS) rates. The five-year ASRS rate is formulated based on the actual observed survival rate against the survival rate of a similar population group that is cancer free [54]. This would adjust for other competing causes of death such as accidents or old age. As shown in Figure 2.6 the five-year ASRS rates of female breast cancer patients in Singapore are 100.1%, 89.5%, 73.3%, and 27.0% for Stage I, Stage II, Stage III,

and Stage IV female breast cancer patients respectively. Therefore, detecting and treating female breast cancer early could be a more effective strategy than preventive measures to reduce the mortality rates.

Figure 2.6: Five-year age-standardised relative survival (ASRS) rate for different stages of female breast cancer from 2013-2017 in Singapore (adapted from [55])

In 2016, the Economist Intelligence Unit (EIU) published a white paper on “Breast cancer in Asia” which agree with this observation [56]. Preventive strategies against female breast cancer may not be effective due to it being a multi-factorial disease and its cause is still not yet fully understood. Hence, the report proposed two solutions to enhance current early detection strategies to mitigate female breast-cancer risk – (1) improve awareness about the risk of breast cancer, and (2) upgrade the current screening processes.

The major limitation of current breast cancer detection techniques is their low uptake. Reasons for this may be attributed to the general public’s perception of breast cancer and a lack of knowledge regarding the diagnostic procedure [57]. Other contributing factors include the worry of overdiagnosis which may inevitably lead to unnecessary expenses and could even discourage other females from participating in the breast cancer

screening program [58]. Therefore, improving the general public's awareness about the risk of breast cancer could be an effective way to reduce their sensitivity towards the risk of overdiagnosis. However, a recent study has shown that despite efforts by the health authorities to promote and encourage breast cancer screening, only about half of the female population have kept up with the recommended screening schedule [59]. Hence, this suggests that improving awareness may have limited effectiveness in motivating the general public to go for regular screening. Alternatively, existing techniques for breast cancer screening can also be reviewed for upgrading.

2.1.1.2 Early Detection Techniques for Female Breast Cancer

There are several well-established clinical diagnostic techniques in use currently for female breast cancer detection and there are summarised in Table 2.1.

Table 2.1: Comparison of different female breast cancer detection techniques

Detection techniques	Principle	Advantages	Disadvantages
Self-examination [60]	Visual inspection and physical touch	Private, in confidence, free	Generally untrained
Clinical examination [61]	Examination by trained medical professionals	More accurate prognosis than self-examination	Emotional barrier
Mammography [62,63]	X-ray imaging technique	Quick and relatively simple	False-positive
Magnetic resonance imaging (MRI) [64,65]	Proton density and relaxation time	Relatively more sensitive	Expensive
Sonography [66,67]	Ultrasound imaging	Relatively cheaper than MRI	Skill dependent

The cost of each technique varies according to its accuracy and skill requirement. Among existing methods of breast cancer detection, the most strongly advocated method is for women to regularly perform a breast self-examination [60]. This method is very simple and easy to be carried out in a private environment, but further tests would still be required by a medical professional to confirm any diagnosis. Alternatively, a medically trained professional can also conduct a clinical breast examination by visually looking at the breast and carefully feel for any abnormalities that could be symptoms of breast cancer [68]. However, patients may not be comfortable with being touched physically by others which forms an emotional barrier leading to a delay in the diagnosis. These two methods are usually not conclusive and would almost always require further testing by other methods to confirm the diagnosis.

Mammography is a non-invasive imaging technique where x-rays are used to inspect the tissue inside the breast [62]. The working principle behind x-ray imaging is based on the differential transmission efficiency of x-rays through different types of tissues to form a contrasting image. Cancerous cells are denser than the normal adipose tissue so they would absorb x-rays more efficiently, and have a relatively lower transmission rate which can then be identified by a bright white spot in the x-ray image as shown in the inset of Figure 2.7. The procedure is typically performed by having the patient position her breast on the mammography unit as illustrated in Figure 2.7. A transparent plate would then press on the breast to spread and even out the tissue, so a more uniformed image can be seen. Due to the relative ease in operating the equipment, mammography has remained highly popular with clinicians. However, the major limitation of mammography is that the image itself may be insufficient to differentiate a normal tumour from a malignant one which often results in overdiagnosis [63].

Figure 2.7: Illustration of a patient undergoing mammography (with permission from [64])

Breast MRI is a technique which employs a very strong magnetic field and radio frequency (RF) pulse to image the internal structure of the breast as shown in Figure 2.8. As the human body is composed of mostly water molecules, hydrogen atoms are abundant. Within the nuclei of these hydrogen atoms are protons which are sensitive to any magnetic field and are randomly orientated [65]. The MRI scanner would first apply a strong magnetic field around the patient, polarizing all the hydrogen atoms within her body in the direction of the field. An RF pulse is then generated at a frequency resonant to the hydrogen atoms which excites and causes them to rotate. When they return to the relaxed energy state, photons with energy corresponding to the energy difference are emitted and can be picked up as a magnetic flux [69]. These signals are measured by dedicated breast coils that encapsulates the breasts and the contrast between various types of tissues would be determined by their respective relaxation time [70]. The result

is a thin two-dimensional (2D) cross-sectional slice of the different tissues within the breast, as shown in the inset of Figure 2.8, the procedure can then be scanned repeatedly to produce different slices of the breasts to produce a detailed three-dimensional (3D) model [71]. The primary advantage of breast MRI has over other techniques is its sensitivity towards soft-tissues and could create images from any direction [72]. The major downside of breast MRI is the high cost which is partly due to the expensive equipment and the relatively low throughput due to the long processing duration.

Figure 2.8: Illustration of a patient undergoing breast MRI (with permission from [73])

Breast sonography is a painless and non-radiative method where high-frequency sound waves or ultrasound is transmitted into the breast. As the ultrasound waves are reflected from the tissue within the breast, a transducer picks up the reflected signal and process it to create a sonograph image of the internal structure of the breast [74]. Sonography is much sensitive to soft tissues as compared to mammography and is also relatively cheaper as compared to MRI [66,67]. However, the quality of the image is highly dependent on the skill of the operator, therefore, it is typically used in conjunction with other diagnostic techniques.

For breast cancer to be detected at an early stage, it is recommended for females to go for periodic breast screening once a year one to two years. Those with high risks are also advised to go for more regular screening [75]. However, the costs of the screenings would inevitably increase the financial burden of the patients [76]. Besides, all the techniques are conducted in a clinical setting and some people may perceive it as too much of a hassle to make frequent visits for the breast screenings [79].

Therefore, there is an urgent need an alternative breast cancer detection technique that is hassle-free, low cost and highly accurate. A biosensor is a potential candidate that has been gaining huge popularity in medical research for diagnostic applications [77]. It is envisioned that future biosensors for breast cancer screening can be made affordable for purchase off the shelves. This would allow everyone to have access to the kits for in situ testing for breast cancer as per their convenience. This should remove the emotional barrier and significantly improve the participation rate of breast cancer screening which would reduce the risk of delayed treatment and result in high survivability. However, the limitations of current state-of-the-art biosensors are the low sensitivity towards the biomarkers and high fabrication cost. These limitations would form part of the research gaps to be fulfilled by the thesis.

2.1.2 Mental and Neurological Disorders

In 2017, mental and neurological disorders collectively contributed about 9.33% to the over global disease burden, as shown in Figure 2.9. This figure has been steadily increasing since 1990 and it is projected to overtake cancer as the second most fatal cause of death after cardiovascular diseases by the next two decades [78]. Countries with an ageing population, such as Japan have already started experiencing the severe economic

impact of this where there is a need to increase its healthcare spending to maintain more hospitals and a rapidly shrinking labour force [79]. Singapore is no exception where the burden of mental and neurological disorder is estimated to be 16.8% of the disease burden in 2017. Additionally, the mental disorders and neurological disorders burdens had grown 70.9% and 104.6% respectively from 1990 to 2017 (refer to Appendix A for the data).

Figure 2.9: Contribution of mental and neurological disorders towards the global disease burden by their DALYs from 1990-2017 (data source [80])

Even though mental disorders and neurological disorders are classified separately in the GBD, there is little distinction between them as their symptoms typically overlap with each other. Neurological disorders, such as Alzheimer's disease, are defined as diseases that affect the nervous system which includes the brain, while mental disorders, such as depression, anxiety and schizophrenia, are typically defined as a psychological illness that impairs a person's cognitive performance, emotions and behaviour regulation [81]. Both disorders typically impair an individual's ability to conduct his/her everyday life which would also result in a higher possibility of disability and mortality as they are not able to attend to their health problems. Therefore, the patients are highly dependent on

their caregiver and families, but this would create additional strain and toll on the economy. Also, these disorders have a huge psychological and emotional impact on the family members of the patient as they must deal with the additional economic burden and manage the added stress [82].

Therefore, various health experts and leaders from WHO have worked together to publish the “Mental Health Action Plan 2013-2020” to recommend various action plans that governments and policymakers can implement to manage for reducing the global mental and neurological disorders burden [83]. According to GBD 2017, the three top contributors to the mental and neurological disorder's burden in Singapore are – (1) headache (3.52%), (2) depression (3.4%), and (3) dementia (2.01%). These three disorders account for more than half of the overall mental and neurological disorders burden and the effectiveness of existing strategies to manage the risks will be analysed.

The most common headache disorders are migraines which constitutes more than 80% of the entire headache disorder burden. Migraines usually cause acute throbbing pain in one side of the head which could last from a few hours to a few days. It can be triggered by external factors such as stress and diet, or hormonal changes in the body [84]. Therefore, migraines can usually be prevented by avoiding triggers. Certain medications such as antidepressants and pain-relief drugs can also help ease pain [85]. Although there are currently no treatment options for migraines, clinical trials conducted in recent years have shown promising results for the elimination of migraine headaches [86]. Headache disorders rarely cause deaths but usually hinders the work productivity and quality of health of the affected people.

Depression is a common mental disorder which is due to extreme sadness which affects as many as 264 million people [87]. Signs of depression include sudden loss of appetite,

insomnia and incoherent speech or thoughts. As these symptoms are very common with other illness or disorders, depression is easily overlooked and often left untreated for a long time which, due to the continual torture, may often lead to suicide [88]. Therefore, it is very challenging to develop early detection techniques for diagnosing depression. Hence, preventive measures such as psychosocial supports and management of social networks could be more effective in reducing the occurrence of depression [89].

Dementia is a condition where a person's cognitive function progressively degenerate at a rate that is faster than expected from normal healthy ageing [90]. Besides affecting the thought process, dementia also impairs the patient's memory, comprehension, communication and orientation skills [91]. The cause and early onset of dementia are still not fully understood which result in it typically being diagnosed in the late stage [92]. In 2015, the estimated number of people affected by dementia is approximately 47 million and its growth rate has also been increasing. By 2030, the number of dementia patients is predicted to reach 75 million and the economic burden of dementia is also projected to exceed US\$ 2 trillion due to the loss in productivity and increasing expenditure on dependency care services [93]. This is also because dementia is a progressive disease that does not cause death in a short period. Therefore, patients tend to spend a long duration in high dependency and disabled state which requires constant care and support.

In response, the WHO has officially recognised dementia as a public health priority in the "Global Action Plan on the Public Health Response to Dementia 2017-2025" [94]. The plan outlines recommendations that countries can adopt to raise awareness of dementia and garner community support for dementia care. Besides, the plan also recognises that there is a research gap in existing techniques to prevent and treat dementia. There are multiple types of dementia, namely, Alzheimer's disease (AD), vascular dementia, frontotemporal

dementia and dementia with Lewy bodies. They are not mutually exclusive and oftentimes, multiple dementias may develop at the same time. The following section reviews in detail the most form of dementia which is AD.

2.1.2.1 Alzheimer's disease

AD accounts for an estimated 60% - 80% of all dementia cases and is caused by damaged neurons within the brain [95]. A normal fully developed human brain contains about 100 billion neurons. As illustrated in Figure 2.10, a neuron has multiple synapses which connect to other neurons for the transfer of information between each other. As signals travel through the brain, a neuronal circuit is created allowing for control of other parts of the body or cognition [96]. However, in patients with AD, the neuronal circuit is broken due to the damaged neurons. As a result, a person's ability to perform his/her daily function is impaired.

Figure 2.10: Anatomy of a neuron (adapted from [96])

As AD is a progressive disease, the damage typically starts small and slowly develop as more neurons in the brain are damaged. In the preclinical stage, the brain can compensate for the damaged neurons and therefore, no visible symptoms would be observed [97].

But as more neurons are damaged, a tipping point is reached, and the brain is unable to compensate anymore. At this point, symptoms such as declined cognitive ability start to show and it is termed as mild cognitive impairment (MCI) stage. However, the change is usually subtle and is most often confused with age-related cognitive decline. The last stage of the disease progression is named as “dementia due to AD”. At this stage, neurons in multiple sections of the brains are damaged which affects a person’s ability to conduct its everyday activities. Dementia due to AD can be further divided into three levels of severity – (1) mild stage, (2) moderate stage, and (3) severe stage [98].

At the mild stage, multiple symptoms are present such as cognitive decline and behavioural changes. However, the person can still conduct his/her daily activities albeit with assistance or supervision to ensure his/her safety. Patients would usually spend the longest time at the moderate stage. At this point, they most like require assistance for conducting his/her daily activities. Communicating becomes a problem and personality changes become more significant. Ultimately, as the disease progressed to the severe stage, basic bodily functions such as body movement are not possible, and the patients would be bed-bound until eventual death.

Once the disease progressed to a later stage, it is almost impossible to reverse its effects or symptoms as there is currently no known method to regenerate damaged neurons. Hence, detecting AD early could be an effective strategy so that preventive measures can be implemented to slow or stop further progression of the disease [99]. However, since there are no visible symptoms during the early onset of AD, it is very challenging to accurately diagnose it with existing detection techniques which may result in a high cost due to overdiagnosis. In this context, the next section will review the effectiveness of existing state-of-the-art detection techniques for the early detection of AD.

2.1.2.2 Early Detection Techniques for Alzheimer's Disease

One of the hallmarks for the early detection of AD is the discovery of the close link between the aggregation of amyloid plaques in the brain and the progression of AD. The amyloid plaques are formed by beta-amyloid ($A\beta$) which is an extracellular peptide that is toxic to neurons. Studies have shown that the accumulation of $A\beta$ plaque in the brain can start 10-20 years before the initial symptoms of AD start to show [100]. Therefore, monitoring the accumulation of $A\beta$ plaque within the brain is a potential method to assess the pathogenesis of AD which is achieved by using neuroimaging. Table 2.2 is a summary of different reported neuroimaging techniques for the detection of $A\beta$.

Table 2.2: Comparison of different neuroimaging techniques for $A\beta$ detection

Detection techniques	Principle	Advantages	Disadvantages
Positron emission tomography [101]	γ -ray emission from positron emission decay	High spatial resolution	Expensive, not readily available
Single-photon emission computed tomography (SPECT) [102]	Direct γ -ray emission and measurement	Lower cost than positron emission tomography, fast	Low spatial resolution
Magnetic resonance imaging (MRI) [103]	Proton density and relaxation time	Non-ionizing, high spatial resolution	Low sensitivity, poor penetration of contrast agent
Near-infrared fluorescence (NIRF) [104]	Optical imaging using fluorescence probes	Non-ionizing, low cost, real-time results	Low penetration depth, poor signal-to-noise-ratio

Conventionally, the detection and diagnosis of AD are conducted through a cognitive assessment. This can be administered by either a specialist or a primary care practitioner. Through this assessment, if there is any decline in cognitive ability the patient will be referred for further check-ups to confirm the diagnosis. However, this method is highly

subjective and not always frequently being carried out [105]. It is also only able to detect AD after symptoms are visible. Neuroimaging techniques could also be used to directly monitor the accumulation of $A\beta$ plaque within a person's brain. There are a few techniques that are either available clinically or are at various stages of clinical trials that could be potentially be used [106]. However, much research is still necessary to develop these techniques for detecting the accumulation of $A\beta$ plaque.

Positron emission tomography uses a minute amount of radioactive substance known as a radiotracer to measure the metabolic activity of the brain. The radiotracer is formed by attaching a radionuclide to a probe that can be metabolized by $A\beta$. It is then injected into the patient's bloodstream which transports it to the brain. As the radiotracer is broken down by the $A\beta$, a positron is emitted which interacts with an electron within the body and produce a pair of gamma photons (γ -ray). The two γ -rays are then picked up by the scanner, as shown in Figure 2.11, which recreates an image map of the brain. Areas with more $A\beta$ would appear brighter on the image map as more radiotracer would bind to it.

free vectors from Vecteezy.com

Figure 2.11: Schematic of positron emission tomography (adapted from [101])

Similarly, single-photon emission computed tomography (SPECT) also use a radiotracer to identify $A\beta$ plaque. However, the radionuclides used in SPECT do not emit positron but instead emit γ -rays directly. These γ -rays are then collected using a γ -ray camera which rotates around the brain to generate multiple 2D projection images. Tomographic reconstruction is then used to reconstruct a 3D model of the brain [102]. As the radionuclides used are more easily synthesised than those used for positron emission tomography, the relative cost of a SPECT scan is also cheaper. However, the spatial resolution is lower as due to the limitation of the γ -ray camera.

Brain MRI can also be used to detect any accumulation of $A\beta$ plaques [103]. Before the scan, a contrast agent is injected into the patient. The contrast agent typically consists of a probe which binds specifically to the $A\beta$ plaque and a heavy metal material to increase its visibility as opposed to the brain tissue. Therefore, the presence of and $A\beta$ plaque is indicated by a high concentration of the contrast agent which will appear as a bright spot in the MRI image. Besides the high cost of a brain MRI scan, the poor penetration depth of the contrast agent is also one of its drawbacks.

Fluorescence imaging is a well-established optical imaging technique that has been widely in used biology and medicine. To achieve greater penetration depth, long wavelengths than those in the visible spectrum were employed which resulted in the development of near-infrared fluorescence (NIRF) imaging [104]. As compared to the other techniques, the equipment necessary for NIRF is much cheaper and easily operated. It also can provide real-time analysis of the result. However, suitable fluorescent probe targeting $A\beta$ plaques must be developed before it can be used for AD diagnosis.

In summary, the existing techniques for diagnosing AD is primarily achieved by using neuroimaging to detect the accumulation of $A\beta$ plaque within the brain. However, the high

cost of the equipment limits their effectiveness in diagnosing AD. Recent studies have revealed that $A\beta$ plaques present in other parts of the body, such as the skin, may also have a possible connection to AD which is also known as “brain-skin connection” as illustrated in Figure 2.12 [107]. Such $A\beta$ plaques could potentially be detected using a biosensor that is attached to the skin which is highly desirable as it would offer a simple and low-cost diagnostic approach for AD diagnosis. Hence, detection of $A\beta$ plaques would also form one of the research objectives of this thesis.

Figure 2.12: Links between brain amyloids and skin amyloids (adapted from [107])

2.1.2.1 Treatment of Alzheimer’s Disease

There are currently no known techniques that are reported to be able to successfully cure AD. To do so, the technique must be able to repair the damaged neurons which is the most challenging bottleneck in the development of a cure. The next best approach is to try and slow or halt the progression of AD by preventing more neurons from being damaged. This could be achieved by using pharmacologic treatments such as medications or non-pharmacologic therapy approach [108]. However, all these techniques are still in the research or clinical trial phase and an effective cure is still far from development. Also, their effectiveness is highly dependent on the detection techniques available to identify people who are genuinely suffering from AD. Hence, this further reinforces the need to develop a biosensor as a cost-effective approach for AD diagnosis.

2.1.3 Cardiovascular Diseases

Cardiovascular diseases are a group of abnormal conditions or diseases which affect the cardiovascular system which is also known as the circulatory system. The cardiovascular system includes the heart, blood vessels, blood and the lymphatic system [109]. Its main function is to supply oxygen and other nutrients to all parts of the body, and the removal of waste materials.

The two greatest contributors to cardiovascular disease burdens are – (1) ischemic heart disease and (2) stroke. Ischemic heart diseases (IHD) occurs when there is reduced blood supply to the heart muscle [110]. The most common reason for this is due to fatty deposits or plaque build-up within the inner walls of the arterial blood vessels which reduce the diameter of the blood vessels. Symptoms may include acute chest pain and shortness of breath. Further progression of IHD may result in a complete block of the artery which can lead to a heart attack. Strokes are caused by the reduction of blood supply to the brain which results in a partial shutdown of the brain. Reasons for this may be due to an ischemic condition (blood supply is blocked) or a haemorrhagic condition (bleeding in the brain) [110]. The signs are usually a sudden impairment of one or more functions of the body such as vision, body movement, and speech etc.

In Singapore, the cardiovascular disease burden has been declined steadily from 18.46% in 1990 to 14.16% in 2017. Also, the age-standardised rate for the DALYs of both IHD and stroke has decreased by over 60% (refer to Appendix D for the data). This shows that the impact of cardiovascular diseases on Singaporeans has weakened slightly over the past two decades. Hence, this indicates a certain level of success in the current strategies for the prevention, detection and treatment of cardiovascular diseases. In this context, this thesis would only focus on the early detection of female breast cancer and AD.

SECTION B

2.2 Biosensors

Biosensors are portable devices that are used to detect or measure the concentration of a biomolecule (analytes) that is present in a sample fluid. A biosensor can achieve this by using special biorecognition elements known as bioreceptors which binds specifically to the analytes [111]. The most-reported method for biosensing is outlined in Figure 2.13 where the bioreceptors are first immobilised on the surface of the sensing site of the biosensor. When the sample fluid flows over the sensing site, the analytes within the fluid would be attracted and bound to the immobilised bioreceptors [112]. This would trigger a biochemical reaction that can be detected by the biosensor.

Figure 2.13: Biosensing detection protocol (adapted from [112])

In recent years, biosensors have been gaining huge interest as a potential candidate to replace traditional laboratory-based techniques for biomedical applications such as detection of health pollutants in the water supply or monitoring of a patient's vital signs [77]. Besides, biosensors could also be potentially used to detect the presence of disease biomarkers in a patient. The major advantage is the ability to provide portable, real-time in situ testing with relatively good sensitivity towards the analytes. This could be used to supplement conventional clinical techniques as the biosensors offer a facile approach to improve the efficiency of existing early detection strategies.

2.2.1 Biomarkers for Disease Detection using Biosensors

In the context of female breast cancer detection, a variety of biomarkers could be invaluable in the diagnostic process. Human epidermal growth factor receptor 2 (HER-2) and Mucin1 (MUC1) are two of the most common glycoproteins which are present in nearly 30% of all breast cancers [113]. Also, autoantibodies related to tumour associated antigens (TAA) have been identified as an effective biomarker. Estrogen receptor alpha ($ER\alpha$) proteins are closely linked to the development of hormone-dependent breast cancer [114]. Other useful biomarkers include circulatory tumour cells, DNA, micro RNAs etc. [115]. All these can be detected using bioreceptors such as aptamers for detection of glycoproteins and antibodies for the detection of proteins [116]. The biomarkers can be found in a patient's psychological fluids such as saliva and milk near the breast.

The most important biomarker for the detection of AD is $A\beta$ plaque. Recent literature has reportedly demonstrated a certain level of success by using antibodies such as an $A\beta$ bioreceptor [117]. However, the major bottleneck limiting the development of biosensors for disease detection is their sensitivity towards the biomarkers. As the concentration levels of the biomarkers are typically in very low concentrations in the psychological fluids of the patients, the biosensors need to have very high sensitivity to ensure the biomarkers are detected accurately. The next sections review different types of biosensors and how their sensitivity can be further enhanced.

2.2.2 Electrochemical Biosensors

One key component of a biosensor is the transducer element which converts the biochemical reaction between the analyte and bioreceptor into a signal that can be measured. Therefore, biosensors can be broadly classified according to their type of

transducer element. Electrochemical biosensors measure the electrical property (current, potential or impedance) across a sensing surface and are relatively inexpensive to fabricate [118]. It can also be designed to be very compact and robust which makes it highly portable. However, the bioreceptors selection for electrochemical biosensors is limited to those which produces an electrical signal such as those which produces free electrons after reacting with the analyte. Hence, the development of electrochemical biosensors is not as flexible due to the limited selection of bioreceptors. Nonetheless, electrochemical biosensors have found huge success due to the relatively low cost and simple design. One such example is the monitoring of blood glucose level in diabetic patients [20].

2.2.3 Optical Biosensors

In recent years, optical biosensors are gaining huge popularity as the demand increases for biosensors with higher sensitivity and ultra-low detection limits [119]. As optical light does not interact with the sensing medium directly, it can measure the signal with relatively low noise [120]. Additionally, optical biosensors offer more flexibility as it could employ different properties of light such as intensity levels, phase difference, or changes in the state of polarization of the reflected or transmitted light as interrogation parameters for sensing applications [121]. Alternatively, the spectral wavelength shift could also be used to indirectly measure any changes in the detected signal [122]. Novel optical biosensing configurations based on the resonant coupling of light such as surface plasmon resonance (SPR) and guided-mode resonance (GMR) allows for even high sensitivity and have been gaining popularity in the recent decade [123,124].

Optical biosensors rely on coupling light into different structures such as waveguides, optical fibres etc. to act as the transducer element. [125]. Due to the similar guiding

principle, waveguide-coupled optical biosensors are mostly replaced by optical fibres designs [126]. Optical fibres are relatively cheap but difficult to integrate for multiplex sensing as the sensing surface are usually at the ends of the optical fibres. Grating-coupled optical biosensors could achieve high sensitivity by exploiting the unique property of light diffraction which generates evanescent waves along the interface. Advantages of grating-coupled optical biosensors are that they can achieve relatively high sensitivity with low noise due to high intensities of the evanescent waves at the localised region near the grating. Furthermore, grating-coupled optical biosensors could also be easily integrated with microfluidic channels which is beneficial in delivering a small amount of the sample fluid for highly accurate sensing [127]. However, a critical limitation is that the currently available fabrication techniques necessary for producing the high-resolution nanoscale grating features necessary for the biosensors are too slow and not economical [128].

2.2.3.1 Surface Plasmon Resonance (SPR) Biosensors

An SPR biosensor uses optical light to excite surface plasmons (SP) waves at a metal-dielectric interface for sensing. At a metal-dielectric interface, free electrons can be excited to oscillate along the interface. The collective oscillation of these electrons is known as SP and can be represented as a transverse magnetic (TM) electromagnetic wave which propagates along the interface in the x-direction as illustrated in Figure 2.14.

Figure 2.14: Schematic representation of an SP and the field distribution (adapted from [129])

The wave vector of this SP wave ($\mathbf{k}_{SP}$) can be expressed as a function of the wave vector of the incident light ($\mathbf{k}_x$) (in the x-direction) which is assumed to be in free space as [130],

$$\mathbf{k}_{SP}(\omega) = \mathbf{k}_x \sqrt{\frac{\epsilon_d \epsilon_m(\omega)}{\epsilon_d + \epsilon_m(\omega)}} \quad (2.1)$$

where ϵ_d is the permittivity of the dielectric layer, ϵ_m is the permittivity of the metal layer and ω is the angular frequency of the light wave. Since $\mathbf{k}_{SP}$ is greater than $\mathbf{k}_0$ by a factor proportional to the effective material permeability, a coupler must be introduced to increase the $\mathbf{k}_x$ value so that the momentum of the incident light matches that of the SP [131]. Figure 2.15 shows how the wave vectors can be modified in two of the coupling configurations. At a specific frequency, the $\mathbf{k}_x$ value of the coupled incident light would match that of the $\mathbf{k}_{SP}$ and therefore the resonance condition is satisfied. At this point, energy from the incident light is resonantly transferred to excite the SP. From Eqn (2.1), since $\mathbf{k}_{SP}$ is influenced by the effective permittivity or refractive index (RI) at the metal-dielectric layer interface. Any RI change at the interface would cause a shift in the resonance condition which can be detected by the SPR biosensor. Hence, an SPR biosensor is also an effective RI sensor.

Figure 2.15: Relationship between the wave vectors of different coupling methods (adapted from [132])

The three major coupling configurations are prism-coupled, grating-coupled, and waveguide-coupled and they are schematically represented in Figure 2.16. Each coupling methods has its own merits and limitations; hence, the coupling design should also be carefully considered with respect to the research objectives.

Figure 2.16: Schematic of (a) prism, (b) grating, and (c) waveguide coupled SPR configuration

(adapted from [132])

In the prism coupled configuration, evanescent waves are generated due to the attenuated total reflection (ATR) of light at the prism-metal interface and the wave vector (k_{EW}) is given as [133],

$$k_{EW} = k_x = k_0 n_{\text{prism}} \sin \theta \quad (2.2)$$

where n_{prism} is the RI of the prism and θ is the angle of incidence of the incident light. In this configuration, SPR sensing could be conducted in either angular or spectral interrogation mode. In angular interrogation mode, laser light with fixed wavelength (λ) is used and the θ is slowly varied until k_{EW} matches that of k_{SP} . As a result, the resonance condition is satisfied and due to the resonant transfer of energy, the reflected light would have a significant reduction in its intensity. Hence, by measuring the reflectance of the light against the angle of incidence, the corresponding resonance angle can be identified by the characteristic sharp dip in the reflectance.

In the spectral interrogation mode, a broadband light source is used while the angle of incidence is fixed. As each λ would have a different corresponding k_x value, a particular λ would match that of k_{SP} . Therefore, by measuring the spectrum of the reflected light using a spectrometer, the resonance wavelength can be identified as it would have the lowest reflectance.

Figure 2.17a shows a typical prism coupled SPR biosensor that is set-up in the Kretschmann configuration. First, bioreceptors are immobilised on the surface of the metal and the reflectance curve is measured. Next, the dielectric sample fluid containing the analytes flows over the metal surface which forms the metal-dielectric interface necessary to support the SP wave. As the analytes pass near the vicinity of the bioreceptors, it would get immobilised on the metal surface. As a result, the RI in the sample fluid near the metal-dielectric interface would be increased. Hence, the k_{SP} value is changed which causes a shift in the resonance signal as shown in Figure 2.17b.

Figure 2.17: (a) Schematic of a prism coupled biosensor in the Kretschmann configuration, and (b) the shift in the SPR reflectance signal due to analyte-bioreceptor binding (adapted from [131])

In a grating coupled SPR setup, light is incident on a grating resulting in the diffraction of the light in many different directions. Each diffracted beam has a different order (m), and the respective x-direction wave vector can be expressed as [134],

$$\mathbf{k}_x^m = \mathbf{k}_0 n_d \sin \theta + m \frac{2\pi}{\Lambda} \quad (2.3)$$

where Λ is the periodicity of the grating. As such, the diffracted beam would have an increased wave vector value which is possible to be matched with $\mathbf{k}_{SP}$ to realise the resonance condition. Similarly, a grating coupled SPR can also be configured in angular interrogation mode or spectral interrogation mode [135]. Since the diffraction angle is highly dependent on the geometry and periodicity of the grating, the grating design can be customised for a specific bioreceptor or analyte to ensure optimal SPR sensitivity. Also, grating coupled SPR biosensors can be made in highly compact devices and also allows the possibility of multiplex sensing in a single biosensor chip by using an array of varying grating features [136].

Waveguide coupled SPR biosensors make use of guided modes of optical light in a waveguiding layer to generate evanescent waves for excitation of the SP. Such an SPR system is typically in spectral interrogation mode but could also be designed for phase interrogation or intensity interrogation by various waveguide designs. As the optical fibre has a similar structure as a waveguide, optical fibre coupled SPR has also been demonstrated as a flexible SPR biosensor [137]. However, due to the scale of the structures, waveguide and optical fibre coupled SPR biosensors typically require high-end fabrication equipment which limits the cost-efficiency of these devices.

Out of all the coupling techniques, grating coupled SPR biosensors offers significant advantages such as flexibility in its design and compactness. This was demonstrated in recent literature where a grating coupled SPR biosensor was developed for the detection of breast cancer-related genes [138]. However, one major limitation identified is the high cost of fabrication for producing the nanoscale grating features. As the dimensions of the

grating features are in the wavelength scale of the optical light, hence, advanced nanoscale fabrication techniques such as e-beam lithography are necessary for producing the high-resolution features [139]. Hence, the later sections would also review existing nanofabrication techniques to identify a more economical approach for producing the grating features necessary for an SPR biosensor.

2.2.3.2 Localised Surface Plasmon Resonance (LSPR) Biosensors

With the recent improvement in nanotechnology, new forms of SPR biosensors based on nanomaterials have been developed which is termed localised SPR (LSPR) biosensors [140]. Metal nanoparticles, that are much smaller than the wavelength of light, is suspended in a dielectric fluid and excited optically. As illustrated in Figure 2.18, the nanoparticles and the fluid would form a metal-dielectric interface and the collective oscillation of the free electrons would form an electron cloud which is described as the localised SP (LSP) [141]. Hence, LSPR biosensors are designed based on the resonant coupling of optical light, as it passes through the nanoparticles, for excitation of the LSP.

Figure 2.18: Schematic illustration of the LSPR (adapted from [142])

The extreme field confinement of localised SP wave makes LSPR biosensors highly sensitive to RI changes at the localised region near the nanoparticles. The geometry, size and material composition of the nanoparticles can also be modified to tune the resonance condition for different targeted applications [143]. However, due to its small size, the

synthesis of nanoparticles is very challenging and requires expensive fabrication techniques, hence, as a trade-off, most of the research on LSPR biosensors have been focused on exploring new nanoparticles designs, geometries and materials to further improve the quality factor [144].

2.2.3.3 Signal Amplification in SPR Biosensors using Nanomaterials

Besides being used in LSPR biosensors, nanoparticles and other nanomaterials including 2D materials such as graphene have been gaining popularity in recent years as a potential method to enhance the signal in SPR biosensors [145,146]. The enhanced SPR biosensor can be developed for sensing of lower concentration of analytes or smaller biomolecules, such as DNA in breast cancer gene screening.

There are two primary schemes where nanomaterials can be used in SPR biosensors for signal enhancement – (1) as amplification tags, or (2) to improve sensing surface. When used as amplification tags, the nanomaterials are first introduced to the sample fluid and attached to the analytes. This increases the mass of the analyte would then induce a larger RI shift and produce a greater SPR signal. Functionalised nanomaterials are also be used to trigger secondary signal amplification by catalytic activity [147].

Alternatively, the nanomaterials could be layered over the sensing surface of the SPR biosensor for coupling between the SP wave to the LSP in the nanomaterials [148]. The increased field confinement would promote better sensitivity and a lower detection limit in the SPR biosensor. This was demonstrated where the surface of a conventional SPR biosensor was covered with a gold (Au) nano-dot array. Their result shows a significant enhancement in the detection limit as compared to a normal SPR biosensor [149]. Therefore, nanomaterials could be a potential avenue to explore to further enhance the signal of the SPR biosensor for disease detection.

SECTION C**2.3 Fabrication Techniques for Producing High-Resolution Nanoscale Features**

Over the recent two decades, nanoscale fabrication techniques have been heavily pushed by the semiconductor industry's demand for smaller features to produce an increasingly dense integrated circuit (IC) chips in accordance to Moore's law [150]. Some of these technologies have been adapted for research and development into the micro-electro-mechanical system (MEMS) and Bio-MEMS industry with potential applications that include the fabrication of micro and nanoscale features or miniaturisation of laboratory process into a single device for use in medicine, sensors, photonics, nanorobots etc. [128].

However, the existing techniques used in Bio-MEMS are typically directly adapted from the IC industry which is primarily designed for use with silicon materials. Therefore, the high cost and bio-incompatibility make the equipment highly unfavourable for fabricating low-cost biosensors. In this context, the following sections will review existing nanofabrication techniques to explore an alternative approach for fabricating the high-resolution grating features necessary for a grating coupled SPR biosensor.

Additionally, an advanced manufacturing technique based on additive manufacturing (AM) will also be reviewed. AM allows complex 3D structures to be fabricated from a computer model in a variety of materials such as polymer or metals in a layer-by-layer fashion. The relative ease of use and versatility of such 3D printers would be highly beneficial for the research and development of biosensors with customised designs.

2.3.1 Photolithography

Photolithography has been an integral part of the semiconductor industry and had evolved over the last decade [151]. It employs light to transfer micro and nano-scale

patterns from a photomask onto a substrate. Light-sensitive chemicals or polymers, known as photoresists, are first coated on the substrate material and when light passes through the photomask, the resulting pattern would be produced in the photoresist. The reaction which occurs when the light is absorbed by the chemicals differs according to the type of photoresist [152]. As outlined in Figure 2.19, negative-tone photoresists become insoluble when exposed to and would remain on the substrate after washing in the developer solution. Positive-tone photoresists behave in the opposite way where exposed regions become soluble in the developer and are washed off [152].

Figure 2.19: Positive-tone vs negative-tone photoresist (adapted from [152])

There are also two methods where the pattern can be transferred to the desired material and the difference between these two methods are summarised in Figure 2.20. For the lift-off method, the desired material is deposited after the pattern is recorded in the photoresist. The photoresist would act as an intermediate masking layer between the material and substrate, hence, when it is removed or lift-off, the material will only remain on the exposed regions which form the desired pattern. Alternatively, the desired material can be first deposited on top of the substrate before the coating of photoresist. The photoresist is now an etch mask which prevents the covered regions from being removed by the etchant. After the removal of the photoresist, the material which is protected remains on the substrate which forms the desired pattern.

Figure 2.20: Lift off method vs etching method (adapted from [152])

As the feature size shrinks further, the minimum resolution of the system approaches the limits bounded by the Rayleigh criterion [153]. It defines the minimum distance to resolve two points from each other as; the distance between the first minimum of the Airy pattern formed by one of the points to the maximum of the other Airy pattern formed by the other point. An Airy pattern is the resulting diffraction pattern formed by a spot of light that is perfectly focused by a lens with a circular aperture [154]. The practical resolution of a projector lens system used in typical photolithography is can be given as, [155]

$$\text{Resolution} = k_1 \frac{\lambda}{\text{N.A.}} \quad (2.4)$$

where, k_1 is the k-factor, λ is the wavelength of the light source, and N.A. is the numerical aperture of the lens system. These three factors govern the resolution of the photolithography system. The k-factor is determined by the mechanical system such as the type of masks, photoresists, process controls and aberrations correction which can be improved using resolution enhancement techniques (RET). The robustness of the mechanical components such as the motors and actuators were redesigned to reduce the effect of noise on the system. The shape of the light source and mask designs were

optimised to allow better tolerance control in areas where smaller feature size is critical. New photoresist technology such as chemically amplified photoresists was also introduced which contains photo-acid generators that perform well in high-resolution patterns [156].

Different approaches had been employed to change the values of these factors to improve the resolution of the system and produce nanoscale features. The most straightforward method is to switch to a light source of a shorter λ such as ultra-violet (UV) which extends beyond the visible spectrum as shown in Figure 2.21. This approach has remained relatively practical until the wavelength reaches the x-rays region [157]. The generation of such high energy rays efficiently was a major technological barrier which makes this method highly unsustainable for high throughput applications. The costs also increase exponentially due to the need for specialised equipment for x-rays delivery.

Figure 2.21: Spectrum of light waves from x-rays to infrared (adapted from [158])

Another method to improve the resolution of the system was to increase the N.A. of the lens system used in the photolithography system, which is given as,

$$\text{N. A.} = n \sin \theta_{\text{cone}} \quad (2.5)$$

where n is the RI of the medium between the lens and the substrate and θ_{cone} is the maximum half-angle of the cone of light as it leaves the lens. Having a high angle means that the photoresist has to be very near to the lens. There are two approaches to increase

the N.A.; first is to increase the RI of the medium between the lens and the substrate using immersion lithography, second is to modify the lens design to maximise the N.A. Immersion lithography works by replacing the medium between the lens and substrate from air ($n \approx 1.0$) to a liquid fluid such as water ($n > 1.33$). This technique, when coupled with hyper N.A. lens, was able to sufficiently improve the resolution of the photolithography system significantly without using light with shorter wavelengths (λ) [159]. However, the immersion fluid needs to be filtered to remove any contaminants such as dust particules which would cause defects.

Photolithography has remained a highly competitive nanofabrication technique for high throughput applications. Coupled with RETs, it is possible to fabricate high-resolution features necessary for the optical biosensors. However, the RETs would also inevitably complicate the fabrication process and increase the fabrication cost of the biosensors.

2.3.2 Interference Lithography

Interference lithography is an advanced fabrication technique that exploits the property of light waves to make sub-wavelength patterns without the use of a photomask [160]. IL is a simple and cost-effective approach to achieve large-scale patterning in a photoresist, and the patterns could then be formed directly for a variety of applications [161]. Even though the patterns are usually limited to periodic features, such features resonate especially well with applications such as photonic crystals, metamaterials, field emitter arrays, biosensing, high-density magnetic data storage, optical trapping and sub-wavelength structures, etc. [162].

IL makes use of the interference of two or more beams from a coherent laser source to generate a standing wave pattern. This pattern can then be recorded in a photoresist,

which can then be transferred to other materials, or used directly as the substrate material. In a simple two-beam IL system, each wave is linearly s-polarized and incident at equal angle θ from the normal axis from the plane of interference as shown in Figure 2.22 [163]. The two light waves would interfere as they propagate, which generates an interference pattern that could then be recorded in a photoresist medium.

Figure 2.22: Interference of two laser beams with the interference fringes contained in the x-y plane

The resulting interference pattern would have a periodicity (Λ) that is given as, [163]

$$\Lambda = \frac{\lambda}{2 \sin \theta} \quad (2.6)$$

From this, we can see that periodicity of the interference patterns would be in the range of the wavelength band of the light source used. The main benefit of IL over the conventional photolithography is that there is no need to fabricate expensive photomasks. Additionally, it can be flexibly tuned to create one-dimensional (1D) grating patterns to 2D arrays or even 3D structures by simply varying the angle of incidence or recording multiple exposures on the photoresist [164]. For example, a simple two-beam IL set-up can only generate 1D line gratings with a single exposure. However, by rotating the sample stage by 90° and exposing a second time, 2D hole/dot arrays with square

symmetry can also be achieved. Multi-beam IL with at least four beams can produce 2D or even 3D patterning in a single exposure [165]. Different lattice patterns have also been reported by employing polarizers to adjust the polarization state of each beam. Two pairs of s-polarized interfering beams would result in a square lattice pattern and a pair s-polarized beams with a pair of p-polarized beams would result in a periodic pattern that is arranged in a hexagonal lattice as demonstrated in Figure 2.23 [163].

Figure 2.23: Simulation results & scanning electron microscope (SEM) images of two pairs of
(a) s-polarized beams, and (b) p-polarized beams (adapted from [163])

Therefore, IL is a powerful and simple technique to fabricate large-area, micro and nanoscale periodic patterns or features. Patterns with different periodicities could be achieved using a single IL system with a simple manipulation of the angle of incidence which is highly desired for modifying the design of the periodicity of the grating for optimising sensitivity when used in an SPR biosensor.

2.3.3 Immersion Interference Lithography

Immersion IL is an alternative method to further reduce the minimum achievable feature size of a conventional IL system. This is achieved by incorporating a medium with a higher RI than air into the interference plane such as a prism or deionised water [166]. As shown in Figure 2.24, the addition of a prism would increase the resulting angle of incidence between each beam as per Snell's Law which is given as [166],

$$n_1 \sin \theta_1 = n_2 \sin \theta_2 \quad (2.7)$$

Figure 2.24: Refraction of the beams as it enters the prism before interfering at the bottom surface

An index-matching liquid can also be introduced at the interface between the prism and the photoresist to ensure good optical contact for transferring the pattern to the photoresist. However, since the RI of the prism would be much higher than that of the index-matching liquid, care must be taken to ensure that the incidence angle is not greater than the critical angle which is defined as [166],

$$\theta_{\text{critical}} = \sin^{-1} \left(\frac{n_{\text{liquid}}}{n_{\text{prism}}} \right) \quad (2.8)$$

If the incidence angle is greater than the critical angle, then the light would be totally reflected off the prism and no interference pattern would be formed. Recent work demonstrated the ability of a two-beam immersion-based IL system using a 364nm laser source with a high RI prism to realise 60 nm periodic grating features which are smaller than 0.2 times the wavelength of the laser source used [167].

Immersion IL is a promising technique that could be added to the IL to improve the resolution of the IL set-up and can be achieved by simply adding a high RI prism. However, the addition of an index-matching liquid may inevitably introduce some particulates or contaminants which may result in defects that could affect the integrity of the patterns.

2.3.4 Near-Field Nanopatterning

Near-field nanopatterning techniques have also been proposed as an alternative method to improve the resolution of the IL system further. Such techniques exploit the near-field optical effect of light at special conditions at an interface to generate sub-wavelength, nanoscale patterns. These approaches can be mainly classified into two categories; namely evanescent waves (EW) and surface plasmon (SP) based interference techniques. When a plane wave is reflected due to the total internal reflection (TIR), EW is generated. Multiple EW could then be made to interfere, and the resulting interference patterns could be recorded in a photoresist as illustrated in Figure 2.25 [168].

Figure 2.25: Schematic of counter-propagating EW interference in a prism (adapted from [169])

The EW based interference technique has been reportedly able to generate 3D surface relief features with minimum feature size smaller than 0.1 times the wavelength of the laser and height-to-width aspect ratio of as high as 10 [169]. The geometries of the

periodic patterns can also be flexibly modified by simply changing the state of polarization and the relative phase difference between the incident plane waves [170].

SP based interference techniques work by employing counter-propagating SP waves instead of EW waves [171]. The SP is generated by directing multiple beams of light on a metal-coated prism as shown in Figure 2.26. As the SP waves interfere with each other, an interference pattern is generated which can be recorded in the photoresist layer. Since the wavelength of the SP is much shorter than the λ of the incident light, therefore, the resulting interference pattern would be much smaller than a conventional IL system.

Figure 2.26: Schematic of counter-propagating SP interference in a prism (adapted from [172])

Simulation results using a finite-difference time-domain (FDTD) analytical method also suggests that an SP based interference system could theoretically achieve sub-30nm periodic features which are less than 0.1 times the wavelength of the laser source [172]. Additional work was done to support this experimentally with good agreement between both the simulation and experimental results [173]. Therefore, near-field nanopatterning is a useful method to further enhance the resolution of a conventional IL system with relatively simple optical components such as a prism.

2.3.5 Additive Manufacturing

Additive manufacturing (AM) is a process that builds 3D structures in a layer by layer method by joining, solidifying or deposition of materials [174]. AM or 3D-printing is a novel form of manufacturing technology, and in the recent few years, a variety of commercial 3D printers with different capabilities had appeared in the market, all possessing their advantages and limitations. AM has the primary benefit of being able to print a model with any level of complexities that are difficult to achieve or impossible to realise using traditional manufacturing technologies.

Out of the many types of AM techniques, liquid-based AM techniques have the best resolution, but the build material is only limited to photopolymers. One such example is the stereolithography apparatus (SLA) printer as shown in Figure 2.27. A UV light source or laser is incident on a 2-axis galvo-scanner which selectively cures the photopolymer contained in a resin tank as per the CAD model. The stage then moves upwards, and the process is repeated for each layer until the desired structure is 3D-printed.

Figure 2.27: Schematic of an SLA printer (adapted from [174])

The major drawback of an SLA printer is that the resolution of the printer is limited by the effective laser spot size formed within the resin tank which is typically around 100 μm . Due to the optical diffraction limit, it is difficult to focus the spot size of the light further to achieve higher resolution.

A PolyJet 3D printer uses multiple print nozzles to selectively deposit small amounts of liquid photopolymer on a build platform to form the layers of the printed part. UV light source is attached to the print head so that the resins are cured as the print head scans around the build platform. The major advantage PolyJet has is the ability to print parts with multiple materials or a specific mixing ratio of different build materials. This allows the potential to print a part that is both rigid and soft in certain locations from a single print job [175]. Such components have the potential of being used in flexible biosensors where it could be soft on the outside to prevent the user from being injured but have rigid internal structures for optimal fluid flow and sensing performance.

Direct laser writing (DLW) is a suitable technique to realise ultra-high-resolution nanoscale 3D printing by using the principle of two-photon absorption (TPA) or sometimes even multi-photon absorption (MPA). Photons of identical or different frequencies are simultaneously absorbed by a molecule which results in excitation energy equalled to the combined sum of the energy of the photon. As such, the absorbed energy is at a higher level which is about the same as the energy of a photon with a much shorter wavelength [176]. Since there is a strong dependency on the spatial and temporal match of the photons, the absorption function is a nonlinear response which results in a tightly focused light beam. The advantage of this is that ability to achieve a very high resolution that is less than a cube of the wavelength (λ^3) which is much smaller than the optical diffraction limit [177].

The working principle of a DLW system is shown in Figure 2.28. To achieve very ultra-high precision, a piezoelectric 3D scanning stage is used which is capable of linear translation in the order of nanometers. The laser is then focused using a 100× microscope objective with a high N.A. to selectively cure a single voxel within the photosensitive material. The stage then moves to a different location, and the process is repeated for each voxel to slowly form the desired 3D model.

Figure 2.28: Schematic of a DLW system (adapted from [177])

The ease of use of a DLW system and its high precision has made it a highly popular AM tool to achieve nanoscale fabrication for research applications. Its high resolution is ideal for fabricating the nanoscale grating features necessary for a grating coupled optical biosensor, however, due to the limited travel range of the piezoelectric stage, the build volume of a DLW system is very limited (about 1 mm³). The writing speed is also very slow which result in very low throughput.

2.4 Graphene as a Substrate for Biosensing Applications

One of the most significant discoveries of the twenty-first century is the successful isolation of one-atomic layer thick 2D carbon (graphene) from graphite as shown in Figure 2.29 [178]. Due to its unique 2D structure, graphene demonstrates high charge

carrier mobility at room temperature that exceeds $2.0 \times 10^5 \text{ cm}^2.\text{V}^{-1}.\text{s}^{-1}$ (more than 100 times that of silicon), high thermal conductivity of about 5,000 W/mK [179]. Besides that, graphene is also about 98% optically transparent in the visible wavelength band which makes it ideal to be incorporated into optical devices [180]. Additionally, reports have shown that graphene biosensors have improved sensitivity due to better absorption of the bioreceptors on the graphene layer [181]. Coupled with its good optical and electrical property, graphene could potentially be developed into an SPR biosensor that can be interrogated both optical and electrically [182].

Figure 2.29: Lattice visualization of (a) single-layer graphene, and (b) graphite (adapted from [183])

2.4.1 Graphene Synthesis Methods

The method for growing graphene has improved significantly over the recent decade [184]. The very first sample of graphene was isolated from graphite using a mechanical exfoliation method which is using scotch tape to repeatedly stick and pull apart the graphite layers [185]. This method was novel but slow and could only yield small flakes of graphene of random layers, nonetheless, it demonstrated the possibility of creating atomically thin films. Since then, many modern methods such as chemical vapour deposition (CVD) had been adopted for the synthesis of high-quality graphene for practical industrial applications.

2.4.1.1 Thermal Chemical Vapour Deposition

Thermal based, chemical vapour deposition (CVD) is a reliable and well-researched method for growing large-area, high-quality graphene. The working principle of a thermal-based CVD is to supply excess heat energy to decompose a carbon feedstock source such as hydrocarbons or polymers. The formation of graphene is then catalysed on the surface of the growth substrate. Suitable catalysts substrates are transition metals such as copper (Cu), nickel (Ni), platinum (Pt) and germanium (Ge) [186,187]. The synthesis is usually done in a low pressure, high vacuum chamber to minimise the chances of impurities in the growth substrate. A typical CVD system is shown Figure 2.30 where methane (CH_4) gas is used as the carbon feedstock and a mixture of hydrogen (H_2) and argon (Ar) gas is added as buffer gases to regulate the growth rate. A copper foil is used as the growth substrate to catalyse the formation of graphene.

Figure 2.30: Schematic of a CVD system for graphene deposition (adapted from [183])

The growth mechanism behind CVD can be described as a two-step process; dissolution and segregation [188]. Dissolution is the dissociation of carbon atoms from a carbon feedstock and being adsorbed into a metal substrate. Segregation is the arrangement of carbon atoms into a 2D honeycomb structure which forms graphene. However, the growth process is non-standard and varies according to the carbon solubility of the metal substrates. Figure 2.31 compares the growth of graphene on two different substrates; one with high carbon solubility and one with low carbon solubility.

Figure 2.31: Graphene growth on (a) high and (b) low carbon soluble metal substrates

(adapted from [188])

In metals with high carbon solubility, such as Nickel (Ni), under high heat, hydrocarbon molecules from the CH_4 decompose into carbon atoms in a process known as dehydrogenation. Due to the high carbon solubility, the carbon atoms would diffuse into the bulk Ni substrate. Once the threshold for nucleation has been reached, the segregation process will begin forming a layer of graphene on the surface of the metal and will only stop once the equilibrium has been reached. In metals with low carbon solubility such as Cu, the growth of graphene is more straightforward. Once dehydrogenation occurs, the carbon atoms would be adsorbed to the surface of the Cu and nucleation occurs immediately. The carbon structure would start growing as more carbon atoms get adsorbed, forming graphene.

The graphene deposited using CVD is usually uniformly deposited throughout the substrate which has enabled the upscaling of the process to meet industrial demands and the lowering of the production cost [189]. However, the process is only possible at

elevated temperatures ($>1000\text{ }^{\circ}\text{C}$) which limits the type of substrates to materials with a high melting point such as metals. Additional process steps are then required to transfer the graphene on to the desired substrate which may also introduce defects such as crumpling, tearing and impurities. As graphene performance degrades very rapidly in the presences of these defects, it is critical to ensure the optimal quality of graphene is transferred [190].

Plasma-enhanced CVD (PECVD) is a promising variant to deposit high-quality graphene at reduced temperatures and low energy consumption [191]. Plasma can be artificially induced in a neutral gas by applying extreme heat or strong electromagnetic fields. Due to the excess energy, the molecular bonding of the gas is easily dissociated, and different chemically active species of the gas are formed which reacts with the substrate surface forming graphene [192]. PECVD has been introduced as an alternative method to enable the growth of graphene at low temperatures as low as $240\text{ }^{\circ}\text{C}$ [193]. Positive results had been reported by different teams and proved promising in achieving high-quality graphene deposition using different plasma sources such as radio frequency (RF) and microwave plasma [191,192]. A typical PECVD system is presented in Figure 2.32 where the plasma is generated using an RF source and the sample is heated using an IR lamp.

Figure 2.32: (a) Schematic of a PECVD system for graphene deposition (adapted from [192])

However, due to the direct interaction of plasma on the substrate surface, disorder is typically introduced into the graphene which is an indication of the presence of defects. There is also a higher deposition rate of amorphous carbon which may affect the quality of the deposited graphene [194]. It is possible to reduce the defect density by optimisation of the parameters, but it cannot be completely resolved. Hence, even though PECVD was able to achieve single to few-layer graphene, its quality was not comparable to that of thermal CVD. However, the potential to grow graphene at low temperatures can allow direct deposition on non-metal substrates which may significantly reduce the development time of graphene-enhanced devices [195].

2.4.2 Electro Control of Graphene-Enhanced SPR Biosensors

Due to graphene's unique optical and electrical properties, its dielectric property can be reported tuned by changing its electron charge density via electrostatic gating [196]. As SPR biosensors are highly sensitive to the optical and electrical properties near the metal-dielectric interface, a change in the charge density would inevitably result in a change in the resonance condition. This means that graphene can potentially be used in the structure of an SPR biosensor to develop a novel SPR interrogation method by applying an external electric field across the metal-dielectric interface.

Recent work has developed a theoretical model and experimentally validated this concept by applying different voltages using a direct current (DC) source to tune the resonance condition [197]. In another work, an electrochemical cell replaces the dielectric medium to allow better modulation of the potential at the metal-dielectric interface [198]. The result from their simulation studies is shown in Figure 2.33 where a clear shift in the resonance angle is observed as the voltage is scanned from -200 mV to +200 mV.

Figure 2.33: Reflection spectra of the SPR biosensor reported by Abayzeed, S. A et. al. when different voltage is applied (adapted from [198])

The primary advantage of an electrically controlled SPR biosensor is that the design can be much simpler as there is no need for highly precise rotation stages which are necessary for angular interrogation or spectrometers which are used for spectral interrogation. The modulation of the voltage can be achieved with very high precision by using relatively low-cost and compact electronics; therefore, the overall cost of such biosensors would be relatively cheaper than those configured in the other interrogation modes. Hence, another focus of this thesis would be to investigate the potential of using a graphene-layered hetero-interface for the development of electrically controlled SPR biosensors.

2.5 Outcome of Literature Review

Based on the intensive review conducted on the Global Burden of Diseases (GBD) cardiovascular diseases, cancers, mental and neurological disorders are three of the significant contributors to the disease burden. This is also an indication of the gap in

current health standards to cope with the current disease burden. This problem would be further amplified in Singapore due to an ageing population as the elderly have a high risk of developing the mentioned diseases. Hence, there is an urgent need to improve current health strategies to manage the risk for all these diseases.

In the case of cardiovascular diseases, the trend in Singapore is decreasing. This suggests that efforts to improve the prevention of cardiovascular diseases coupled with advancement in medical technology to treat cardiovascular diseases have been relatively successful. Therefore, it is not a priority for this thesis.

Cancer is challenging to manage as it can develop in any part of the body which makes it difficult to detect early. Of all forms of cancer, female breast cancer has the highest incidence rate globally. It also has the highest mortality rates in women, both worldwide and in Singapore. Despite such high mortality rates, breast cancer has very promising survival rates for it is detected and treated early. Therefore, it is critical to improve the uptake of breast cancer screening for the early detection of breast cancer. The critical gap in existing breast cancer detection techniques lies in the perception of women and their lack of understanding regarding the process. Besides, most women may be too self-conscious to go for breast screening. Therefore, there is an urgent need to develop a less invasive technique for the early detection of breast cancer.

The global mental and neurological disorders burden is a rapidly growing problem, hence, there is an urgent need to address the existing strategies to help manage its risk. Out of all mental and neurological disorders, dementia is projected to have one of the highest financial burdens and has already been declared by WHO as a public health priority. The most common cause of dementia is Alzheimer's disease (AD). Efforts to treat AD have found little success and the risk factors of AD are still not fully understood to develop

effective preventive measures. Nonetheless, the early detection of AD proved to be a potentially effective method for early intervention which may help slow down or halt its progression. However, existing techniques for detection of AD is primarily based on neuroimaging which is expensive and have high chances of overdiagnosis. Hence, there is an urgent need to develop an alternative, low-cost method for the early detection of AD based on non-neuroimaging techniques.

Biosensors could be a suitable candidate to provide highly sensitive and in situ detection of disease biomarkers. This is highly desired in female breast cancer screening where the biosensors would enable women to conduct the test in a private setting as per their convenience. Another benefit is with the improvement of existing fabrication techniques, the cost per biosensor may be further reduced to allow for more regular screening. This would allow the early onset of both breast cancer and AD to be detected at the earliest instance which would result in better chances of survival.

Currently, most biosensors are electrochemical based as it is cheap and relatively easy to fabricate. However, in recent years, optical biosensors have also been gaining popularity as it has better sensitivity. Out of the many different optical biosensors, SPR biosensors are highly attractive due to its excellent sensitivity and flexibility in design which can be suited for a wide variety of biomedical applications. However, one key gap which is preventing the mass adoption of SPR biosensors in clinical settings is its high cost due to the expensive equipment needed to produce the features necessary for the biosensors which are at the scale of the wavelength of optical light.

State of the art nanofabrication techniques are well-established in the semiconductor industry such as advanced lithography can be adopted for the research and development of the sensors. However, the equipment may be too expensive, and the materials may not

be biocompatible or suitable for biosensing applications. Interference lithography (IL) could be a potential candidate to fabricate the high-resolution features necessary for the optical biosensors with relatively low cost. Moreover, IL also has the flexibility of producing periodic features with customised design and geometry to suit different biosensor design. This is especially true as the grating features necessary for grating coupled SPR biosensors can be easily fabricated using IL. Therefore, IL could be a useful alternative method to mass-produce biosensors at a low-cost.

Furthermore, AM techniques are also discussed as a potential method for fabricating biosensors economically. The main benefit of AM is their ability to produce complex hollow structures which could be used as microfluidic devices or channels to contain the sample fluid for sensing. This is highly desirable as the sample volume can be greatly reduced and the sample preparation steps can be integrated directly into the biosensor chip itself. However, the main drawback of AM techniques is their low resolution. Therefore, a hybrid system combining both IL and AM could be developed to maximise the benefits of both systems. For example, the high-resolution features can be first fabricated using IL while the AM system can take over to finish the remaining less critical features of the biosensor. This could form part of the potential future work to extend the work of this thesis.

Besides investigating the fabrication of SPR biosensors, another key area of focus is to improve the accuracy of the biosensors so that it can deliver highly accurate results which are comparable to other well-established detection techniques. One proposed method is to introduce nanoparticles which are commonly used in localised SPR (LSPR) biosensors. By adding nanoparticles in the structure of the SPR biosensors, the surface plasmon (SP) wave which are used for sensing can be further confined to a localised region near the

sensing surface which would amplify the optical signal. Also, the surface area would be increased which means more bioreceptors can be immobilised on the surface and increase the active sensing sites. This would allow a smaller concentration of the analytes to be detected which would be useful to detect the early onset of diseases where the concentration level of the analytes is very low.

Another approach to improve the sensitivity of the SPR biosensor is to integrate a layer of graphene. As graphene has better biocompatibility, it should allow more bioreceptors to be immobilised which would result in better sensitivity as there are more analytes from the sample fluid would be attracted to the sensing surface and signal would be increased. Additionally, due to the novel optical and electrical properties of graphene, the plasmonic response of a graphene-enhanced optical SPR biosensor could be tuned by controlling its charge carrier density which can be achieved by applying a biased voltage across the graphene-layered metal-dielectric interface of the SPR biosensor. This could lead to the development of an electrically controlled SPR biosensor which can be produced with less sophisticated electronics and can greatly reduce the cost of the SPR biosensors.

This literature review critically analysed the different female breast cancer and AD detection techniques available in clinical practice and discuss the need for a biosensor as an alternative diagnostic tool. Out of the many types of biosensors, optical SPR biosensor has been identified as the most ideal type of biosensor for the targeted application. The research gap identified is in the fabrication techniques employed to produce the high-resolution features necessary for the SPR biosensors. Therefore, this forms one of the objectives of this research, which is IL based fabrication of nanoscale grating features as targeted features in grating coupled optical SPR biosensors. One key design consideration

of the SPR biosensor is to keep the spectral response of the optical signal in the visible to near-IR wavelength band which may be more cost-effective to integrate with existing technologies.

Another research gap identified is to improve the accuracy of optical SPR biosensors so that it is comparable to current well-established detection techniques. Therefore, the second research objective is to improve the sensitivity of the optical biosensor using nanoparticles. Since nanoparticles have been widely used in LSPR biosensors, it is envisioned that nanoparticles could potentially be used with conventional SPR biosensors to amplify the optical signal for the detection of very low concentrations of analytes. This would allow the biosensors to detect the early onset of diseases where the concentration level of the analytes is less significant and can also improve the patient's confidence in the test results.

Lastly, the third research objective is to develop a graphene layered heterostructure for use in the SPR biosensor. The few critical challenges anticipated is to synthesise single layer graphene and to perfect the transfer of the graphene onto the desired substrate. The graphene layered hetero interface could be developed into an electrically controlled SPR biosensor which could bring more flexibility in the future development of biosensors.

CHAPTER 3

OPTICAL INTERFERENCE LITHOGRAPHY – FABRICATION OF NANOSCALE GRATING FEATURES FOR SPR BIOSENSORS

This chapter investigates the potential of using optical interference lithography (IL) to fabricate nanoscale grating features for use in a grating coupled SPR biosensor. The phenomenon of light wave interference is first studied by theoretical formulation and simulation to understand the critical parameters and their effects on the quality of the features fabricated by the IL. Two different types of IL systems based on a diffraction grating and single-input multiple-output (SIMO) optical fibre splitter are experimentally validated. The nanoscale grating features were then developed into a grating coupled SPR biosensor to demonstrate its potential for the fabrication of biosensors.

3.1 Theoretical Formulation to Generate Interference Patterns

Light can be described as a transverse-electromagnetic wave which propagates in a straight line in space with a distinctive electric field and magnetic field that oscillates orthogonal to each other. The oscillations are perpendicular to the propagation direction and their oscillation speed is defined as the frequency (f) which is described as the number of cycles per second. The distance between each cycle is known as the wavelength (λ) of the light wave.

In the context of IL, the light waves are described by transforming Maxwell's equation with the assumption that it is propagating in isotropic, linear, non-dispersive, non-conducting, and homogeneous materials. Since the features formed by interference

patterns are usually much smaller than the beam diameter, the interfering beams are assumed to be plane waves and the electric fields (**E**) and magnetic fields (**H**) can be expressed with the respective simplified sinusoidal solutions [199],

$$\mathbf{E}(\mathbf{r}, t) = \mathbf{E}_0 \cos(\omega t - \mathbf{k} \cdot \mathbf{r} + \phi_0) \quad (3.5)$$

$$\mathbf{H}(\mathbf{r}, t) = \mathbf{H}_0 \cos(\omega t - \mathbf{k} \cdot \mathbf{r} + \phi_0) \quad (3.6)$$

where, **E**₀, **H**₀ is the respective complex electric and magnetic field amplitude vectors, **k** is the wave vector which determines the propagation direction and wavelength of the light wave, **r** is the position vector; ω is the angular frequency of the wave, t is the time, and ϕ_0 is the phase angle.

As illustrated in Figure 3.1, interference of light waves occurs when the optical path of two or more light waves with the same λ , intersects with each other at a particular angle, θ with respect to the normal axis.

Figure 3.1: Illustration of two interfering plane waves with the same wavelength but at an angle to each other and the resulting interference pattern (adapted from [199])

The result is a superposition of the two waves and the total electric field ($\mathbf{E}_T$) in the interfering region can be described as the sum of the individual electric fields of the two plane waves [199],

$$\mathbf{E}_T(\mathbf{r}, t) = \mathbf{E}_1(\mathbf{r}, t) + \mathbf{E}_2(\mathbf{r}, t) \quad (3.7)$$

where, $\mathbf{E}_1(\mathbf{r}, t)$ and $\mathbf{E}_2(\mathbf{r}, t)$ are the electric fields of the two plane waves respectively.

By substituting Eqn (3.5) into Eqn (3.7), the total electric field $\mathbf{E}_T$ can be written as,

$$\mathbf{E}_T(\mathbf{r}, t) = \mathbf{E}_1 \cos(\omega t - \mathbf{k}_1 \cdot \mathbf{r} + \phi_0) + \mathbf{E}_2 \cos(\omega t - \mathbf{k}_2 \cdot \mathbf{r} + \phi_0) \quad (3.8)$$

and by assuming the amplitude of both waves to be equal, (i.e., $\mathbf{E}_1 = \mathbf{E}_2$), Eqn (3.8) can be written equivalently as,

$$\begin{aligned} \mathbf{E}_T(\mathbf{r}, t) &= \mathbf{E}_1 [\cos(\omega t - \mathbf{k}_1 \cdot \mathbf{r} + \phi_0) + \cos(\omega t - \mathbf{k}_2 \cdot \mathbf{r} + \phi_0)] \\ &= \mathbf{E}_1 2 \left[\cos \frac{(2\omega t - (\mathbf{k}_1 + \mathbf{k}_2) \cdot \mathbf{r} + 2\phi_0)}{2} \cdot \cos \frac{((\mathbf{k}_1 - \mathbf{k}_2) \cdot \mathbf{r})}{2} \right] \end{aligned} \quad (3.9)$$

The intensity (I) of the resultant wave is thus,

$$\begin{aligned} I(\mathbf{r}, t) &= |\mathbf{E}_T(\mathbf{r}, t)|^2 = \mathbf{E}_T(\mathbf{r}, t) \cdot \mathbf{E}_T^*(\mathbf{r}, t) \\ &= \mathbf{E}_1^2 4 \left[\cos^2 \frac{(2\omega t - (\mathbf{k}_1 + \mathbf{k}_2) \cdot \mathbf{r} + 2\phi_0)}{2} \cdot \cos^2 \frac{((\mathbf{k}_1 - \mathbf{k}_2) \cdot \mathbf{r})}{2} \right] \end{aligned} \quad (3.10)$$

and the time-averaged intensity can be written as,

$$\begin{aligned} I(\mathbf{r}) &= \mathbf{E}_1^2 4 \left\langle \cos^2 \frac{(2\omega t - (\mathbf{k}_1 + \mathbf{k}_2) \cdot \mathbf{r} + 2\phi_0)}{2} \cdot \cos^2 \frac{((\mathbf{k}_1 - \mathbf{k}_2) \cdot \mathbf{r})}{2} \right\rangle \\ &= 4 \mathbf{E}_1^2 \left[\frac{1}{2} \cdot \cos^2 \frac{((\mathbf{k}_1 - \mathbf{k}_2) \cdot \mathbf{r})}{2} \right] \\ &= 2 \mathbf{E}_1^2 \left[\cos^2 \frac{((\mathbf{k}_1 - \mathbf{k}_2) \cdot \mathbf{r})}{2} \right] \end{aligned} \quad (3.11)$$

Therefore, the intensity distribution of the resultant wave is a cosine-squared function and from Eqn (3.11), the maximum intensity is observed only when,

$$\cos^2 \frac{((\mathbf{k}_1 - \mathbf{k}_2) \cdot \mathbf{r})}{2} = 1 \quad (3.12)$$

or when,

$$\frac{((\mathbf{k}_1 - \mathbf{k}_2) \cdot \mathbf{r}_m)}{2} = m\pi, \quad m \in \mathbb{Z} \quad (3.13)$$

where $\mathbf{r}_m$ is the position of the m^{th} number of maximum intensities and m is an integer ($\mathbb{Z}$). As the magnitude of the two wave vectors are equal (i.e. $|\mathbf{k}_1|=|\mathbf{k}_2|$), Eqn (3.13) can also be written as,

$$\begin{aligned} \frac{(\mathbf{k}_1 \cdot \mathbf{r}_m) - (\mathbf{k}_2 \cdot \mathbf{r}_m)}{2} &= m\pi \\ [(k_1 \sin \theta) - (-k_1 \sin \theta)]r_m &= 2m\pi \\ 2(k_1 \sin \theta)r_m &= 2m\pi \\ \frac{2\pi}{\lambda} \sin \theta r_m &= m\pi \\ r_m &= \frac{m\lambda}{2 \sin \theta} \end{aligned} \quad (3.14)$$

From this, the distance between the positions of two adjacent maxima can be derived by calculating the difference in the position of the first maximum (r_1) and the position of the second maximum (r_2) [163],

$$r_2 - r_1 = \frac{(2)\lambda}{2 \sin \theta} - \frac{(1)\lambda}{2 \sin \theta} \quad (3.15)$$

which gives the fringe-to-fringe spacing or periodicity (Λ) of the interference pattern as,

$$\Lambda = \frac{\lambda}{2 \sin \theta} \quad (3.16)$$

This agrees with the theoretical values found in the literature which was given previously in Eqn (2.6). From this, we can see that it is possible to achieve an interference pattern with a periodicity that is smaller than the wavelength of the incident light source. The periodicity can be easily tuned by varying the θ between the two plane waves. By exposing a photosensitive material to the interference pattern, IL can easily write nanoscale, periodic structures without the need for photomasks.

3.1.1 Critical Factors for Forming Interference Patterns

One of the most critical factors for consideration when working with IL systems is the contrast (C) of the interference pattern. This is described as the intensity ratio between values of the maximum ($I_{\max}$) and minimum ($I_{\min}$) intensity of the interference pattern which is defined as [199],

$$C = \frac{I_{\max} - I_{\min}}{I_{\max} + I_{\min}} \quad (3.17)$$

The contrast is highly sensitive to the polarization orientation of the electric fields of the interfering beams and is defined as the vector dot product of the two polarization vectors of $\mathbf{E}_1$ and $\mathbf{E}_2$ which is given as [200],

$$C = |\mathbf{e}_1 \cdot \mathbf{e}_2^*| \quad (3.18)$$

where $\mathbf{e}_1$ and $\mathbf{e}_2$ are the polarization vectors of the two plane waves respectively. From this, we can see that for a pair of s-polarized light, the contrast is always equal to 1 as the angle between the two polarization vectors is always 0, regardless of the incidence angle. However, for the p-polarization case, the angle between the two polarization vectors changes in relation to the angle of incidence and contrast can then be expressed as,

$$C_{p-p} \propto |\cos 2\theta| \quad (3.19)$$

This shows that minimum contrast will be observed when the incidence angle is at 45° and the two polarization vectors are orthogonal. Hence, interference would not happen if the polarization states of each beam are orthogonal or perpendicular to one another. Figure 3.2 shows the variation in contrast with different interference angles for the two polarization pairs. Hence, s-polarized light waves are typically preferred to be used for IL as having good contrast in the interference pattern is ideal for producing high-quality grating features with well-defined lines and edges.

Figure 3.2: Interference contrast plots of a pair of s-polarized and a pair of p-polarized light

Another important factor to be considered for the formation of the interference patterns is the coherence length (L_c) of the light source used which is given as [201],

$$L_c = \sqrt{\frac{2 \ln 2}{\pi}} \left(\frac{\lambda_0^2}{\Delta \lambda} \right) \quad (3.20)$$

where λ_0 is the centre wavelength and $\Delta \lambda$ is the spectral bandwidth of the light source. The interference pattern could only be successfully formed if the optical path difference (OPD) between the two interfering beams is lesser than the L_c of the light source. This means that having a light source with high coherence length is ideal as it would be easier to align the beams to ensure the OPD is within the limits of the L_c . Also, the L_c limits the

maximum size of the interference pattern. Hence lasers with long L_c are typically preferred to be used in IL.

3.1.2 Theoretical Simulation for Validation of the Interference Pattern

Beside solving Maxwell's equation using the theoretical formulation, it is also possible to use theoretical simulation tools to assist in visualisation and confirmation of the interference pattern. Finite-difference time-domain (FDTD) is a well-developed numerical analysis technique used primarily for finding approximate solutions to Maxwell's equation which is widely used to model electromagnetic wave interactions. The intensity distribution of interfering waves can be simulated directly to verify the interference pattern. Figure 3.3 shows the simulated interference pattern formed by a pair of s-polarized, 351 nm light source at a 45° angle of incidence.

Figure 3.3: Simulated intensity distribution showing the interference pattern

The periodicity of the simulated interference pattern can then be measured by taking the line profile plot in the x-direction which is shown in Figure 3.4. The peak centres can then be easily identified by a fitting program and the average distance between the peaks

would correspond to the periodicity which is calculated to be approximately 248.2 nm (refer to Appendix E for the full calculation steps).

Figure 3.4: Line profile plot of the simulated interference pattern

For comparison purposes, the periodicity can also be calculated using Eqn (3.16) by substituting in the same parameters which give,

$$\Lambda = \frac{351 \text{ nm}}{2 \sin(45^\circ)} \approx 248.2 \text{ nm} \quad (3.21)$$

Hence, the simulated interference pattern generated using FDTD methods is comparable to the theoretical formulated model. This confirms that FDTD is a highly accurate tool to predict the expected interference pattern of an IL system. The main benefit of using FDTD simulation is the ease in using the software and relatively short time needed to solve the model. Additionally, more complex interference patterns formed by multi-beam IL can also be easily simulated as shown in Figure 3.5. The simulated interference patterns are consistent with the results from the literature where different lattice patterns were generated by adjusting the state of polarization independently for two pairs of interfering beams [163].

Figure 3.5: Simulated interference pattern of (a) a pair of s-polarized light with a pair of p-polarized light, and (b) two pair of s-polarized light

Therefore, FDTD simulation is a useful technique to verify the resulting interference pattern of an IL system. This could potentially be used for the development of IL systems for the fabrication of more complex periodic 2D and 3D features.

3.2 Experimental Validation of IL System

To keep within the limits of the coherence length, most IL systems use a single light source and employ beam splitters to split the light beam into the desired number of sub-beams. There are a variety of methods for beam splitting and they are illustrated in Figure 3.6.

Figure 3.6: Beam splitting by (a) partially reflecting mirror, (b) cube-beam splitter, (c) optical fibres, and (d) diffraction gratings

A partially reflecting mirror is formed by coating a thin layer of reflecting material over a transparent substrate or sometimes, pellicles of the reflecting material can be directly mounted on a frame. When light is incident on the material, a portion of the light passes through while another portion of the light is reflected off the material. As a result, the incident light is split along two light paths. A cube beam splitter is formed by attaching two prisms on one of their faces. The interface would first be coated with a partially reflective material to achieve the same effect as a partially reflective mirror. Alternatively, multiple optical fibres can also be spliced together to create a single-input-multiple-output (SIMO) optical fibre [202]. As light enters one end of the optical fibre, it would be split into multiple sub-beam as they exit from each end of the spliced optical fibre. A diffraction grating can also work like a beam splitter where incident light is diffracted into many individual sub-beams.

Each method has its own merits and shortcomings when used in an IL system. The following sections will discuss two different IL system, one based on a diffraction grating and another based on a SIMO optical fibre. The primary objective of developing an IL system is to fabricate high resolution, nanoscale grating features which will be developed into a grating coupled SPR biosensor.

3.2.1 Diffraction Grating-Based IL System

The diffraction grating-based IL system is well reported in the literature from previous research work done by the group and the schematic of the system is illustrated in Figure 3.7 [203]. The diffraction grating is a custom fabricated phase cross grating (Ibsen Photonics Pte Ltd) which diffracts a perpendicularly incident light into a single zeroth-order beam and four first-order beams at 45° from the normal axis. The laser source is an argon-gas laser (Coherent Inc., Innova® 90C) configured to emit circularly polarized

laser light at a 364 nm wavelength in continuous wave mode. After the light is diffracted, UV mirrors were then used to direct the beams onto the sample stage to generate the interference pattern. Linear polarizers and attenuators were also added along the optical path to ensure the maximum contrast is achieved in the interference pattern. A hard stop was also added to block the zeroth-order beam since only two beams are necessary for producing the 1D grating pattern. To record the interference pattern, the sample must first be prepared by coating a layer of photoresist (refer to Appendix F for the detailed steps on sample preparation and recording of the interference pattern). After exposing the photoresist to the interference pattern, the photoresist is developed and inspected under a scanning electron microscope (SEM) to confirm the quality of the fabricated features.

Figure 3.7: Schematic of grating-based IL system

The primary advantage of this IL system is that multiple beams can be achieved with just a single optical component. This can simplify the alignment process as there are lesser optical components. Additionally, the phase, intensity, and polarization of each beam can also be independently controlled to produce different patterns. The mirrors can also be tilted to achieve a desired angle of incidence (θ_i) as modelled in Figure 3.8a. Each degree of tilt or rotation in the mirror (θ_{tilt}) towards the sample, would increase the incidence

angle of the beams on the mirrors (θ_{mi}). Since the first-order beams diffract from the grating at a fixed angle of 45° , therefore, θ_{mi} can be expressed as,

$$\theta_{mi} = 45^\circ + \theta_{tilt} \quad (3.22)$$

According to the laws of reflection, the angle of reflection of a light ray leaving a surface would always be exactly equal to the angle of incidence of the incident light ray [204]. Therefore, the relationship between the reflectance angle of the beam leaving the mirror surface (θ_{mr}) and θ_{mi} can be given as,

$$\theta_{mr} = \theta_{mi} \quad (3.23)$$

Hence, the relation between θ_{tilt} and θ_i can be established by simple trigonometry and substituting Eqn (3.22) and Eqn (3.23) where,

$$\begin{aligned} \theta_i &= 90^\circ - (\theta_{mr} + \theta_{tilt}) \\ &= 90^\circ - (45^\circ + \theta_{tilt} + \theta_{tilt}) \\ &= 45^\circ - 2\theta_{tilt} \end{aligned} \quad (3.24)$$

Therefore, it can be concluded that every degree of increment in θ_{tilt} would reduce the θ_i by double the amount. Substituting this into Eqn (3.16), we can establish the relationship between θ_{tilt} and the periodicity of the resulting interference pattern formed, which is plotted in Figure 3.8b,

$$\Lambda = \frac{364 \text{ nm}}{2 \times \sin(45^\circ - 2\theta_{tilt})} \quad (3.25)$$

By using this formula, it is easy to adjust and system to achieve a targeted periodicity. This also suggests that a diffraction grating-based IL system has the potential to be used for the fabrication of periodic features ranging from a few hundreds of nanometres (nm) to a few micrometres (μm).

Figure 3.8: (a) Changing angle of incidence with mirror tilt, (b) plot of periodicity against mirror tilt

To validate the IL system, the targeted periodicity is set to 500 nm. Therefore, based on Eqn (3.25), the tilt angle of the mirrors is calculated to be approximately 11.8°. After adjusting the mirror, the interference pattern is recorded in the photoresist and SEM image of the sample is shown in Figure 3.9a. The image is further analysed in ImageJ to extract the plot profile of the grating features which is presented in Figure 3.9b.

Figure 3.9: (a) SEM image of the grating pattern and (b) plot profile showing the position of the peaks

From the plot profile, the position of the peaks can be easily identified, and the periodicity of the grating features can be estimated from the average distance between each peak to be approximately 526.8 nm. This value is near the targeted value of 500 nm and the

reason for error can be mainly attributed to the low precision of the rotation stage which only has a precision of 1° . Additionally, it is important that the sample stage and diffraction grating are all perfectly parallel to the xy-plane so that all the angles are referenced exactly to the same datum. Nonetheless, the results validated the capability of the diffraction grating-based IL system in producing nanoscale grating features.

One major limitation of the diffraction grating-based IL system is the sheer amount of optomechanical and optical components which is modelled in Figure 3.10. Many of these components (such as the kinematic mounts) are necessary to allow the beams to be perfectly aligned and to allow minor adjustments if needed. However, due to the proximity of the components, there is limited space to access the sample stage or the adjustment knobs of the mounts, making it difficult to align the beams. The components could be spaced further apart, but then the height of the system would also have to be increased proportionally.

Figure 3.10: Model of the diffraction grating-based IL system

This limitation is especially prevalent when the angle of incidence is adjusted to realised patterns with different periodicity as illustrated in Figure 3.11a. Due to the trigonometric relationship, the height difference (Δz) from the grating to the sample plane where the beams interfere varies according to the angle of incidence. This distance is significantly smaller at a larger angle of incidences as compared to the smaller angle of incidences. To demonstrate this, the distance between the two mirrors is fixed at 50 cm while the mirrors are tilted to vary the angle of incidence. The relationship between Δz and θ_{tilt} is plotted in Figure 3.11b which shows that to tilt the mirror in the range of $\pm 20^\circ$, the travel range required for Δz would be from approximately 25 cm to 250 cm. This would be impractical as the optomechanical components would be unnecessarily bulky. Additionally, it would be difficult to align the optics over such a long distance as the alignment error would be magnified significantly.

Figure 3.11: (a) Mirror tilt resulting in different height, (b) plot of height difference against mirror tilt

Therefore, there is a trade-off between having enough space to access the components and the flexibility in achieving different patterns using the IL system. Hence, the diffraction grating-based IL system may not be suitable for the mass production of grating features for SPR biosensors as it is not as versatile.

3.2.2 Flexible Optical Fibre-Based IL System

Besides serving as a beam splitter, a SIMO optical fibre would also provide a flexible medium for beam delivery. The main benefit of this is that the end faces of the optical fibre can be repositioned easily for delivery of the sub-beams at a desired angle of incidence without the use of mirrors or reflectors. Additionally, the polarization and intensity of each of the sub-beams can also be independently controlled to maximize the contrast and generate different patterns. Hence, an optical fibre-based interference lithography (OFIL) system would greatly enhance the versatility of an IL system and greatly reduce its complexity. The schematic model of the proposed OFIL system is shown in Figure 3.12.

Figure 3.12: Schematic of the developed OFIL system

A diode-pumped solid-state laser (CNI Co., Ltd.) with 405 nm emission wavelength was selected as the light source whose long coherence length (> 1 m) is ideal for IL. The optical fibre is designed to be single-mode for 405 nm and the input end is split, using a custom-fabricated fused fibre splitter (FONT Co.), into two output ends with a 50:50 splitting ratio.

The input end was first mounted on a fibre launch system with 3-axis adjustability (Thorlabs Inc.) to ensure maximum coupling of laser light into the optical fibre. A 0.10 NA 4× microscope objective (Olympus Corp.) was also used to tightly focus and couple the laser light into the optical fibre. Each of the output ends was then mounted on separate optical rails which are attached to individual rotation stages that are aligned on the same rotation axis. Therefore, the interference plane would always be at the same distance from the fibre output end regardless of the rotation angle which reduces the need to realign the laser beam after each adjustment. A cage system was also installed at each optical rail for mounting of different optical components as shown in Figure 3.13. Optical components, such as polarizers and lens, can be easily added to the system in a controlled way to reshape the beams for achieving the desired contrast, type of interference pattern and pattern area. The cage system makes it easy to add or remove the optical components and simplify the alignment process with improves the ease of use of the OFIL system.

Figure 3.13: Model of the cage system used in the OFIL system

In the system, a fibre collimator was attached directly to the fibre end face to collimate the laser beam as it exits the optical fibre. A series of plano-convex lenses were then used to expand the diameter of the sub-beams to approximately 5 mm. A linear polarizer was

also added to ensure the sub-beams are s-polarized for the best contrast in the interference pattern. The versatility of this system is demonstrated by fabricating 1D grating features with different periodicities by rotating the optical rails to realise different angles of incidence. Table 3.1 shows the targeted periodicity (Λ_t) values in the interference pattern and the necessary angle of incidence was calculated using Eqn (3.16). The achieved or measured periodicity (Λ_m) of the fabricated 1D grating features is also given in the third column for comparison purposes. These values are calculated by analysing the line profile plot of the SEM images. Based on the measured periodicity values, it is possible to derive and estimate the actual angle of incidence for generating the interference pattern which is given in the last column. There is very little error between the two values which suggest that the rotation stages are sufficiently accurate enough for generating patterns ranging from 500 nm to 1,500 nm.

Table 3.1: Required angle of incidence for the targeted periodicity and the actual achieved periodicity

Targeted periodicity (Λ_t)	Required angle of incidence, θ	Achieved / measured periodicity (Λ_m)	Estimated angle of incidence
500 nm	23.89°	514 nm	23.2°
1,000 nm	11.68°	980 nm	11.9°
1,500 nm	7.27°	1,646 nm	7.1°

The SEM images of the different 1D grating features and the respective line profile plots extracted from the SEM images are shown in Figure 3.14. In the figure, each column is a set of three images with the corresponding Λ_t values labelled at the top while the respective Λ_m values are given at the bottom of the figure. The images in the top rows show the features at 3,500x magnification while the images in the middle row are at a higher magnification of 20,000x. The respective line profile plot of the grating features is extracted from the SEM images and is given at the images at the last row. The grating

features are observed to be uniformly distributed throughout the entire patterned area and have relatively good contrast. Hence, this demonstrates the potential of the OFIL system as a highly versatile method for the fabrication of grating features with a wide range of periodicity.

Figure 3.14: SEM images of the grating features at (a)-(c) 3,500 $\times$, and (d)-(f) 20,000 $\times$ magnification.

(g)-(i) Line profile plot of the respective grating features extracted from the SEM images.

The Λ_t of the grating features is labelled at the top of each column while the Λ_m is given at the bottom.

A possible explanation for the difference between the Λ_t and Λ_m values of the 1D grating features could be attributed to the zero error with respect to the interference plane and due to the accuracy of the rotation stage (1 $^\circ$ resolution). One possible method to reduce

this error is to replace the rotation stages with motorised rotation stages to achieve more precise control of the angle of incidence. Different optical components such as a flat-top beam shaper can also be added to modify the beam profile to achieve a more uniform intensity distribution in the interference pattern which is ideal for IL.

3.3 Grating Coupled SPR Biosensor for Sucrose Sensing

The potential of using the IL as an alternative method to fabricate low-cost biosensors was demonstrated by developing the grating features in a grating coupled SPR biosensor. For demonstration, a grating couple SPR biosensor was developed for sucrose sensing. Before the grating features were fabricated, theoretical studies were first conducted to optimise the grating design for optimum coupling of light and sensitivity in the biosensor. Next, the grating features were fabricated using the OFIL system and developed into a grating coupled SPR sucrose biosensor.

3.3.1 Parametric Study for Optimisation of Grating Coupled SPR Biosensor Design

As discussed previously, the resonance condition for a grating coupled SPR biosensor is given by combining Eqn (2.1) and Eqn (2.3) which gives,

$$\mathbf{k}_{\text{SP}} = \mathbf{k}_x^m$$

$$\mathbf{k}_0 \sqrt{\frac{\varepsilon_d \varepsilon_m(\omega)}{\varepsilon_d + \varepsilon_m(\omega)}} = \mathbf{k}_0 n_d \sin \theta + m \frac{2\pi}{\Lambda} \quad (3.26)$$

The main variables influencing the resonance condition is the effective permittivity of the metal-dielectric interface. Since the metal coating remains unchanged, therefore, $\mathbf{k}_{\text{SP}}$ is only sensitive to the relative permittivity of the dielectric layer (ε_d) which has the following relationship,

$$n_d = \sqrt{\epsilon_d \mu_d} \quad (3.27)$$

where μ_d is the permeability of the dielectric material. Therefore, any change in the RI of the dielectric medium (n_d) would also affect $\mathbf{k}_{SP}$ and result in a shift in the resonance condition. Hence an SPR biosensor is effectively a RI sensor.

Due to the dispersion of light, each wavelength would diffract at a different angle, hence, a grating coupled SPR system has the advantage of being able to be configured for both angular and spectral interrogation mode as shown in Figure 3.15. In angular interrogation mode, the wavelength of the light would be fixed by using a laser source while the angle of incidence is slowly scanned. Due to this, the angle in which the light diffracts is changed which increase in the magnitude of the wave vector along the interface direction. When the wave vector increases until it matches that of the SP, the resonance condition is satisfied which results in a drop in the reflectance of the diffracted light.

In wavelength interrogation mode, a broadband light source is used while the angle of incidence remains constant. A spectrometer would then be employed to measure the spectrum of the reflected or transmitted light after it is incident on the grating. As each wavelength of light has a distinctive wave vector, the angle in which the individual wavelengths of light diffract is also different. Hence, there would be a particular wavelength would have a wave vector that perfectly matches that of the SP. Therefore, the resonance wavelength can be easily identified by monitoring the spectrum of the reflected light as the reflectance at that wavelength would be significantly reduced as compared to the other wavelengths.

Figure 3.15: Grating coupled SPR biosensor in (a) angular, and (b) spectral interrogation mode

The primary advantage of spectral interrogation over angular interrogation mode is the ability to achieve real-time sensing as the entire optical spectral signal can be captured within a very short sampling time. Hence, spectral interrogation mode is selected for the design of the grating coupled biosensor. This chapter will primarily focus on spectral interrogation mode while the spectral interrogation design will be discussed in the next chapter.

The spectral response is targeted to be in the wavelength band from visible to near-infrared (NIR) as the light source and spectrometers in this wavelength range are readily available and easy to align. In this context, a parametric study was undertaken using FDTD simulations to understand the effects of the various geometric parameters of the grating features on the performance of the SPR biosensor. The grating geometric parameters are defined in Figure 3.16.

Figure 3.16: Definitions of the important geometric parameters of a grating

In the FDTD simulations, the material for the grating and the underlying metal film was chosen to be gold (Au) which is conventionally used for SPR biosensors due to its good biocompatibility. As shown in Figure 3.17, the light source was set to incident normally (purple arrow indicates the propagation direction) on the grating features and the spectrum of the reflected light is monitored. The light source is also set to be p-polarized (blue arrow indicates the polarization angle) from 400 nm to 1,000 nm. The background (black region) is in a dielectric medium with a refractive index (RI) of 1.33 to simulate a sample fluid which is deionised water (DI water) and substrate (red region) is set as silicon (Si).

Figure 3.17: FDTD simulation screen in (a) x-z view, and (b) perspective view

One critical figure of merit used to assess the performance of the SPR biosensor is the coupling efficiency between the optical light and the SP at the point of resonance. Ideally, the coupling should be maximised to have a very high signal to noise ratio so that the resonance wavelength can be easily identified. Hence, the following parametric study aims to investigate the most optimum grating geometry and dimensions to ensure the SPR signal can be easily identified for sensing.

The simulation is to determine the minimum thickness necessary for the metal film layer and the simulation result is shown in Figure 3.18a. As can be seen from the figure, if the

layer is too thin (30 nm), it would not be possible to support SP as the waves would penetrate through the metal film and reach the substrate layer which results in poor coupling efficiency. As the layer thickness increases, the coupling efficiency also increases until it reaches the maximum (50 nm). Therefore, the optimum metal film thickness is set to 50 nm as there is no extra benefit in using excess material.

Next, the second set of simulation is to determine the optimum grating height and the result is shown in Figure 3.18b. From the result, the grating height should ideally be around 250 nm to 260 nm for maximum coupling efficiency. However, it is also important to note that the grating height would also affect the resonance wavelength.

Figure 3.18: Simulated reflectance plot with varying (a) metal film thickness, and (b) grating height

The next set of simulations is to determine the periodicity of the grating feature and the result is shown in Figure 3.19a. Based on Eqn. (3.26), we can expect the resonance condition to change significantly with varying grating periodicity. This is confirmed by the simulation result where the resonance wavelength increases with increasing periodicity. Therefore, choosing the appropriate periodicity is important as it would control the operating wavelength of the SPR biosensor. Since the IL system has

demonstrated its potential in fabricating grating features with 500 nm and the expected resonance wavelength is also in the targeted wavelength range, therefore, the periodicity of the grating is fixed at 500 nm.

Finally, the last set of simulation for the parametric study is to determine the duty cycle of the grating and the result is given in Figure 3.19b. From the result, a duty cycle of 0.5 gives the maximum coupling efficiency. A higher or lower duty cycle would reduce the coupling efficiency which is indicated by an increased reflectance at the resonance wavelength. The targeted duty cycle of grating features can be easily modified by controlling the exposure dose of the laser beam in the IL system.

Figure 3.19: Simulated reflectance plot with varying (a) grating periodicity, and (b) duty cycle

Based on the optimised parameters, the sensitivity of the SPR biosensor can also be estimated by increasing the RI of the sample fluid to simulate the increasing concentration of sucrose. For the biosensor to be effective, its sensitivity should ideally be as high as possible which is defined as the shift in the resonance wavelength shift for each increase in refractive index unit (RIU).

For comparison purposes, the optimised Au grating design will be compared against a grating made of photoresist material. A photoresist grating would be beneficial in

reducing the fabrication steps for producing the SPR biosensors. Therefore, this simulation will be used to benchmark the theoretical sensitivity between the two materials when used in a grating coupled SPR biosensor.

The simulation was first conducted with a sample fluid that has a RI of 1.33 and repeated with a RI of 1.34. Next, the Au grating is replaced with a photoresist grating and the simulation is repeated. The simulated reflectance plot of both grating designs is presented in Figure 3.20 where the solid lines are for a RI of 1.33 and the dashed lines correspond to a RI of 1.34.

Figure 3.20: Simulated reflectance plots for the two different grating designs
at RI = 1.33 (solid line) and RI = 1.34 (dotted line)

For both designs, the resonance wavelength can be identified by the sharp dip in the reflectance. However, the coupling efficiency of the photoresist grating is very poor as compared to the Au grating design. This could be due to a variation in the refractive indices of the different layers. Nonetheless, the sensitivity of each grating design is then calculated by dividing the wavelength shift by the RI change in the sample fluid and given in Table 3.2.

Table 3.2: Respective resonance wavelength and sensitivity of the different grating designs

Grating design	Resonance wavelength, λ_1 (nm) [$n_1 = 1.33$]	Resonance wavelength, λ_2 (nm) [$n_2 = 1.34$]	Sensitivity, $\frac{\lambda_1 - \lambda_2}{n_1 - n_2}$ (nm/RI)
Au grating	696.848	701.601	475.3
Photoresist grating	718.109	720.610	250.1

From the table, the photoresist grating design has a lower sensitivity as compared to the Au grating design. This suggests that having a high coupling efficiency would also have a direct relation to the sensitivity of the biosensor. Next, the next step would be to fabricate the grating features as per the optimised design and transfer the pattern into the Au material for experimental validation of the SPR biosensor.

3.3.2 Experimental Validation of the Grating-Coupled SPR Biosensor

A two-step lift-off process was used to fabricate the Au grating features as outlined in Figure 3.21. First, 50 nm layer of Au is sputtered over the Si with a 3-5 nm layer of chromium (Cr) adhesion. Next, an approximately 1 μm thick layer of photoresist is spin-coated over the Au layer and the OFIL system is used to generate a 1D grating interference pattern with 500 nm periodicity. To control the duty cycle of the Au grating feature, the exposure dose of the laser beam is varied to achieve a duty cycle of about 0.5. After the desired pattern is formed in the photoresist, the second layer of Au which is approximately 250 nm thick is deposited over the patterned photoresist. The Au would fill the gaps in between the grating features and thus, the desired Au grating features are fabricated. Subsequently, the photoresist is lift-off in an acetone bath which removes the photoresist grating pattern and the desired Au grating pattern is left on the Si substrate.

Figure 3.21: Au grating fabrication process using the lift-off method

The Au grating was then developed as an SPR biosensor in the spectral interrogation mode as shown in Figure 3.22. A broadband halogen light source is first collimated and directed through a polarizer and a cube beam splitter to incident TM polarized light normally to the surface of the Au grating. The reflected light from the Au grating is then collected by a collector lens which is coupled to a spectrometer (USB4000, Ocean Optics, Inc.). The fluidic cell is formed from polydimethylsiloxane (PDMS) (SYLGARD™ 184 silicone elastomer, Dow Inc.) using soft lithography.

Figure 3.22: Schematic model of the grating coupled biosensor in spectral interrogation mode

Fluids with different concentrations of sucrose were then introduced into the fluidic cell through the inlet and outlet of the fluidic cell. As the fluid covers the surface of the grating, the reflection spectra are captured and shown in Figure 3.23a. From the figure, a sharp dip in the reflection spectra is observed at the resonance wavelength for all the sample fluids and there is a redshift with increasing sucrose concentration. This confirms that the sucrose concentration has a direct effect on the refractive index of the sample fluid which would affect the resonance condition at the metallic-dielectric interface formed by the gold and sucrose fluid. The experiment was repeated 3 times to determine the average resonance wavelength values and the respective sucrose concentration values were then converted into the respective refractive indices as shown in Figure 3.23b [205]. From this, the sensitivity of the SPR sensor can be estimated from the linear fit to be 267.29 ± 9.91 nm/RI unit.

Figure 3.23: (a) Spectrum of the reflected light for each sucrose concentration,
(b) plot of the observed resonance wavelength against the refractive index

To confirm that the reflectance is indeed due to the SPR effect, a separate control experiment was also performed. For the first controlled experiment, the polarization was changed from p-polarized to s-polarized light. The spectrum of the reflected light is

plotted in Figure 3.24a. For the s-polarized light, there is no distinctive drop in the reflectance which indicates that there is no resonant coupled of light to excite the SP. This is because the polarization direction is now perpendicular to the grating direction, and therefore, the diffracted light would have a wave vector that is orthogonal to the interface and cannot excite the SP.

Next, the experiment was repeated by taking the reflection spectrum of DI water directly on an Au coated Si substrate without any grating features. The results of this experiment are given in Figure 3.24b. For both p-polarized and s-polarized light, the reflectance curve is flat and the distinctive drop in reflectance is absent. This confirms that the grating features are indeed able to couple incident light to excite the SP. Therefore, this demonstrates the potential of using the OFIL to fabricate grating features which can be developed into a grating coupled SPR biosensor.

Figure 3.24: Spectrum of the reflected light in s-polarization and p-polarized for the
(a) substrate with Au grating, and (b) substrate without any Au grating

3.4 Summary and Discussions

In summary, the versatility and robustness the OFIL system is demonstrated by the fabrication of grating patterns with periodicities ranging from 514 nm to 1,646 nm in an

area of approximately 5 mm in diameter. The patterned area could also be easily varied by adjusting the distance between the lens or using a lens with different focal length without affecting the alignment of the laser beams. As a result, the time needed to readjust and realign the optics for realising different interference pattern or a specific design is significantly reduced. Additionally, the laser beam is expanded only to the desired pattern area which results in minimal energy losses as the unnecessary regions are not patterned. Therefore, it is envisioned that the OFIL system could be easily integrated with other micro and nanoscale fabrication processes such as additive manufacturing techniques which would allow greater versatility and efficiency in the fabrication of micro–nanoscale features for the mass production of photonic devices, such as biosensors and lab-on-a-chip devices for medical and healthcare applications which could be one of the potential future research directions.

Also, the grating features fabricated by the OFIL system were developed into a grating coupled SPR biosensor for sucrose sensing. FDTD simulations were first used to optimise the grating design for maximum sensitivity and the theoretical sensitivity is 475.3 nm/RI. Using the OFIL system, the desired grating features were fabricated and coupled with a PDMS fluidic cell to form the SPR biosensor. The SPR biosensor can sense different concentrations of sucrose with a sensitivity of approximately 267.29 nm/RI. This value is much lower than the theoretical value which could be attributed to variation in the duty cycle of grating features.

As the optical fibre used in the OFIL system is single-mode, the laser beams exiting the optical fibre would have a beam profile that is similar to a Gaussian beam profile. Therefore, the intensity distribution of the interference pattern is not constant throughout the entire region which results in the centre region receiving higher exposure

dose as compared to the peripheral areas. This deviation in exposure dose would cause the grating features to have varying linewidths which would prevent the coupling of light for excitation of the SP wave. This problem could potentially be minimised by using a flat-top beam shaper to redistribute the intensity to be more uniformed (flat-top profile) and achieve a flat phase front.

CHAPTER 4

GOLD NANO-URCHINS ENHANCED SPR BIOSENSORS FOR DISEASES DETECTION

This chapter investigates the potential of using nanomaterial to further enhance the field confinement of surface plasmons in an SPR biosensor. First, a conventional prism coupled SPR biosensor was studied using theoretical simulation and experimental validations. A pair of highly precise rotation stage was employed to ensure good repeatability and high accuracy in the angular interrogation mode. The next section compares different geometries of nanomaterials and the effect on amplifying the shift in the resonance angle when coupled to an SPR biosensor. For demonstration, an LSPR biosensor based on Au nano-urchins was developed into an $A\beta$ sensor which could be used for the early detection of AD. Furthermore, the Au nano-urchins was also integrated into a nanomaterial enhanced SPR biosensor with improved sensitivity for the detection of $ER\alpha$ which is a common female breast cancer marker.

4.1 Conventional Prism Coupled SPR Biosensor

Due to its good stability and relative ease in fabrication, prism coupled SPR biosensor configuration has found huge success and popularity in biomedical research. Though most of the commercially available SPR biosensors are based on the prism coupled design, its bulky size limits its portability. Nonetheless, with the improvement in nanofabrication technologies, it may soon be possible to fabricate prism coupled SPR biosensors using integrated optics design. This way, the biosensor would be more portable and still be highly sensitive with a relatively low manufacturing cost.

A prism coupled SPR biosensor can be set up in either Otto configuration or Kretschmann configuration [131]. In the Otto configuration, the sample fluid is sandwiched between the prism and a metal substrate, however, it is challenging to realise the required separation gap in the fluid as it is very small. Alternatively, in the Kretschmann configuration, a thin layer of metal is coated directly on the prism and the sample fluid flows across the metal-coated surface. This metal layer thickness is within the near-field region ($< \lambda/3$) so that the evanescent wave (EW), generated due to the attenuated total reflection (ATR) of light, can penetrate through it and excite the surface plasmon (SP) wave at the other side of the metal layer.

For resonance to be achieved, the wave vectors of the EW and the SP must be propagating the same direction and have equal momentum (i.e. $\mathbf{k}_{SP} = \mathbf{k}_{EW}$). If this condition is met, then the energy from the EW would be transferred to excite the SP. Hence, Eqn (2.1) and Eqn (2.2) are equal and the resonance condition can be given as,

$$\mathbf{k}_{SP} = \mathbf{k}_{EW}$$

$$\mathbf{k}_0 \sqrt{\frac{\epsilon_d \epsilon_m(\omega)}{\epsilon_d + \epsilon_m(\omega)}} = \mathbf{k}_0 n_{\text{prism}} \sin \theta \quad (4.1)$$

The resonance condition in a prism coupled SPR biosensor can be observed in either the angular interrogation mode or spectral interrogation mode. In the angular interrogation mode, the angle of incidence is the varied parameter while the reflectance is measured using a photodetector. Alternatively, a spatially sensitive photodetector can also be used to detect the shift in resonance angle. When the resonance condition is satisfied, energy from the incident light is transferred to generate the EW which couples resonantly with the SP resulting in a loss of energy in the reflected light. Therefore, the resonance angle

is identified as the point with the lowest reflectance. In the spectral interrogation mode, a broadband light source is incident on the prism and the reflected spectrum is measured using a spectrometer. As the spectral interrogation method has been discussed in detail in the previous chapter, this chapter would focus on the angular interrogation technique instead.

4.1.1 Theoretical Simulation of a Prism Coupled SPR Biosensor

Before the prism coupled SPR biosensor is validated experimentally, FDTD simulation was used to determine the optimal thickness of the metal layer to ensure the greatest coupling efficiency. This is the most important parameter as the metal layer must be thick enough to support the propagating of the SP wave while also be thin enough so that the EW can be able to couple through to excite the SP wave. Au is selected to form the metal layer as it has the best biocompatibility and is relatively stable in atmospheric conditions. A BK7 prism is modelled with varying thickness of Au and illuminated with a p-polarized, 632.8 nm wavelength light source. The angle of incidence is varied in steps of 0.01° with a background RI is set to 1.00 and the result is shown in Figure 4.1 where the various SPR reflectance plots are simulated with different Au thickness from 10 nm to 100 nm.

Figure 4.1: Simulated SPR reflectance plots with varying Au thickness (nm)

From the graphs, 50 nm thick Au has the best coupling efficiency where the reflectance is the lowest at the resonance angle, hence, the Au thickness is selected to be 50 nm. Next, the sensitivity of the prism coupled SPR biosensor can be estimated by repeating the simulation with a background RI of 1.01. The SPR reflectance plots of both background RI are plotted in Figure 4.2 and a clear shift in the resonance angle can be observed. When the RI was 1.00, The resonance angle was 46.75° when the RI was 1.00 and it was shifted to 47.91° when the RI was increased to 1.01. Therefore, it can be concluded that the resonance of a prism coupled SPR biosensor should increase with increasing RI in the sample fluid and the theoretical sensitivity can be defined as the resultant shift in the resonance angle per change in the RI which is estimated to be $116^\circ/\text{RI}$.

Figure 4.2: Simulated SPR reflectance plots showing the shift in resonance angle due to different RI

4.1.2 Experimental Validation of a Prism-Coupled SPR Biosensor

Instead of coating the Au layer directly on the prism, the Au is deposited on a BK7 (RI of 1.515 at 632.8 nm) glass slide and is attached to the BK7 prism using a refractive index matching fluid (Series A Refractive Index Liquid, $n_D = 1.5000 \pm 0.0002$, Cargille Laboratories) to improve the optical contact between the two surfaces. This way, the

prism can be mounted permanently to the SPR system and the glass slide can be swapped easily. This approach is much more cost-effective as only the glass slides are swapped out for each sample and the flat surface is less challenging for preparing the sample.

A 50 nm thick gold (Au) layer is then sputtered on the glass slide with a thin layer of chromium (Cr) as the adhesion layer. The sputtering equipment is first calibrated to determine the required deposition time for achieving the targeted thickness. This is accomplished by the deposition of multiple samples with various deposition durations. The thickness of each sample is then verified using atomic force microscopy (AFM) which gives the relationship between the thickness of the metal film with respect to their respective deposition time. Based on the linear fit, the deposition rate is calculated to be around 6 nm/min and the deposition duration necessary for depositing 50 nm Au is determined to be around 8 min. Based on the AFM measurement as shown in Figure 4.3a, the step height is measured to be around 50 nm and the roughness of the Au film is also given in Figure 4.3b which shows relatively good uniformity.

Figure 4.3: (a) AFM image of Au film near the edge and the step height measurement, and
(b) roughness measurement of the Au film

The Au coated glass slide is then coupled to the prism coupled SPR system as illustrated in Figure 4.4. A linearly polarized helium-neon (He-Ne) gas laser with approximately

632.8 nm emission wavelength is selected as the light source and a linear polarizer is used to ensure that the light is p-polarized so that it can be used for excitation of the SP. To vary the incidence angle of the light, the prism is attached to a prism table which is mounted on a rotation stage. The intensity of the reflected light is then measured by a photodetector which is mounted on a separate rotation stage and the detected intensity value is displayed on a power meter.

Figure 4.4: Schematic illustration of the SPR set-up in Kretschmann configuration

The two rotation stages (Standa Ltd.) are mounted on a special mount which allows both rotation stage to rotate about the same axis with full 360° continuous travel range as shown in Figure 4.5. They are also motorised and can achieve a minimum step size of 6.75 arcsec with a repeatability of 40 arcsec. To minimise any errors and to ensure high repeatability, a LabVIEW program was also developed to interface the two rotation stages and the power meter for measuring the SPR reflection curves (refer to Appendix G for the LabVIEW program).

Figure 4.5: Model of the prism coupled SPR biosensor system

The plot of the reflectance against the angle of incidence, θ of the incident laser light is plotted in Figure 4.6. showing the resonance angle to be approximately 46.6° . For comparison purposes, the experiment was repeated by using an s-polarized light.

Figure 4.6: Measured reflectance against the angle of incidence for p-polarized and s-polarized light

From the graph, there is a sharp drop in the reflectance when the light is p- polarized. The drop in reflectance is absent when the light is s-polarized, and the reflectance instead remains high after a certain angle which is consistent with the phenomenon of total

internal reflection of light in a prism. Therefore, this indicates that it is indeed possible to couple p-polarized light to excite the SP in the prism coupled configuration.

The system is relatively simple and easy to align and requires limited optical components. The resonance angle can also be observed and identified. However, prism coupled SPR biosensor has been reported to have poor detection limit and is unable to detect an ultra-low concentration of analytes or smaller biomolecules such as DNA. Therefore, the later sections of this chapter will introduce a potential signal amplification method for a prism coupled SPR biosensor by using nanomaterials. Typically, nanomaterials are used in LSPR biosensor, however, recent reports have demonstrated the potential of using integrating nanomaterials into SPR biosensors to improve their sensitivity and detection limits.

4.2 Signal Enhancement using Nanomaterials

As discussed in the previous chapters, nanomaterials such as nanoparticles can realise localised confinement of electric field which are also known as localised surface plasmons (LSP). The electric field intensities of LSP are typically orders of magnitudes greater than surface plasmon (SP) waves. Therefore, the enhanced field confinement would allow LSPR biosensors to achieve relatively lower detection limits and better sensitivity as compared to conventional SPR biosensors.

From literature, the field enhancement of the nanoparticles is heavily influenced by its geometric shape and size. For example, nanoparticles with urchins-like features would have significantly higher field enhancement factors as compared to nano-spheres. This is because the electric field would be higher concentrated at the tips of the sharp features which means that any change in the surrounding medium would induce a greater shift in the resonance condition. This would improve the sensitivity of the biosensor as the shift

in the resonance condition is amplified. Additionally, the size of the nanoparticles would also influence the resonance condition and field enhancement factor.

For demonstration, FDTD simulations were used to simulate the electric field intensities in Au nano-urchins as compared to Au nano-spheres when excited optically. To start, both Au nano-spheres and nano-urchins were modelled with a particle size of 100 nm and the minimum mesh size of 0.2 nm was selected for the simulation as it has an extinction wavelength near to the laser used in our SPR system (632.8 nm). These nanoparticles were first excited using a broadband light source from 400 nm – 700 nm to determine their respective extinction spectrum as shown in Figure 4.7a. The peak extinction is at 608 nm and 637 nm for Au nano-spheres (Figure 4.7b) and Au nano-urchins (Figure 4.7c), respectively. This value would correspond to the resonance wavelength of the nanoparticles, where the electric field enhancement is the greatest, which would be used to determine their respective field enhancement factor.

Figure 4.7: (a) Theoretically simulated extinction spectrum for (b) Au nano-sphere, (diameter = 100 nm) and, (c) Au nano-urchin (tip-to-tip distance = 100 nm)

Frequency domain power monitors were used for the study of electric field intensity around the structures when excited by a plane wave source which corresponds to their respective resonance wavelength values. Figure 4.8 shows the electric field intensity of Au nano-spheres and nano-urchins, respectively. From the result, the field enhancement factor of Au nano-urchin is 1050 which is more than 7 times greater than the Au nano-sphere value of 135. Therefore, Au nano-urchins would be a more effective nanomaterial design as a higher field enhancement factor should lead to lower detection limits. The greatest electric field intensity is also observed at the tips of the spiky nanostructures which suggests that the geometric shape of the Au nano-urchins is highly advantageous in LSPR biosensors.

Figure 4.8: Simulated electric field intensity and the field enhancement factor of the (a) Au nano-sphere, and (b) Au nano-urchin (refer to Appendix H for the unscaled results)

With this information, the next step of the simulation study is to investigate the influence of the size of the Au nano-urchins on the electric field enhancement factor. The extinction spectra of Au nano-urchins with different particle sizes were first simulated to determine their individual resonance wavelength as shown in Figure 4.9. It is observed that there is

an increase in the resonance wavelength with a corresponding increase in the particle size. Therefore, it is important to choose an excitation wavelength equal to the resonance wavelength to maximise the electric field enhancement factor of the nanoparticle.

Figure 4.9: Theoretically simulated extinction spectrum for Au nano-urchins with varying particle sizes

The electric field intensities of the Au nano-urchins with varying particle sizes were then simulated by fixing the excitation wavelength at their respective resonance wavelength. Based on the results as shown in Figure 4.10, as the size of the Au nano-urchins decreases from 100 to 80 nm and 60 nm, there is a corresponding drop in the enhancement factor. The same is also true as the size increases from 100 nm to 120 nm. Therefore, it can be concluded that Au nano-urchins with a particle size of 100 nm gives the highest field enhancement factor which would be ideal for biosensing applications.

Figure 4.10: Simulated electric field intensity of Au nano-urchins with an average particle size of (a) 60 nm, (b) 80 nm, (c) 100 nm, and (d) 120 nm (refer to Appendix H for the unscaled results)

4.2.1 Experimental Validation of the Gold Nano-Urchin LSPR Biosensor for Alzheimer's Disease Detection

Therefore, to demonstrate the potential of Au nano-urchins as an effective nanoparticle for biosensing, an Au nano-urchins based LSPR biosensor was developed for sensing of the fibrillation kinetics of beta-amyloid 1-42 ($A\beta_{1-42}$). Rapid aggregation of $A\beta_{1-42}$, which is a protein abundant in the central nervous system of humans, results in the formation of soluble oligomers known as Amyloid β -derived diffusible ligands (ADDLs) [206]. Proteolytic activities of β and γ secretase on amyloid proteins leads to the formation of

$A\beta_{1-42}$ fibrils which trigger the onset of Alzheimer's Disease (AD) [207]. Therefore, it is crucial to detect $A\beta_{1-42}$ fibrillation at a very early stage so that preventative measures such as medications or therapies can be introduced at an early stage to slow down or halt the progression of AD.

Au nano-urchins with an average particle size of 100 nm were purchased commercially (Sigma Aldrich). Based on the datasheet provide from the supplier, the density of the Au nano-urchins is 0.1mM which is approximately 3.84×10^9 particles/mL and is suspended in phosphate-buffered saline (PBS) solution. The purchased Au nano-urchins were first characterised using transmission electron microscopy to confirm its average particle size and its urchin-like features. The TEM image is shown in Figure 4.11 and from the image, the average particle size of the nano-urchins is confirmed to be approximately 100 nm. Additionally, from the image, the urchin-like features could be observed along the edges of each particle. The overlapping contrast also indicates that the features are three dimensional. The urchin features on the surface of the Au nano-urchins are also randomly orientated which would eliminate any possible lattice resonance effect.

Figure 4.11: TEM image of the Au nano-urchins

The experimental setup of the LSPR biosensor is illustrated in Figure 4.12. A stabilized broadband light source (360 nm to 2600 nm) is used as the excitation source and the output is coupled directly to an optical fibre. A lens is then used to focus the light on the sample which is contained within a cuvette. As the transmitted light passes through the sample, it is collected by a collector lens which focuses the light into an optical fibre that is coupled to a spectrometer.

Figure 4.12: Schematic model of the LSPR biosensor

The primary advantage of this system over commercially available spectroscopy instruments is the relative ease in reconfiguring the setup according to different requirements such as replacing the spectrometer or light source with one that has a different wavelength range and the possibility of moving the focal spot to other regions within the sample. To obtain the absorbance spectrum, the transmission value of the cuvette and PBS solution is first measured to be used as the reference transmission data. After which, the reference cuvette is replaced with the sample cuvette and the transmission is measured again. The transmittance (T) of the sample can then be derived

by subtracting the baseline of the reference transmission data. The absorbance (A) can then be simply calculated by Beer-Lambert law by using the formula $A = -\log_{10}(T)$ [208].

Figure 4.13: Absorption spectra of the sample (Au nano-urchins in PBS with Aβ₁₋₄₂) at various incubation times

The Aβ₁₋₄₂ peptide sample for the biosensing experiment was purchased from Abcam (purity >95%). The Aβ₁₋₄₂ solution was prepared by mixing 1 mg of Aβ₁₋₄₂ in 100 μL of sodium hydroxide (NaOH) solution and diluted to a concentration of 1 mg/mL by adding PBS. The diluted samples were then aliquoted to 100 μL parts and were stored at -200°C. Samples for the biosensing study were then prepared by mixing 50 μL of Aβ₁₋₄₂ solution with 500 μL of Au nano-urchins. To prevent the Au nano-urchins from being clustered together in lumps, the Au nano-urchins were first sonicated for more than 10 mins before being mixed with the Aβ₁₋₄₂ solution. The solution was further diluted to 1 mL by PBS. To

sense fibrillation stages of $A\beta_{1-42}$, the absorbance study of the sample is performed at various time intervals. Excitation over the range 500 nm to 800 nm is used to monitor the change in absorption of Au nano-urchins (650 nm peak) in the presence of $A\beta_{1-42}$. The spectrum data is recorded in every 5-minute interval for a total time of 90 minutes. Figure 4.13 shows the respective absorption spectra of the sample for the different $A\beta_{1-42}$ incubation times. A gradual reduction in Au nano-urchin absorbance is observed with increased incubation time. To identify the LSPR absorption peak of Au nano-urchins with the $A\beta_{1-42}$ sample, baseline correction is done based on the absorption spectrum of PBS.

There are two absorption peaks observed which corresponds to the Au nano-urchins peak and the mutual absorption contribution from PBS and $A\beta_{1-42}$. The absorbance peak intensity near to 650 nm corresponds to the LSPR peak of Au nano-urchins as shown in Figure 4.14a and it is used as the sensing parameter for the sensing of $A\beta_{1-42}$ fibrillation. The absorption peak observed near the wavelength range of 741 nm is attributed to mutual absorption contribution from PBS and $A\beta_{1-42}$ as shown in Figure 4.14b. With an increase in the incubation times, a decrease in absorbance is observed. The shift in the LSPR absorbance peak is due to the addition of $A\beta_{1-42}$ and NaOH which alters the RI of the sample fluid. It is observed from the graph that the LSPR peak intensity remains almost constant until 10 minutes which indicates the lag phase of $A\beta_{1-42}$. At this stage $A\beta_{1-42}$ monomers slowly start getting aggregated. Since the solution lacks agglomerates, the LSPR intensity of Au urchins is not getting affected at this stage. Rapid reduction in LSPR peak intensity is observed in the time interval from 10 minutes to 70 minutes suggesting the growth phase of $A\beta_{1-42}$. Many $A\beta_{1-42}$ fibrils are formed at this stage and these fibrils tend to bind around Au nano-urchins hindering their plasmonic response. This resulted in the reduction of LSPR peak intensity. As more and more fibrils surround Au nano-

urchins, LSPR intensity gets rapidly reduced. After 70 minutes, absorbance values remain constant indicating the steady phase where $A\beta_{1-42}$ fibrillation has almost completed. It is inferred from the results that LSPR peak of Au nano-urchins can be used as an efficient sensing tool for the sensing of $A\beta_{1-42}$ fibrillation.

Figure 4.14: Optical absorption spectrum of (a) Au nano-urchins, and (b) $A\beta_{1-42}$ and PBS

Figure 4.15a shows the normalized LSPR peak intensity values extracted from Figure 4.13 to highlight the three fibrillation stages of $A\beta_{1-42}$. For comparison purposes, fibrillation kinetics of $A\beta_{1-42}$ was also tested using Thioflavin T (ThT) assay which is the standard method used to confirm the activity of $A\beta_{1-42}$ peptide. ThT emission gets enhanced when it is bound to $A\beta_{1-42}$ fibrils. This emission feature of ThT is used for the detection of various fibrillation stages of $A\beta_{1-42}$. 100 μ M of ThT stock solution is first diluted to 1 μ M with de-ionised water and mixed with 100 μ l of $A\beta_{1-42}$ solution. Emission studies were performed then at an excitation wavelength of 410 nm which corresponds to the absorption peak of ThT. Fluorescence intensity was measured every 10 minutes. Figure 4.15b shows the emission plot of ThT at various incubation times of $A\beta_{1-42}$. The growth process of $A\beta_{1-42}$ was observed to show three phases – lag phase, growth phase and steady phase. A slight increase in emission intensity until 40 minutes indicates the self-assembly

of $A\beta_{1-42}$ monomers during lag phase. After 40 minutes, a sharp increase in emission intensity is observed which is due to the binding of a large number of $A\beta_{1-42}$ fibrils to ThT during the growth phase. After 60 minutes, the fibrillation reaches a steady phase beyond and there is no notable increase in ThT emission. Though ThT can sense $A\beta_{1-42}$ fibrillation, due to the possible disadvantage of cross-reactivity and non-specific binding, label-free LSPR based sensing can be a better option for biosensing. The LSPR peak intensity changes even at a small interval of 5 minutes suggesting the sensitivity of LSPR peak of Au nano-urchins for biosensing applications.

Figure 4.15: (a) LSPR sensing of various fibrillation stages of $A\beta_{1-42}$ in comparison with (b) kinetics study of $A\beta_{1-42}$ fibrillation using ThT assay

Further, Field Emission Scanning Electron Microscopy (FESEM) was used to inspect the actual formation of $A\beta_{1-42}$ fibril. The $A\beta_{1-42}$ are mixed with Au nano-urchins at incubation times of 5 minutes (lag phase), 40 minutes (growth phase) and 90 minutes (steady phase) respectively. It is clear from Figure 4.16a that $A\beta_{1-42}$ fibrils are in the lag phase. Few small fibrils of length around 400 nm are observed in lag phase which indicates the beginning of monomer aggregation. Au nano-urchins are observed as a white layer in the background. Nucleation of monomers to fully grown fibrils appears to occur at various

regions in Figure 4.16b indicating the growth phase. From Figure 4.16c it is inferred that the fibrils have been elongated and almost the whole area is covered with fibrils length of 2 μm to 3 μm indicating the steady phase of $A\beta_{1-42}$ fibrillation. The radial growth of beta-amyloid fibrils is observed to be consistent with the growth of beta-amyloid fibrils that are reported in the literature [209].

Figure 4.16: SEM images of $A\beta_{1-42}$ mixed with Au nano-urchins at an incubation time of (a) 5 minutes (lag phase), (b) 40 minutes (growth phase), and (c) 90 minutes (steady phase).

The red circles indicate Au nano-urchins attached to $A\beta_{1-42}$ fibrils.

Due to the fibrillation of $A\beta_{1-42}$, there may be an effect of change in refractive index which reduces the LSPR coupling efficiency in the Au nano-urchins. Therefore, FDTD simulations are performed to study LSPR intensity decay during the fibrillation process. Increase in fibrillation is represented as an increase in effective refractive index in FDTD

simulations. Electric field calculations are performed between Au nano-urchin spikes and the separation between the spikes is considered as 5 nm. The excitation wavelength is set at 632.8 nm. Figure 4.17 shows the electric field calculations between the spikes for various refractive indices values of fibrils (from 1.6 to 1.9 in steps of 0.05). Reduction in LSPR field distribution around the urchin spikes is observed with increase in refractive indices of fibrils and the results agree with the experimental observations.

Figure 4.17: FDTD simulations of electric field intensities in between Au nano-urchin spikes distributed on beta-amyloid fibrils with various refractive index values (a) 1.6, (b) 1.65, (c) 1.7, (d) 1.75, (e) 1.8, (f) 1.85, (g) 1.9 respectively (approximation for increasing beta-amyloid fibrillation)

To further validate the results, the LSPR experiment was also repeated using Au nanospheres of diameter 100 nm to confirm that Au nano-urchins LSPR biosensors would have better sensitivity towards beta-amyloid. The experimental parameters such as sample concentrations are kept constant with that used in the Au nano-urchins LSPR biosensor. Figure 4.18 shows the comparison between Au nano-urchins and Au nanospheres LSPR biosensors for the study of beta-amyloid fibrillation at nanomolar concentration level. It is observed the Au nano-spheres LSPR biosensor do not show much change in optical absorption as compared to Au nano-urchins at various fibrillation

stages of beta-amyloid fibrillation. Therefore, Au nano-spheres have limited potential to be developed for nanomolar concentration sensing of beta-amyloid fibrillation.

Figure 4.18: Comparison between the sensing of $A\beta$ fibrillation at the nanomolar concentration level using Au nano-urchins and Au nano-spheres

The major advantage of using Au nano-urchins in beta-amyloid fibrillation sensing lies in the peripheral diagnostics for Alzheimer's disease (AD). According to the previous literature, tissue-level changes of AD are detectable years before their clinical expressions [210]. These changes in beta-amyloid proteins are reflected in the body fluids as these proteins are excreted and expressed in various body regions of the fluids from the central nervous system. There are reports of non-invasive detection of beta-amyloid from body fluids such as saliva and blood, hence peripheral diagnostics of AD in a non-invasive way is highly significant in early-stage detection of beta-amyloid fibrillation. In the present work, LSPR sensing with Au nano-urchins is mainly targeting future peripheral diagnostics applications rather than in vivo applications. For in vivo applications, further optimization of aspect ratio is required to cross the blood-brain

barrier. Though the cytotoxicity of Au nanoparticles is not fully studied, they are reported to be more biocompatible when compared with other metal nanoparticles. Au nanostructures are also known to induce nanoparticle-induced endothelial leakage which will be helpful for the transportation of nanomedicine. With further optimization of the aspect ratio of Au nano-urchins, these structures may be useful for in vivo applications.

One of the advantages of using Au nano-urchins for beta-amyloid fibrillation sensing is the possibility of fibrillation sensing at low concentrations due to the enhanced localized plasmon resonance. Therefore, there is a scope for further improvement in the sensing concentration by changing the density and thickness of spikes over the surface of Au nano-urchins which will have a huge influence on the localized SPR field enhancement. Though optical dyes such as ThT and Congo red can give similar information on the fibrillation kinetics of beta-amyloid, concentration for accurate sensing is observed to be higher which can adversely affect the capability of optical dyes in early-stage beta-amyloid fibrillation sensing.

In conclusion, an LSPR biosensor for sensing of $A\beta_{1-42}$ fibrillation using Au nano-urchins was developed. The LSPR absorbance peak of Au nano-urchins is observed to be highly sensitive to structural changes of $A\beta_{1-42}$ during the fibrillation growth process even at nanomolar concentration. The enhanced localized electric field generation at the spiky structures over Au nano-urchins and high sensitivity of LSPR to ambient conditions enable them to act as an efficient biosensor. It is envisioned that this work can lead to the realization of highly sensitive label-free and real-time sensing of beta-amyloid for AD detection.

4.3 Gold Nano-Urchin Enhanced SPR Biosensor for Breast Cancer Detection

As discussed previously, nanoparticles have been demonstrated as a potential method to further improve the detection limit or sensitivity of conventional SPR biosensor. This can be achieved by simply immobilising the nanoparticles on the sensing surface of the SPR biosensor so that the SP wave can be coupled to the nanoparticles to excite the LSP. In this context, an Au nano-urchins enhanced SPR biosensor was proposed as shown in Figure 4.19. A conventional Au SPR biosensor is selected for its good biocompatibility and affinity with the Au nano-urchins. A layer of anti-ER α would also be immobilised over the Au nano-urchins for label-free detection of ER α which is a commonly used biomarker for the diagnosis of breast cancer. A microfluidic cell will also contain the sample fluid during the SPR measurement.

Figure 4.19: Schematic illustration of the Au nano-urchins enhanced SPR biosensor

To better understand the effect when Au nano-urchins are coupled to the SPR biosensor, FDTD simulations were once again used to validate the electric field intensity distribution across the metal-dielectric interface. For comparison purpose, the first simulation is based on the conventional SPR biosensor without the addition of the Au nano-urchins as shown in Figure 4.20a. The Au thickness is set to 50 nm based on the optimisation study conducted in the earlier section of this chapter. The light source for the simulation is

selected to be 632.8 nm wavelength which is close to the theoretically simulated resonance wavelength and is also the emission wavelength of the He-Ne laser used in our SPR system developed in the earlier section of this chapter. For comparison, two different models are also added where a single particle of 100 nm Au nano-sphere is added to the dielectric interface of the Au layer as shown in Figure 4.20b, and a single particle of 100 nm Au nano-urchin is added to the other model as shown in Figure 4.20c.

Figure 4.20: FDTD simulation window of an (a) conventional SPR biosensor,

(b) Au nano-spheres, and (c) Au nano-urchins enhanced SPR biosensor.

(d) Electric field intensity simulation of the SPR sensor without Au nano-urchins, (e) with Au nano-spheres, and (f) with Au nano-urchins (the 50 nm gold film layer is located above $y = 0$)

The electric field intensity (along the x-z plane) of each model, at their respective resonance angles, is then simulated to determine the maximum electric field enhancement of all the biosensors configurations. Figure 4.20d shows the electric field intensity of a conventional SPR biosensor while Figure 4.20e and Figure 4.20f shows the electric field intensity distribution of the Au nano-sphere and Au nano-urchin enhanced SPR biosensor, respectively. There is a significant enhancement to the maximum electric field (1.78 to 160) when the Au nano-sphere is added and the electric field increased further (160 to 2×10^4) when the Au nano-sphere is replaced with the Au nano-urchin. The surface plasmon wave generated by the Au film is coupled to the nanomaterials leading to localized confinement of the surface plasmon wave. This could be a possible explanation behind the mechanism for the significant electric field enhancement in the proposed SPR sensor.

However, it is important to note that in the Au nano-sphere enhanced SPR biosensor, the electric field is only confined to the region near the interface of the Au film and the Au nano-sphere. In comparison, for the Au nano-urchin enhanced SPR biosensor, the electric field is highly concentrated all around the spikes of the Au nano-urchins. This could explain why the Au nano-urchin have a better field enhancement factor as compared to the Au nano-sphere. Another possible reason is that the excitation wavelength (632.8 nm) is very close to the extinction wavelength of the Au nano-urchin (637 nm) as compared to the Au nano-sphere (607 nm) which allow for better coupling.

4.3.1 Experimental Validation of Gold Nano-Urchins Enhanced SPR Biosensor

The Au nano-urchins enhanced SPR biosensor are experimentally validated in the prism coupled setup in the angular interrogation configuration as detailed in the first section of

this chapter. However, the prism was replaced with a material with high refractive index (N-SF11) and the Au layer is sputtered directly on the prism. Next, Au nano-urchins with an average particle size of 100 nm were purchased commercially (0.1 mM concentration suspended in phosphate-buffered saline (PBS) solution, Sigma-Aldrich) and immobilised on the Au surface by first functionalizing the Au surface using EDC/NHS coupling with poly(ethylene glycol) bis(amine) (DAPEG) as detailed in [211].

The Au surface was then imaged using scanning electron microscopy (SEM) as shown in Figure 4.21. The Au nano-urchins are observed throughout the entire area and the distribution of Au nano-urchins is random with some aggregation in some particles. However, the majority of the particles are randomly spaced on the Au surface with few μm inter-particle spacing. The orientation of the Au nano-urchins on the substrate is also random.

Figure 4.21: SEM image of the Au nano-urchins after immobilisation on an Au substrate

The anti-ER α antibodies (1:100 dilution in PBS, ab16660, abcam) are then bound to the Au surface by dipping the prism in a solution containing the antibodies for more than 12 hours to functionalise the sensing surface. The microfluidic cell is fabricated from

polydimethylsiloxane (PDMS) as described in the previous chapter. Estrogen receptor alpha ($ER\alpha$) (recombinant protein, concentration: 4 μ g at 0.15 mg/ml, ab82606, abcam) is specifically chosen to work with the anti- $ER\alpha$ antibodies. Upon delivery of the $ER\alpha$, PBS is added, and it is aliquoted into different concentrations before being stored at -20 °C. Samples were then taken out of the freezer and used immediately.

Before the start of the measurement, PBS is first injected into the microfluidic cell which is also used as the buffer solution. The analyte is then introduced into the microfluidic cell and the reflectance is then measured in rotation steps of 0.1°. The measurement is repeated every 5 min for 30 min to average out the measurement error. Based on the obtained SPR reflectance curve, the resonance angle can be identified by the angle where there is a sharp drop in reflectance.

By comparing the respective resonance angles obtained for the various $ER\alpha$ concentration against the control experiment, it is possible to calculate the resultant shift in the resonance angles due to the presence of $ER\alpha$. The plot of the resonance angle shift against the $ER\alpha$ concentration would then indicate the SPR biosensor sensitivity. To keep the data consistent, the maximum shift in resonance angle values for each sample were used.

To further validate the results, the $ER\alpha$ sensing experiment was repeated with a 100 nm diameter Au nano-spheres enhanced SPR biosensor and a conventional Au film SPR biosensor without any nanoparticles. Figure 4.22. shows the combined plot of the resultant shift in the resonance angles with respect to the various $ER\alpha$ concentrations for the three different biosensor configurations. The sensitivity of the SPR biosensors can be determined from the slope of the linear fit. For the Au nano-urchins enhanced SPR

biosensor, the slope is approximately $6.7^\circ/\text{nM}$ while the slope for the Au nano-spheres enhanced SPR biosensor is $5.6^\circ/\text{nM}$. The conventional SPR biosensor has a slope of approximately $2.5^\circ/\text{nM}$. Therefore, it is evident that both the nanoparticles enhanced SPR biosensor has a better sensitivity than the conventional SPR biosensor. Furthermore, the Au nano-urchins enhanced SPR biosensor has the best sensitivity out of the three biosensor configurations which support the findings from the theoretical simulations.

Figure 4.22: Plot of the shift in resonance angle against the various ERα concentrations for the different SPR biosensor configurations

Based on the obtained results, it is shown that Au nano-urchins showed high field enhancement factor compared to other nanoparticles of different geometry leading to best sensitivity. The theoretical results are validated experimentally. The addition of a layer of anti-ERα antibodies which acts as the bioreceptor in the developed biosensor created a gap between the Au nano-urchins and the ERα analytes. This reduces the effectiveness of the localized field confinement as it is only at the surrounding regions near the tips of the Au nano-urchins and leads to a lower than expected sensitivity in the developed SPR biosensor. Nonetheless, the Au nano-urchins enhanced SPR biosensor still showed the best sensitivity out of the three different biosensor configurations discussed.

An Au nano-urchins enhanced SPR biosensor was developed to enhance the sensitivity for the sensing of $ER\alpha$. Different concentrations of $ER\alpha$ was sensed by the developed SPR biosensor to validate its sensitivity. A significant improvement in the SPR signal was observed when comparing the developed Au nano-urchins SPR biosensor against an Au nano-spheres SPR biosensor and a conventional SPR biosensor. This was also verified using theoretical FDTD simulations. It is envisioned that in the future, the Au nano-urchins SPR biosensor can be developed into a highly sensitive $ER\alpha$ biosensor which could be potentially used for the early detection of breast cancer.

4.4 Summary and Discussions

In summary, different nanoparticle designs were investigated to assess their ability to amplify the biosensing signal in a prism coupled SPR biosensor. Based on the theoretical simulations, Au nano-urchins have an electric field enhancement factor that is more than 7 times that of Au nano-spheres. Additionally, different sizes of Au nano-urchins were also simulated, and it was observed that Au nano-urchins with a particle size of 100 nm gives the highest electric field enhancement when excited at a wavelength of 637 nm.

Based on this, an Au nano-urchins LSPR biosensor was developed and compared against an Au nano-spheres for sensing the fibrillation of beta-amyloid ($A\beta$) which could potentially be used for detecting the early onset of Alzheimer's Disease. Due to its sharp urchins-like features, the Au nano-urchins LSPR biosensor is highly sensitive and can detect $A\beta$ at the nanomolar concentration levels. Further optimization of the Au nano-urchins geometry or aspect ratio can be conducted to target the possible application of in vivo sensing of $A\beta$ within the brain by penetrating the blood-brain barrier. It is envisioned

that this can be developed into a label-free biosensor which can detect and sense the concentration of $A\beta$ in the brain with high sensitivity and in real-time.

Furthermore, the Au nano-urchins were also integrated with a conventional SPR biosensor to amplify its biosensing signal and improve its detection limit. The theoretical simulations reveal that the Au nano-urchins enhanced SPR biosensor has an electric field enhancement factor that is few orders of magnitudes better than a conventional SPR biosensor (2×10^4 and 1.78 respectively). Therefore, it should be possible to increase the sensitivity of the biosensors for the detection of lower concentrations of analytes. For demonstration, the Au nano-urchins SPR biosensor was developed for sensing of $ER\alpha$ which is a commonly used breast cancer biomarker. Both the Au nano-urchins and Au nano-spheres enhanced SPR biosensors demonstrated significantly better sensitivity as compared to the conventional Au SPR biosensor. Additionally, the Au nano-urchins enhanced SPR biosensors has the best sensitivity which suggests that it has the most optimal shape and geometry for the detection of $ER\alpha$. In the future, such biosensors can be developed for the detection of breast cancer in women.

Therefore, in conclusion, this chapter demonstrated the potential of using nanoparticles to enhance the biosensing signal of SPR biosensors. In the future, nanoparticles can also be integrated with highly compact, grating coupled SPR biosensor for improving its biosensing signal. Nonetheless, further optimisation studies are still necessary to maximise the signal amplification of the nanoparticles when coupled with a grating coupled SPR biosensor.

CHAPTER 5

GRAPHENE LAYERED HETEROSTRUCTURE FOR ELECTRICALLY CONTROLLED SPR BIOSENSORS

This chapter investigates the potential of using a graphene layered heterostructure for the development of electrically controlled SPR biosensors. The first section of this chapter discusses the advantages and disadvantages of two common methods of synthesising high-quality monolayer graphene. This is followed by the steps needed to transfer the graphene onto the desired substrate to realise the graphene layered heterostructure. The last section demonstrates the potential of using the graphene layered heterostructure in an SPR biosensor which is electrically controlled.

5.1 Graphene Synthesis

As discussed previously in the literature review, there are multiple methods which can be adopted for the synthesis of graphene and they each have their benefits and shortcomings. The most critical factor affecting the quality of the graphene is the number of graphene layers and the size of the individual graphene flakes. As graphene is too thin and transparent, it is challenging to use conventional microscopy to investigate its quality, hence, Raman spectroscopy is typically used instead. Raman spectroscopy is a unique optical spectroscopy technique which can measure the molecular vibrations and determine the crystal structures of the sample.

Information on the number of the graphene layer is critical as there is a huge difference in the electrical properties between monolayer graphene and few-layer-graphene (FLG). A single continuous sheet of monolayer graphene would have relatively higher electrical

properties which are highly desired as compared to FLG which would have a similar atomic structure as graphite. Hence, Raman spectroscopy is an ideal tool to measure the number of graphene layer which is used to assess the quality of the graphene synthesised using the deposition system.

For graphene, the respective Raman spectrum is recognized by the presence of a G peak ($\sim 1580\text{ cm}^{-1}$) and 2D peak ($\sim 2700\text{ cm}^{-1}$) [212]. The G peak is related to the hexagonal structure of sp^2 carbon atoms and the ratio of the intensities of the 2D peak to the G peak would indicate the number of graphene layers. A ratio of greater than 2 would suggest single-layer graphene, and a ratio of about 1 indicates bilayer graphene. Any values lesser than 1 would indicate few-layer-graphene (FLG) [212]. A D peak ($\sim 1350\text{ cm}^{-1}$), indicates defects in the graphene structure such as disorder within the graphene or the presences of doping. Additionally, the full width at half-maximum (FWHM) and a shift in the peak position would also provide further information on the disorder and doping characteristic of the graphene [213].

In this context, this chapter investigates and compare two different chemical vapour deposition (CVD) methods for synthesising graphene; thermal CVD and plasma-enhanced CVD. Raman spectroscopy will be used primarily to comment on the number of graphene layers synthesised from both CVD methods and microscopy techniques will be employed to determine the size of the graphene flakes.

5.1.1 Thermal Chemical Vapour Deposition Method

The thermal CVD method is illustrated in Figure 5.1. A quartz tube with an outer diameter of 40 mm and a wall thickness of 3 mm was assembled horizontally. This would serve as the reaction chamber to contain the substrate during the deposition process. The low

pressure is accomplished with a rotary vane vacuum pump (Edwards Ltd) that is connected to one end of the quartz tube. The other end of the quartz tube was attached to a pressure regulator. Methane (CH_4) gas is selected as the carbon feedstock and hydrogen (H_2) gas is mixed in a buffer to regulate the growth speed of the graphene. The flow rate of each gas is controlled individually by digital mass-flow controllers (Alicat Scientific Inc.). A ceramic heater is then positioned over the quartz tube to provide the heat energy necessary to decompose the carbon feedstock to catalyse the growth of graphene on the substrate.

Figure 5.1: Schematic of the CVD system

Copper (Cu) is selected as the growth substrate as it has very low carbon solubility which makes it relatively easy to grow high-quality monolayer graphene. It is also readily available as a smooth foil as it is a material that is commonly used in industrial manufacturing processes. The copper foil is first trimmed to size and cleaned in an acetone bath with sonication followed by a quick rinse in DI water. It is then cleaned in isopropanol alcohol (IPA) bath with sonication. After the copper foil is cleaned, it is rinsed again with DI water and dried on a hot plate at about 110°C . It is then placed on a graphite boat and positioned within the quartz tube at the centre of the ceramic heater to ensure a uniform heating profile across the entire substrate. Next, the pressure within the tube is evacuated to below 1.0×10^{-2} mbar. After the desired pressure is achieved, H_2 gas flow

is introduced at 2 Standard Cubic Centimetres per Minute (SCCM) which is maintained constant throughout the entire deposition process. The heater is then turned and the temperature within the chamber is slowly increased until it reaches 1,000°C. The temperature is then stabilised at roughly 1,050°C and CH₄ gas is introduced into the chamber at a flow rate of 35 SCCM. After 30 min, the CH₄ gas supply is turned off and at the same time, the heater is shifted away from the substrate location to quickly quench the substrate to halt the growth of graphene.

After the temperature within the chamber is cooled down to room temperature, the H₂ gas supply is turned off and the chamber pressure is equalised. The Cu foil is then removed and investigated using scanning electron microscopy (SEM) to determine the size of the individual graphene flakes as can be seen from Figure 5.2. The boundary between each graphene flake is observed and they can be seen throughout the entire surface of the Cu foil. Each graphene flake is approximately 50µm to 100µm long in both directions.

Figure 5.2: SEM image of the Cu surface after deposition in the CVD system

There is a noticeable difference in the contrast between the individual graphene flakes which suggests that there is a variance in their conductivity. This may be due to the formation of additional layers of graphene flakes which increased the electrical resistance at that region. However, it may also simply be due to the variation in the thickness of the Cu foil. Hence, it would be better to use Raman spectroscopy to further confirm the exact number of graphene layers after deposition by the CVD system.

Raman spectroscopy is a highly sensitive tool for gathering information on the atomic structure of graphene. However, it is challenging to directly measure the Raman spectrum of graphene on the Cu foil as the surface plasmon emission of Cu would result in a background signal that saturates the Raman signal from the graphene [214]. This may be avoided by using a laser source with lower excitation wavelength, but our Raman spectroscopy equipment is limited to 632.8 nm which makes it difficult to eliminate the photoluminescence effect of Cu. Therefore, the synthesised must first be transferred from the Cu foil to another such as Si or glass (SiO_2) before it can be characterised using Raman spectroscopy. The transfer process will be explained in detail in the later section of this chapter as it is also necessary to transfer the deposited graphene onto different substrates to form the desired graphene-enhanced devices.

The characterisation of graphene is done on Si wafer substrate as it has a highly orientated crystalline structure which results in a single Raman peak at around 520 cm^{-1} . The Raman spectrum collected from the graphene after it is transferred on the Si is shown in Figure 5.3a. From the graph, there are two distinct peaks can be observed which corresponds to the “G” peak and “2D” peak of graphene. This confirms the presence of graphene and the intensity of the “2D” peak is also more than twice the intensity of the

“G” peak which suggests that the graphene is indeed monolayer. Additionally, a 2D Raman mapping of an area ($\sim 35\mu\text{m} \times 30\mu\text{m}$) is conducted to investigate the uniformity of the graphene. The “G” peak and “2D” peak are measured during the 2D Raman mapping and the ratio between their intensities is plotted in a 2D colour map as shown in Figure 5.3b. In the figure, the regions which are green and red correspond to monolayer graphene while blue areas would suggest a greater number of graphene layers. Therefore, this suggests that the monolayer graphene can be found on the majority of the region, however, there are certain spots where there FLG. This could be due to overlapping between the boundaries of the graphene flakes.

Figure 5.3: Raman spectrum of a CVD graphene on Si - (a) single point, (b) 2D Raman mapping

Nonetheless, the results demonstrate that graphene can be grown successfully using the thermal CVD approach and the graphene is uniformly deposited throughout the entire surface of the growth substrate. The graphene also remains relatively undamaged after the transfer process which suggests that it was possible to use this method for the development of a graphene layered heterostructure. The structures can then be employed in electrically controlled graphene-enhanced SPR biosensors.

5.1.2 Plasma-Enhanced CVD Method

Compared to the thermal-CVD method, the plasma-enhanced CVD method can synthesise graphene with significantly reduced growth temperature and duration. This is very practical and highly desirable as graphene could potentially be directly deposited on materials with a lower melting point such as polymers.

Conventional PECVD method involves the placement of substrates within plasma volume which usually results in the growth of vertical graphene on the substrates due to the high electric field caused by plasma sheath formation [215]. Therefore, to synthesise large-area, horizontal graphene, a novel method of remote plasma-assisted CVD was proposed and developed wherein the heated reaction chamber is separated and placed downstream from the plasma generation region

As illustrated in Figure 5.4, the plasma is created by mounting two parallel copper ring electrodes, kept at 15 cm apart, on the outer surface of the quartz tube which is upstream of the heated CVD reaction chamber. A radio frequency (RF) power source (CESAR RF Generator – Model Number 1320) with an operating frequency of 13.56 MHz was used to generate an RF plasma and an auto-matching network was employed to match the source impedance to the plasma impedance. Before deposition, the Cu foil is first prepared in the same manner as the standard CVD process and placed on a graphite boat within the reaction chamber. Next, the quartz tube is sealed, and the pressure is evacuated to about 1.0×10^{-2} mbar. H_2 gas is then introduced into the chamber and the temperature is slowly raised to 1040 °C for 15 minutes to etch the surface of the copper to improve the quality of the deposited graphene. Thereafter, the temperature of the reaction chamber is reduced to and maintained at 1000 °C, 800 °C, 600 °C and 400 °C, respectively to

investigate the lowest deposition temperature. Once the desired chamber temperature is achieved, CH_4 gas is introduced into the reaction chamber at 10 SCCM flow rate, while at the same time, the RF power supply is switched on and set to an output power of 100W. After 15min, the methane supply and the RF power supply are turned off, and the reaction chamber is rapidly cooled down to room temperature under the flow of H_2 .

Figure 5.4: Schematic of the PECVD system

Due to the strong interaction between the feedstock gases and the RF plasma, it is therefore important to characterise the plasma parameters as it would have a strong influence on the quality of the synthesised graphene. Since the plasma parameters are governed by the physical parameters, it is necessary to quantify these parameters (for example, the dimensions of the quartz tube, electrode space, gas used, RF power, etc.) to ensure the repeatability of the experiment. Optical emission spectroscopy (OES) is preferred in this study since the measurements are non-invasive and can provide an indication of the concentration of the different species present in the plasma. The plasma temperature can also be estimated by examining the wavelengths of photons emitted by atoms or molecules during their transition from an excited state to a lower energy state. This OES study was achieved by using a spectrometer (USB4000, Ocean Optics, Inc.) to

measure the spectrum of the light emitted during the generation of the RF plasma. The spectrum formed in the CH₄/H₂ gas mixture at an RF power of 100 W is given in Figure 5.5. Different distinctive lines were found in the 386 – 390 and 430 – 435 nm range which was not present when the spectrum was captured in 100% H₂ plasma environment. This indicates that these lines correspond to the CH species found in the CH₄/H₂ plasma.

Figure 5.5: Optical emission spectrum of the RF plasma at 100 W formed in a CH₄/H₂ environment

The plasma temperature is then calculated from the spectra using the line ratio method assuming that the plasma is in local thermal equilibrium (LTE) and that the spectra are optically thin [216]. The intensity of the spectral line is given by [217],

$$I_{ij} = \frac{hcA_{ij}g_jn}{\lambda_{ij}U(T)} e^{\left(\frac{E_j}{kT}\right)} \quad (5.1)$$

where I_{ij} and λ_{ij} is the intensity and wavelength corresponding to transition from i to j respectively, h is the Planck's constant, c is the speed of light, n is the number density of emitting species, $U(T)$ is the partition function, A_{ij} is the transition probability between

level i and j , k is the Boltzmann's constant, T is the excitation temperature, g_i is the statistical weight of upper energy level and E_j is the upper energy level in eV unit. Taking the intensity ratio of two spectral lines of the same species and ionization stage, the line ratio can be expressed as,

$$\frac{I_1}{I_2} = \frac{g_1 A_1 \lambda_2}{g_2 A_2 \lambda_1} e^{[-\frac{(E_1 - E_2)}{kT}]} \quad (5.2)$$

where I is the intensity, g is the statistical weight, A is the transition probability, λ is the wavelength and E is the energy of excited state in eV. Based on Eqn (5.20), the electron temperature of the plasma can then be estimated from the corresponding H_α and H_β spectral lines which are marked in Figure 5.5 to be approximately 1.38 eV.

The morphology and size of graphene crystals of the deposited graphene on the Cu foil were then observed using SEM, and the respective images are shown in Figure 5.6. For the 1000 °C sample, there is overcrowding of particles on top of the graphene flakes which could be attributed to the over-sourcing of the carbon radical due to both plasma dissociation and a high CVD reaction chamber temperature. The 800 °C sample shows uniformly distributed large graphene crystallite flakes ($\sim 100 \mu\text{m} \times 100 \mu\text{m}$), with no overcrowding. At 600 °C, the results are similar to the 800 °C sample. However, for the 400 °C sample, the deposited carbon seems to be very different from the observation of particles and net-like features on the substrate. This suggests that the energy of the plasma at this temperature may be insufficient to breakdown the carbon feedstock gas for the deposition of defect-free graphene. Additionally, the spatial uniformity of the graphene flakes on the surface of the Cu foil was also investigated by repeating the SEM measurements at a different region of the Cu foil along the direction of the gas flow. The respective SEM images of the different samples are also given in Figure 5.6.

Figure 5.6: SEM images of the surface of the Cu substrate at different locations at (a)-(b) 1000 °C, (c)-(d), 800 °C, (e)-(f) 600 °C, and (g)-(h) 400 °C deposition temperatures

The results showed that the features of the graphene observed on the surface of the samples are consistent throughout the entire surface of the Cu foil. This suggests that the growth is uniform and there is a potential to scale up the deposition method for synthesis of graphene on a larger scale.

Raman spectroscopy was then used to characterise the quality of the graphene and the respective Raman spectra of the sample deposited at different temperatures are shown in Figure 5.9. The synthesised graphene for all samples was first transferred on to a Si substrate before the Raman spectrum is measured. The presence of the “2D” peak in the, confirms the ability of remote plasma-assisted CVD method to synthesize graphene at temperatures as low as 600 °C. However, the “2D” peak is not observed for the 400 °C sample which confirms the observation made in the SEM image where particles and net-like features were observed. Additionally, the “D” peak is also observed for all the samples which indicate the presence of disordered carbon in graphene.

Figure 5.7: Raman spectra of the samples grown at the respective temperatures

Figure 5.8 shows the magnified Raman spectra in the range of 1500 to 1700 cm^{-1} with the splitting of “G” peak into “G” ($\sim 1580 \text{ cm}^{-1}$) and “D prime (D’)” (at a higher wavenumber

of about 1620 cm^{-1}) Raman peaks. The presence of “D” peak can be attributed to (i) strained graphene and (ii) disordered graphene due to the presence of defects and edges. The intensity of “D” peak is highest in the sample synthesized at $1000\text{ }^{\circ}\text{C}$ and lowest in the sample synthesized at $600\text{ }^{\circ}\text{C}$ which suggests the least defects in $600\text{ }^{\circ}\text{C}$ sample. A shift in the “G” peak position to higher wavenumber value with increasing growth temperature is also indicative of a greater number of graphene layers. The higher temperature, coupled with plasma, causes accelerated reaction rates and excess amounts of activated carbon and these two factors resulted in the formation of multilayer graphene with more defects and disorder.

Figure 5.8: Magnified view of the Raman spectra of the samples grown at different temperatures showing the respective “G” peak and “D” peak

The intensity ratio between the Raman peaks for the respective samples are also plotted in Figure 5.9 and can be used to assess the level of disorder in the graphene. It can also provide an estimation of the number of graphene layers. A smaller I_D/I_G ratio indicates a lower level of disorder which suggests that the $800\text{ }^{\circ}\text{C}$ sample has the least disorder. The number of graphene layers can be estimated from the I_{2D}/I_G where a value greater than

two corresponds to high-quality monolayer graphene while a value lower than two would indicate multi-layer graphene formation. Since the I_{2D}/I_G ratio for all the samples is less than two, it can be concluded that the graphene deposited using the remote plasma-assisted method is indeed multi-layered.

Figure 5.9: Plot of the relative intensity of the “G” band with respect to the “D” and “2D” band

The remote plasma-assisted CVD method has demonstrated its ability for the growth of horizontal graphene at a reduced temperature as low as 600 °C. Both SEM and Raman results show the large area graphene growth with an average graphene flake size of approximately 100 $\mu\text{m} \times 100 \mu\text{m}$.

To validate the effect of plasma on the graphene growth, conventional thermal CVD without plasma was also used to synthesize graphene at 1000 °C and 800 °C. Figure 5.10 shows the Raman spectra of the deposited material on each sample after it was transferred on the Si substrate. For the 1000 °C, the “G” peak and characteristic “2D” peak of graphene were observed which is consistent with the findings from the previous section. However, the Raman spectrum of the 800 °C sample reveals that the conventional thermal CVD is not able to synthesise graphene at such low temperature.

Figure 5.10: Raman spectra of the deposited material from thermal-CVD at 1000 °C and 800 °C

Therefore, this proves that the reduced temperature graphene synthesis is indeed only possible with the plasma-enhanced CVD approach. With further optimisation of the plasma parameters such as the pressure, power, and concentration of the carbon precursor feedstock, it may be possible to synthesise graphene at even lower temperatures using the remote plasma-assisted technique.

For demonstration, a graphene-based electrochemical biosensor for glucose sensing was fabricated from the graphene synthesised using the remote plasma-assisted CVD method. First, the graphene was synthesized at 600 °C and transferred on to a flexible polyethylene terephthalate (PET) substrate. Next, two metal contact pads, at a distance of approximately 10 mm apart, were added over the graphene surface. The graphene surface was then cleaned thoroughly by soaking the substrate in a DI water bath for about 8 h. Next, the substrate is placed in a solution containing a concentration of 1 mg/dL of glucose oxidase (GOx, Sigma-Aldrich) for about 10 min to allow the immobilization of GOx on the graphene surface. This would be used as a biorecognition molecule for the label-

free detection of glucose in a sample fluid. After the graphene surface is functionalized with GOx, a direct current (DC) source is applied using a source-meter (2400, Keithley) via the metal contact pads while the corresponding voltage is measured. A polydimethylsiloxane (PDMS) microfluidic chip is then placed over the graphene surface and glucose solution (β -D-glucose 99% purity, Cayman Chemical) is then injected into the microfluidic chip. The schematic of the graphene based electrochemical glucose biosensor is given in Figure 5.11.

Figure 5.11: Schematic illustration of the graphene electrochemical glucose biosensor

Using the source meter, a -1 mA current is applied through the biosensor via the contact pads while the corresponding voltage is measured. The current is then increased in steps of 0.1 mA until it reaches +1 mA. After the measurement is completed, fresh DI water is injected into the PDMS microfluidic chip to refresh the GOx. A new batch of the solution with a higher glucose concentration is then injected into the microfluidic chip and the measurement was repeated. For comparison, the resulting current-voltage (I-V) curves for the different glucose concentrations are plotted in Figure 5.12.

Figure 5.12: I-V characteristic curves of the graphene with varying glucose concentration,

From the I-V curve, the resistance of the graphene can be obtained by linear curve fitting and the resulting relationship between the glucose concentration and resistance is plotted in Figure 5.13. The resistance when only GOx is present with DI water is estimated to be 7.66 k Ω . The resistance decreased to 6.31 k Ω in the presence of 100 mg/dL of glucose solution and further decreased to 4.92 k Ω when the glucose concentration is increased to 200 mg/dL. The reduction of the resistance can be attributed to the improvement to the electron-transfer rate across the surface of the graphene. This is because the GOx would oxidise the glucose contained in the sample fluid forming gluconolactone which then hydrolyses into gluconic acid. During this process, H⁺ ions are produced, and free electrons are generated during this process. The increase in electrons would then result in a great charge density which is detected as a reduction in the measured resistance across the graphene interface. By establishing the relationship between the concentration of the glucose solution and the measured resistance across the graphene, the graphene layered substrate can effectively function as a label-free glucose biosensor.

Figure 5.13: Change in the measured resistance across the graphene
with respect to the varying glucose concentration

In summary, a remote plasma-assisted CVD method was demonstrated for the synthesis of graphene at a reduced temperature as compared to conventional thermal CVD methods. The characteristic “2D” peak of graphene is observed from the Raman signal which confirms the successful synthesis of graphene. The synthesised graphene was then further developed into an electrochemical biosensor for the label-free sensing of glucose concentration in a sample fluid.

5.2 Transfer of Graphene

The transfer of graphene from the Cu foil to the desired substrate is done using the wet chemical etching method which is schematically presented in Figure 5.14. First, a layer of polymethyl methacrylate (PMMA) (950 PMMA A7, MicroChem) which is approximately 1.4 μm thick is spin-coated on top of the graphene at 1000 rpm for 45 s and baked at 160 $^{\circ}\text{C}$ for 4 min to fully cure. The PMMA layer protects the graphene layer from breaking apart during the later etching steps [218]. The Cu layer, which is approximately 20 μm

thick, is then etched from the underside by floating the PMMA/graphene/Cu stack on an iron(III) chloride (FeCl_3) solution. After 20 - 30 min, the Cu is completely etched, leaving behind the PMMA/graphene stack which floats on top of the solution. The PMMA/graphene stack is then carefully transferred to a DI water bath to rinse off any residual FeCl_3 solution. This is repeated multiple times and the PMMA/graphene stack is then scooped up from the solution using the desired substrate. The substrate is then kept in an oven at 50 °C for about 8 h to improve the adhesion of graphene and remove any moisture caught between the graphene and substrate. Lastly, the PMMA layer is removed in an acetone bath leaving the graphene on the surface of the desired substrate.

Figure 5.14: Graphene transfer process using a wet chemical etching method

To confirm the successful transfer to the graphene, Raman mapping of an area of approximately $35\ \mu\text{m} \times 25\ \mu\text{m}$ is performed on the graphene that has been transferred on to

a Si substrate. Figure 5.15a shows the Raman map of the centre region of the Si substrate after the graphene transfer process where the ratio of the characteristic graphene “2D” peak to its baseline value is highlighted in red. The red colour can be observed throughout the entire region which suggests that most of the graphene has been successfully transferred to the substrate. To further certify the assertion made, Raman mapping was repeated near the graphene edge (refer to Figure 5.15b), where the lower region is the graphene layer while the upper region is uncovered bare Si. The region without any graphene does not have the characteristic “2D” peak, hence, it is dark in colour. This confirms that it is indeed possible to transfer high-quality graphene with very high coverage using the wet chemical etching transfer method.

Figure 5.15: Raman 2D mapping of the (a) middle portion of the substrate surface,
and (b) edge of graphene after the transfer process

(colour scale corresponds to the ratio of the intensity of the “2D” peak to its baseline value)

Furthermore, graphene has also been successfully transferred on to a variety of non-metallic, polymeric substrates. These substrates are optically transparent and highly flexible, such as polyethylene terephthalate (PET) (MacDermid, Autotex), or flexible and stretchable substrates such as polydimethylsiloxane (PDMS) (SYLGARD™ 184 silicone elastomer, Dow Inc.). This could potentially be used for the development of graphene

layered heterostructures which are highly desired in optical biosensors as the optical signal would be able to transmit through the substrate easily and the flexibility/stretchability of the substrate is ideal for the development of wearable devices. Figure 5.16 shows the respective Raman spectra of the PDMS and PET surface, before and after the graphene transfer process.

Figure 5.16: Raman spectra of graphene transferred on (a) PDMS, and (b) PET clearing showing their “2D” peak

The characteristic “2D” peak of graphene could be observed in both spectra as highlighted in red which confirms the presence of graphene on the polymeric substrates. With the good electrical property of graphene and its excellent biocompatibility, it is envisioned that in the future, wearable graphene-enhanced optical biosensors could be potentially developed for real-time patient health monitoring.

5.3 Electrically Controlled Graphene Enhanced SPR Biosensor

Besides being used as a highly biocompatible substrate for biosensors, graphene was reported as a suitable material for supporting SP waves in optical SPR biosensors. However, the SP waves supported by graphene is typically excited optically by light in the

mid-infrared to far-infrared wavelength which then becomes difficult to be monitored. Hence, by layering the graphene within the layers of the heterostructure, the SPR biosensor can be excited using visible light while the excellent electrical properties of graphene can enhance the electrical tunability.

A layer of ion-gel is added to the heterostructure to achieve the electrical interrogation of the proposed heterostructure. The significance of the ion-gel is that it is a unique polymer where an ionic conducting liquid has been immobilized within. In the presence of an electric field, the positively and negatively charged ions within the polymer would migrate to opposite regions of the electric field which would induce a change in the localized region near the surface plasmons which and result in a change in the measured optical signal [219].

The schematic of the proposed graphene layered heterostructure structure is shown in Figure 5.17a and is configured for angular interrogation mode in an SPR system. A source-meter (Keithley, 2400) is used to apply an external voltage across the layers of the heterostructure to modulate the propagation of surface plasmons which are generated at the Au/graphene/ion-gel interface as shown in Figure 5.17b. When an external voltage is applied (at Ag), the ion within the ion-gel migrate either towards or away from the ion-gel/graphene interface depending on the polarity of the applied voltage with the magnitude of ion movement depending on the amount of voltage applied. This induces a change in the chemical potential of the localized region near the Au/graphene/ion-gel interface affecting the conductivity of the Au/graphene layer that will influence the resonance condition for the excitation of surface plasmons upon the laser light incidence. Therefore, due to the applied voltage, a change in the reflectance at the resonance angle

and shift in resonance angle are expected as schematically shown in Figure 5.17c. The polarity of the applied voltage is expected to control the direction of the change in the reflectance where a positive voltage would increase the reflectance while a negative voltage would decrease the reflectance as pictorially presented in Figure 5.17d.

Figure 5.17: (a) Schematic of the proposed heterostructure coupled to the prism, (b) surface plasmons at the Au/graphene/ion-gel interface, and (c, d) expected modulation of the heterostructure reflectance under externally applied voltage

5.3.1 Photoinduced Electric Field from Excitation of Surface Plasmons

The photoinduced electric field of the plasmonic device was also formulated theoretically to support the finding that the graphene-layered heterostructure has a greater electric field as compared to the heterostructure without the graphene layer. In a conventional plasmonic device, under laser illumination, surface plasmons are generated at the Au/air interface. Negative charges are indefinitely compressed on one side of the interface

creating a layer of positive charges on the other side which provides the restoring force for electron oscillations. Let h be the width of the positive layer, which is given by,

$$h = \frac{\sigma_{Au}}{\rho} \quad (5.1)$$

where σ_{Au} is the surface charge density induced by the optical field and ρ is the background positive charge density.

The electric field generated due to the oscillating electric field (U) is directly proportional to the product of applied light intensity (I) and width of the positive layer (h), and inversely proportional to the light-induced surface charge density (σ_{Au}), which is given as,

$$U \propto \frac{Ih}{\sigma_{Au}} \quad (5.2)$$

With the addition of the graphene layer over the Au film, the surface charge density (σ) will now have contributions from both the Au and the graphene layers and is given as,

$$\sigma = \sigma_{Au} + \sigma_C \quad (5.3)$$

where σ_C is the surface charge in the graphene layer which is induced by the optical field.

The addition of the graphene layer also changes the width of the positive charge layer formed (h'). According to the previous reports, charge penetration gets highly enhanced when the graphene layer is added on to a metal surface which increases the width of the positive layer ($h' > h$). Hence, the electric field generated at the Au/graphene interface (U') can thus be modified as,

$$U' \propto \frac{Ih'}{\sigma_{Au} + \sigma_C} \quad (5.4)$$

where h' is the width of the positive layer at the Au/graphene interface.

Since the contribution of graphene towards surface charge density is relatively low compared to that from Au. Hence $\sigma_{Au} + \sigma_C$ can be approximated as σ_{Au} .

If the laser intensity remains constant, then the increase in the width of the positive layer in the presence of graphene would result in an increase in the voltage generated ($U' > U$). Thus, the addition of graphene layer on Au leads to a greater photoinduced electric field. This can be confirmed by measuring the photoinduced electric field using a nano-voltmeter (2181A, Keithley) as shown in Figure 5.18 to measure the voltage across the heterostructure. A shutter is added to the laser path to block the laser illumination to modulate the excitation of surface plasmons (SP). For comparison purposes, the voltage will be measured in a graphene-enhanced plasmonic device and the plasmonic device without the graphene layer.

Figure 5.18: Schematic of the voltage potential measurement setup

Before starting the voltage measurement, the reflectance plots of the two devices are used to identify their respective critical angle and resonance angle as shown in Figure 5.19. From the figures, the critical angle (angle where intensity is maximum) and resonance angle (angle where intensity is minimum) can be observed which suggests that the SP are

excited. For the device with only the Au layer, the critical angle is 40.1° while the resonance angle is 45.6° . With the addition of graphene, the critical angle shifted to 40.4° and the resonance angle is now 46.1° . The shift could be attributed to the addition of the graphene layer over the Au which modified the effective permittivity at the Au/graphene/air interface.

Figure 5.19: Reflectance plots of the Au substrate (a) without graphene, and (b) with graphene layer.

The voltage measurement is then conducted at four different angles, namely (i) before the critical angle: 30° , (ii) at their respective critical angle: 40.1° or 40.4° , (iii) at their respective resonance angle: 45.6° or 46.1° and, (iv) after the resonance angle: 60° . The measurements were also repeated with the shutter turned off and on.

The difference in the voltage potential between the two laser states for the respective substrates is then calculated and plotted in Figure 5.20. By comparing the difference in the voltage potential with and without incident laser light, it is possible to determine if there is an electric field generated due to the excitation of SP. From the figures, there is a clear increase in the voltage potential of both substrates at their respective resonance angles. When the angles are out of resonance (i.e. at 30° , 40.1° or 40.4° and 60°), there is no significant change in the voltage potential which suggests that there is no

photoinduced electric field formed at these angles. However, there is an increased voltage potential when the substrate is at the resonance angle which confirms that the formation of SP also generates a photoinduced DC electric field which explains the voltage potential across the Au substrate. Additionally, there is a significantly larger difference in the voltage potential of the substrate with graphene as compared to the substrate without graphene. This suggests that there is an enhancement in the photoinduced DC electric field due to the presence of the graphene. This would be beneficial for the development of an electrically interrogated SPR biosensor device as it would be more sensitive towards an externally applied electric field and can be tuned easily.

Figure 5.20: The difference in the measured voltage potential, ΔV , between the state where the laser is turned on and off at the various angles of incidence. The two plots compare the Au substrate (a) without graphene, and (b) with graphene.

From the figures, there is a clear increase in the difference between the voltage potential of both substrates at their respective resonance angles. When the angles are out of resonance, there is no significant change in the voltage potential which suggests that there is no photoinduced DC electric field formed at these angles. However, there is a significant increase in the difference in the voltage potential when the substrate is at the

resonance angle which confirms that the formation of SP generates a photoinduced electric field. Additionally, there is a larger difference in the voltage potential of the substrate with graphene as compared to the substrate without graphene. This suggests that there is an enhancement in the photoinduced electric field due to the presence of the graphene. This would be beneficial for the development of the electrically controlled SPR device as it would be possible to use a larger electric field for the control of the SP.

5.3.2 Theoretical Modelling of Electrically Controlled SPR Biosensor

A theoretical model was developed to explain the electrical control of the surface plasmons in the heterostructure which is shown in Figure 5.21. The model consists of the four layers which are the glass prism, metal Au layer, monolayer graphene, and the ion-gel layer. For comparison purposes, three different heterostructure configurations will be compared – (i) Gl/Au/Air, (ii) Gl/Au/C/Air, and (iii) Gl/Au/C/IG (where Gl represents glass, Au for Au, C for graphene and IG for ion-gel). For simplicity, the light diffusion effects are ignored.

Figure 5.21: Schematic of the theoretical model

Since the prism used is a right-angle prism, the incidence angle (θ) of the light at the Gl/Au interface can be calculated by using Snell's law which gives,

$$\theta_1 = \pi/4 - \sin^{-1}(\sin(\pi/4 - \theta)/n_{Gl}) \quad (5.5)$$

where θ_1 is the incidence angle of the laser normal to the sample surface and n_{Gl} is the refractive index of the glass.

The reflectance of the laser as it is incident on the Au layer can be calculated by solving Maxwell's equations. For Transverse Magnetic (TM) polarized light, the respective fields in each layer are given as,

$$E_x(x, z, t) = E_x(z)e^{i\beta x}e^{-i\omega t} \quad (5.6)$$

$$E_z(x, z, t) = E_z(z)e^{i\beta x}e^{-i\omega t} \quad (5.7)$$

$$H_y(x, z, t) = H_y(z)e^{i\beta x}e^{-i\omega t} \quad (5.8)$$

The equations can be modified by representing the two propagating waves with momentum $\vec{k} = (\beta, 0, \pm k)$, where $k^2 + \beta^2 = \epsilon \mu_0 \omega^2$ is the light dispersion in each layer which gives,

$$E_x(z) = \frac{k}{\omega \epsilon} (A^+ e^{ikz} - A^- e^{-ikz}) \quad (5.9)$$

$$E_z(z) = \frac{k}{\omega \epsilon} (A^+ e^{ikz} - A^- e^{-ikz}) \quad (5.10)$$

$$H_y(z) = (A^+ e^{ikz} + A^- e^{-ikz}) \quad (5.11)$$

As each layer is a different material, there would be different ϵ , k , A^+ and A^- values which correspond to each material and they will be identified by their respective subscripts. However, for the field to match across all the layers, the frequency ω and the projection of momentum along the interface, β is assumed to be constant. The remaining constraints due to field matching conditions at each interface require continuity for $E_x(z)$ and $H_y(z)$.

The only exception is the graphene layer which is treated as an infinitely thin 2D conducting layer which a surface charge density $J = \sigma_C E_x(z_C)$, where σ_C is the graphene's planar conductivity, and $E_x(z_C)$ is the x-component of the electric field at the graphene layer's position z_C . Therefore, the field matching condition is modified to include the surface charge density which becomes $E_x(z_C^+) = E_x(z_C^-)$ and $H_y(z_C^+) = H_y(z_C^-) - J$.

To obtain the theoretical reflectance plot of the Gl/Au/C/Air heterostructure, the equations can be solved by using $k_{Gl} = \sqrt{\epsilon_{Gl} \mu_0} \omega \cos \theta_1$, and $\beta = \sqrt{\epsilon_{Gl} \mu_0} \omega \sin \theta_1$ which gives,

$$\frac{k_G}{\epsilon_{Gl}} (A_{Gl}^+ e^{ik_{Gl}d} - A_{Gl}^- e^{-ik_{Gl}d}) = \frac{k_{Au}}{\epsilon_{Au}} (A_{Au}^+ e^{ik_{Au}d} - A_{Au}^- e^{-ik_{Au}d}) \quad (5.12)$$

$$A_{Gl}^+ e^{ik_{Gl}d} + A_{Gl}^- e^{-ik_{Gl}d} = A_{Au}^+ e^{ik_{Au}d} + A_{Au}^- e^{-ik_{Au}d} \quad (5.13)$$

$$\frac{k_{Au}}{\epsilon_{Au}} (A_{Au}^+ - A_{Au}^-) = -\frac{k_{air}}{\epsilon_{air}} A_{air}^- \quad (5.14)$$

$$A_{Au}^+ + A_{Au}^- = A_{air}^- + A_{air}^- \frac{\sigma_C k_{air}}{\omega \epsilon_{air}} \quad (5.15)$$

where d is the Au layer's thickness (in this case it is 50 nm).

Therefore, the reflectance can be expressed as [220],

$$R_{Gl/Au/C/air} = \left| \frac{A_{Gl}^+}{A_{Gl}^-} \right|^2 = \left| \frac{r_{Gl/Au}^{TM} + r_{Au/C/air}^{TM} e^{2ik_{Au}d}}{1 + r_{Gl/Au}^{TM} r_{Au/C/air}^{TM} e^{2ik_{Au}d}} \right|^2 \quad (5.16)$$

with

$$r_{Gl/Au}^{TM} = \left(\frac{k_{Gl}}{\epsilon_{Gl}} - \frac{k_{Au}}{\epsilon_{Au}} \right) / \left(\frac{k_{Gl}}{\epsilon_{Gl}} + \frac{k_{Au}}{\epsilon_{Au}} \right) \quad (5.17)$$

$$r_{Au/C/IG}^{TM} = \left(\frac{k_{Au}}{\epsilon_{Au}} - \frac{k_{IG}}{\epsilon_0} - \frac{k_{Au}}{\epsilon_{Au}} \frac{k_{IG}}{\epsilon_{IG}} \frac{\sigma_C}{\omega} \right) / \left(\frac{k_{Au}}{\epsilon_{Au}} + \frac{k_{IG}}{\epsilon_{IG}} - \frac{k_{Au}}{\epsilon_{Au}} \frac{k_{IG}}{\epsilon_{IG}} \frac{\sigma_C}{\omega} \right) \quad (5.18)$$

The reflectance in the other heterostructure configurations can be obtained in the same method by calculating the momenta in the remaining layers from the light dispersion relation in each layer. By using $\epsilon_{\text{Au}}/\epsilon_0 = -8 + 3.5i$, $\epsilon_{\text{GI}}/\epsilon_0 = 2.3$, and $\epsilon_{\text{IG}}/\epsilon_0 = 1.1 + 0.2i$, the theoretical reflectance plot of the respective heterostructure configurations is shown in Figure 5.22. Note that the graphene conductivity is assumed to be $\sigma_c = 5(1 + i)10^{-4}$ which has a similar order of magnitude as the experimentally obtained graphene. However, the precision of the theoretical model is insufficient to provide a quantitative estimation of the graphene conductivity. Additionally, the permittivity of the other layers might also be slightly altered in the actual samples

Figure 5.22: Theoretically calculated reflectance plots for the various heterostructures

When a voltage is applied through the heterostructure, the free ions within the ion-gel would migrate to opposite surfaces of the ion-gel and cause the chemical potential of the graphene to be altered which influence its conductivity. Based on the experimental results which are given in Figure 5.23a, the reflectance increases when a positive voltage

is applied through the ion-gel and it increases with increasing voltage. Additionally, there is almost no shift in the resonance angle, hence, by fitting the theoretically obtained results with the experimental results, it is observed that only the real part of the graphene conductivity is altered while any change in the imaginary part would cause the resonance angle to shift. Based on literature, it is expected that the real part of the conductivity of graphene at infrared frequencies decreases with increasing chemical potential [221]. Therefore, when a positive voltage is applied, the chemical potential would increase which leads to an increase in reflectance as shown in Figure 5.23b.

Figure 5.23: (a) Experimentally obtained plot showing the change in the reflectance due to an externally applied voltage through the ion-gel, and (b) theoretically calculated change in the reflectance due to a change in conductance of the graphene layer

Experimental validation of theory is discussed in the following section to prove the electrical tunability of the proposed graphene layered heterostructure.

5.3.3 Experimental Validation of Electrically Controlled SPR Biosensor

The graphene layered heterostructure is set up in angular interrogation mode in a prism coupled SPR configuration as illustrated in Figure 5.24. The excitation of SP is achieved using a p-polarized helium-neon (He-Ne) laser with an emission wavelength of at

approximately 632.8 nm. The prism is fixed on a prism table and mounted on a motorized rotation stage to vary the incidence angle while another rotation stage is used to simultaneously rotate the photodetector to measure the reflectance of the laser from the prism. The control of the rotation stage and the collection of data from the photodetector is done via a LabVIEW program interface to ensure high repeatability.

Figure 5.24: Schematic of the experimental setup.

The fabrication of the Au layer and the graphene layer has been described in detail in the previous sections. The fabrication steps to prepare the ion-gel film is illustrated in Figure 5.25. 5 ml of acetone and 5 ml of N, N-dimethylformamide solution was first mixed and then added to 1 g of P(VDF-HFP) to prepare the precursor solution. The prepared P(VDF-HFP) solution is then mixed with 4 ml of [EMIM]⁺ and [TFSI]⁻ to form the precursor ion-

gel sol. The ion gel solution then poured on a glass slide and dried overnight in a vacuum oven at 60 °C to remove the residual solvent which forms a free-standing ion-gel film.

Figure 5.25: Schematic of the fabrication steps for the preparation of the ion-gel film

The fabricated ion-gel film is then characterized using the I-V curve as shown in Figure 5.26a. From the graph, two distinctive regions can be clearly observed which is also marked in the graph as “region I” and “region II”. In “region I” which is from approximately -0.6 V to 0.5 V, the change in the current is small and vary linearly which may be due to the electrostatic charge accumulation between the electrodes and the ion-gel interface. When the voltage increases past a certain point ($V_g > 0.5$ V, $V_g < -0.6$ V), the behaviour of the ion-gel changes, which is defined as “region II”. In this region, after the threshold voltage, there is a sudden increase in current. This could be due to an electrochemical reaction in the ion-gel film which causes non-linearity in the I-V measurement. The applied potential difference between Ag and Au electrode causes the negative [TFSI]⁻ ions and [EMIM]⁺ ions within the ion-gel layer to migrate to opposite surfaces within the ion-gel. Figure 5.26b describes the scenario where the Ag surface is

positively charged while the Au surface is negatively charged. The $[\text{TFSI}]^-$ ions would be attracted to the Ag surface while the $[\text{EMIM}]^+$ ions would be attracted to the Au surface. When the polarity of the voltage is reversed, the Ag surface would be negatively charged, and the Au surface would be positively charged as shown in Figure 5.26c. The ions would then migrate within the ion-gel layer and be attracted to the opposite surface.

Figure 5.26: (a) I-V curve of the ion-gel film, (b, c) schematic of the migration of ions within the ion-gel layer due to the externally applied electric field

After obtaining the free-standing ion-gel film, it is trimmed to the desired size and transferred over the graphene. Lastly, a layer of conducting silver (Ag) ink is added over the exposed surface of the ion-gel layer to form the electrical contact pad. A source-meter (2400, Keithley) is then connected to the Ag and Au surface to apply an external voltage across the heterostructure.

After the Au/graphene/ion-gel hetero-interface layered structure is fabricated, it is then coupled to the prism to first determine its resonance angle. Figure 5.27a shows the

experimentally recorded reflected laser intensity as a function of incident laser angle for the designed heterostructure; results corresponding to plasmonic devices having Au/graphene and Au only interfaces are also included for comparison. From the curve, a characteristic sharp drop in reflectance is observed which is identified as the resonance angle. For the Au/air interface, the resonance angle is approximately 45.6° and when the graphene layer is added, the resonance angle increased to 46.1° . When the ion-gel layer is added to heterostructure, the resonance angle shifted to around 48.5° . The increase in resonance angle is expected as the addition of more layers increases the effective refractive index near the Au surface and influence the resonance condition.

Next, the SPR device is fixed at the resonance angle and the intensity of the laser reflected from the prism is constantly monitored over time with increasing applied voltage (at Ag) with polarity reversal. After an initial period of 30 s, a +0.1 V voltage is applied using the source-meter for 30 s. Subsequently, the polarity of the voltage is reversed (i.e. a -0.1 V is applied) for another 30 s. The voltage is then increased and reversed in steps of 0.1 V (30 s positive, 30 s negative) until the voltage reaches -1 V. For comparison purposes, the reflected intensity measurements were conducted on the plasmonic device with the graphene-integrated heterostructure as well as for a heterostructure without graphene as shown in Figure 5.27b. From the graphs, there is a clear influence of the applied voltage on the intensity of the reflected light. When a positive voltage is applied (at Ag), an increase in the reflected intensity is observed with a relatively higher increase in reflected intensity at higher positive voltages. The reflectance also responds to a negative voltage where the intensity drops according to the amount of negative voltage applied. This confirms the active tunability of surface plasmons using electro-ionic control. Additionally, the relative change in intensity is plotted against the change is applied

voltage (at Ag) as shown in Figure 5.27c. From the graphs, it is evident that the presence of graphene allows for better tunability of the surface plasmons as the change in intensity is greater in the heterostructure with graphene. Therefore, this confirms that the graphene can indeed improve the sensitivity of the electro-ionic control of the developed plasmonic device. At a voltage difference of >0.6 V, it is observed that the change in reflectance for the graphene-enhanced heterostructure is non-linear in relation to the change in applied voltage.

Figure 5.27: (a) Normalized reflectance plots of the respective heterostructures, (b) plot of the reflected laser intensity over time (an additional axis has been added below the graph to show the change in applied voltage (at Ag) across the heterostructure) (c) plot of the change in intensity against the change in applied voltage

A multi-level optical switch was demonstrated using the proposed Au/graphene/ion-gel heterostructure for the electro-ionic control of a plasmonic device in electro-optical interrogation mode. The real part of the electrical conductivity of the heterostructure was modulated by an external electric field, which resulted in the intensity control of the reflected laser light. This result correlates well with the theoretical model that was developed based on the proposed heterostructure. Furthermore, the addition of the graphene enhances the sensitivity of the device towards an externally applied electric field, which is demonstrated both theoretically and experimentally. The advantage of this device is that it can also be interrogated with any light source in the visible to NIR wavelength band for better integration into any existing integrated optical devices. It is envisioned that in the future, this device could allow better and more precise control over the intensity of optical light used in subwavelength scale integrated optical devices for a wide variety of applications such as optical communications, spectroscopy and optical sensing.

5.4 Summary and Discussions

In summary, a graphene layered heterostructure was proposed for the development of electrically controlled SPR biosensors. Different methods for the synthesis of graphene was discussed and experimentally validated. The synthesised graphene was characterised using SEM and Raman spectroscopy to determine its quality and to optimise the deposition parameters.

After high-quality monolayer graphene was synthesised, it was then transferred on to a variety of substrates for the development of different devices. The graphene was transferred onto a PET substrate which was subsequently developed into a flexible

electrochemical biosensor which was able to sense varying concentrations of glucose solution by first immobilising GOx on the surface of the graphene.

Additionally, graphene was also transferred on to the surface of the Au coated glass slide which was then coupled to a prism coupled SPR system. A layer of ion-gel was then added to the graphene surface to form a graphene layered heterostructure which can be interrogated both optically and electrically. It is envisioned that such heterostructures would allow the development of compact SPR biosensors using integrated optical devices.

CHAPTER 6

CONCLUSION AND FUTURE WORK

This chapter concludes the thesis and highlights the major contributions of the research work. The last section recommends some future work which can be undertaken to further extend the research work.

6.1 Conclusion

From the latest result published from the Global Burden of Disease (GBR) from 2017, the three primary contributors to the global burden of diseases as (1) mental & neurological disorders, (2) cardiovascular diseases, and (3) cancers. Furthermore, according to the data, the primary cause for mental & neurological disorders is Alzheimer's Diseases (AD) while the most diagnosed type of cancers is female breast cancer. On the other hand, the data also revealed that the trend of cardiovascular diseases is gradually decreasing over the years. Hence, in this context, an in-depth literature review was conducted on the currently available techniques for the early detection of AD and female breast cancer in an effort to understand the limitations of the current art which forms part of the main research objectives of this thesis.

Since AD is a neurological disorder, current clinical methods rely on neuroimaging to assess a patient's brain activity to determine the extent of the damage done by the disease. However, these methods are very expensive and would be too costly to be employed for screening for all patients regularly. As a result, AD is usually diagnosed after symptoms started showing which would already affect the patient's quality of life. Even then, there is no known cure to completely reverse the damage done by AD which means that

patients would have to live the rest of their lives in a state of ill health. Hence, there is an urgent need to develop a low-cost alternative for more effective screening of AD. This would allow the early onset of AD to be detected and allow preventative measures to be implemented early to slow down or halt its progression.

Female breast cancer is the most prevalent form of cancer and is the deadliest cancer among women. Despite such high mortality rates, the data has shown that the survival rates of female breast cancer are very promising if the cancer is detected and treated at the early stages. However, to detect the early onset of breast cancer, the patients need to go for periodic clinical screenings which may be perceived as a hassle. Some countries have also introduced a breast cancer screening program to encourage more people to sign up for the screening program, but the uptake did not even meet half of its target. Hence, there is an urgent to improve existing breast cancer detection techniques so that it is more easily accessible.

A biosensor is a promising candidate to provide low-cost and real-time testing of diseases biomarkers which could be useful for the early detection of AD and female breast cancer. Patients can use the biosensors to test themselves at their convenience which should greatly improve the effectiveness of the screening program. Therefore, a review on biosensors was also conducted on the state-of-the-art biosensors. Electrochemical biosensors are well-established and can be produced at very low cost. However, it lacks the sensitivity which could be achieved using optical biosensors, especially those based on the surface plasmon resonance (SPR) effect. But, the fabrication cost of such optical biosensors is relatively high due as it demands very high-resolution features in the order of the wavelength scale of the optical light. Hence, part of the main objective of this thesis

was to investigate nanofabrication techniques for the low-cost fabrication of optical biosensors.

Since optical components and devices operating in the visible to near-infrared (NIR) are the most common and relatively less expensive, therefore, the operating wavelength range of the proposed optical biosensor is targeted to be in this range to ensure the cost is minimised. From the literature review, it is determined that interference lithography (IL) would be a potential method for the fabrication nanoscale grating features which could be used in a grating coupled SPR biosensor. In this context, theoretical formulations of the interference of light waves were conducted to confirm the possibility of achieving sub-wavelength scale features using IL. This was also validated using theoretical simulations based on the finite-difference time-domain method. From the theoretical method, it is clear that the periodicity of the interference pattern is largely influenced by the angle of incidence of the interfering light beams, hence, the capability of IL is its versatility for the fabrication of multiple patterns. This was demonstrated experimentally where two different IL configurations were set up. The first IL set up employs a diffraction grating which made the beams difficult to adjust. Hence, another IL set up based on a single-input multiple-output (SIMO) optical fibre. The versatility and robustness of the optical-fibre IL (OFIL) system were demonstrated by the fabrication of grating patterns with different periodicities ranging from 514 nm to 1,646 nm. The patterned area is approximately 5 mm but could also be easily adjusted by moving the lens or changing the lens with one that has a different focal length. As a result, it is easy to readjust the optics for realising different interference pattern or to achieve a specific design. Additionally, the OFIL system is a highly energy-efficient fabrication process as the laser beam is expanded only to the desired pattern area with minimal losses as the beam is focused to

the regions to be pattern without illuminating the unnecessary regions. The grating features fabricated by the OFIL system were developed into a grating coupled SPR biosensor for sucrose sensing by coupling it to a fluidic cell to contain the sample fluid. The sensitivity of the SPR sucrose biosensor is approximately 267 nm/RI and can be further improved with proper optimisation of the grating geometry or using a spectrometer with higher resolution.

Another critical requirement of using biosensors for diseases diagnostics is that the sensitivity of such biosensors is still lacking as compared to well-established laboratory-based detection techniques. Hence, there is also a need to further improve the sensitivity of optical biosensors for highly accurate detection of diseases. Through the literature review, it is reported that the addition of nanomaterials, such as nanoparticles, to the sensing surface of the optical biosensors, is a viable method to amplify the signal of SPR biosensors. In this context, a series of theoretical FDTD simulations were used to investigate different nanoparticle designs to assess their ability to amplify the signal in a prism coupled SPR biosensor. The results show that gold (Au) nano-urchins have an electric field enhancement factor that is more than 7 times that of Au nano-spheres. For demonstration, an Au nano-urchins LSPR biosensor was developed and compared against an Au nano-spheres LSPR biosensor for sensing the fibrillation of beta-amyloid ($A\beta$) which could potentially be used for detecting the early onset of AD. Due to its sharp urchins-like features, the Au nano-urchins LSPR biosensor is highly sensitive and can detect $A\beta$ at the nanomolar concentration levels. Additionally, the Au nano-urchins were also integrated with a conventional prism coupled SPR biosensor to investigate the amplification of the biosensing signal and determine the improvement in the detection limit. The theoretical FDTD simulations reveal that the Au nano-urchins enhanced SPR

biosensor has an electric field enhancement factor that is few orders of magnitudes better than a conventional SPR biosensor (20,079 and 1.78 respectively). Therefore, it should be possible to detect much lower concentrations of analytes using the enhanced SPR biosensor. For demonstration, the Au nano-urchins SPR biosensor was developed for sensing of ER α which is a commonly used breast cancer biomarker. The sensing surface was first immobilised with a layer of ER α antibodies which is a commonly used bioreceptor for the label-free detection of ER α . The enhanced SPR biosensor shows an increase in its biosensing signal as compared to a conventional SPR biosensor which suggests that its detection limit is improved. Therefore, it should be possible to detect even lower concentrations of ER α using the enhanced SPR biosensor.

Lastly, the thesis also investigated the potential of using graphene to develop an electrically controlled SPR biosensor. The advantage of this is that such biosensors could be made at even lower cost as compared to conventional optical biosensors as there is no need for highly sensitive optical components. Two methods for the synthesis of graphene were first investigated and compared against each other. Firstly, a thermal CVD approach was investigated where graphene was synthesised using very high temperatures. The quality of the graphene grown using this method was of very high quality with very clear “2D” Raman peaks which corresponds to the presence of graphene. However, the high temperatures (> 1000 °C) used in this method was not desirable which limited to growth substrates to metals. A PECVD approach was also investigated where the high temperature was replaced with a plasma to synthesise the graphene. The result shows that it was possible to grow graphene at a relatively low temperature (600 °C). However, the plasma also inevitably introduced some defects into the graphene which may not be ideal. The grown graphene was also successfully demonstrated to be transferred from the

growth substrates onto the desired substrates. The Raman spectroscopy of the graphene after transferred on to the substrates shows very clear 2D peaks. Nonetheless, this method allows the development of graphene layered heterostructure which is further developed into an SPR sensor. The addition of a layer of ion-gel allows the graphene-enhanced SPR sensor to be interrogated both optically and electrically where the SPR signal was tuned by an externally applied electric field across the heterostructure.

The future work planned for improving the current work in this report to meet the targeted research objectives are detailed in the later sections.

6.2 Major Contributions

- a) Development of a diffraction grating coupled interference lithography (IL) system for the low-cost fabrication of nanoscale grating features
- b) Conceptualisation and development of a flexible optical fibre IL (OFIL) system for a more versatile fabrication method of grating features with a wide range of priorities ranging from 514 nm to 1,646 nm.
- c) Development of a grating coupled SPR biosensor fabricated using the OFIL system for the sensing of sucrose concentration
- d) An Au nano-urchins LSPR biosensor was developed for the sensing of $A\beta$ which is a common AD biomarker. The Au nano-urchins LSPR biosensor has a field enhancement factor that is 7 times greater than an Au nano-spheres LSPR biosensor
- e) An Au nano-urchins enhanced SPR biosensor was developed for the sensing of $ER\alpha$ which is a common female breast cancer biomarker. The Au nano-urchins enhanced SPR biosensor has amplified SPR signal and achieved a lower detection limit as compared to conventional SPR biosensor which would allow the detection of smaller concentrations of $ER\alpha$ and improve its accuracy.

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- f) A remote plasma-assisted CVD method for the synthesis of horizontal graphene at a reduced temperature was demonstrated. The synthesised graphene has the characteristic “2D” Raman peak which indicates the presence of graphene.
- g) The synthesis of graphene was further developed into a graphene layered heterostructure which is further developed into a novel SPR biosensor that is electrically controlled. The graphene layered SPR biosensor shows a change in the SPR intensity when an external electric field was applied across the interface.

6.3 Recommendations for Future Work

This research primarily focuses on optical SPR biosensors for the early detection of diseases. In order to extend the scope of the research, the following recommendations are discussed as future work directions.

- Integration of the developed flexible optical fibre interference lithography system with other laser-assisted additive manufacturing (AM) methods for highly efficient fabrication of grating coupled SPR biosensor
- Optimise the beam profile of the IL system using beam shapers to achieve more uniform beam intensity
- Improve biosensing sensitivity of grating coupled SPR biosensor by using nanoparticles such as nano-urchins or nano-spheres
- Develop an electrically controlled grating coupled SPR biosensor by adding a layer of graphene to the grating coupled SPR biosensor
- Extend the capability and accuracy of the biosensor by fabricating multiple sensing channels for multiplex sensing or detection of diverse disease markers
- Fabrication of periodic structures for guided-mode resonance (GMR) applications

6.3.1 Integration of OFIL with Additive Manufacturing Methods

The OFIL system can be integrated with a laser-based 3D printing system such as (stereolithography) SLA or direct laser writing (DLW) to maximise the capability of both systems in a hybrid manufacturing system as illustrated in Figure 6.1.

Figure 6.1: Schematic illustration of the advanced laser-assisted 3D printing system

Both the OFIL system and a laser-based 3D printer (SLA or DLW) operates with a laser in the UV or near UV wavelength range to record the pattern in a photopolymer. Hence, the single-input multiple-output (SIMO) used in the OFIL system can be employed to combine both systems into a hybrid system. In the proposed system, a single 405 nm laser is coupled to a 1×3 optical fibre splitter where two of the optical fibres are coupled to the OFIL system while the remaining optical fibre can be coupled to the 3D printing system. A 3-axis stage can also be added to record the pattern across a wider area. The primary benefits of the proposed 3D printing system are the relative ease and flexibility in reconfiguring the OFIL system for the fabrication of a range of periodic nanoscale

patterns. Additionally, the fabrication of the rest of biosensor features, such as the fluidic channels, can be easily achieved using the 3D printer which greatly reduces the fabrication time. The flowchart explaining the fabrication process is given in Figure 6.2.

Figure 6.2: Flowchart of the proposed 3D printing process

First, the desired biosensor is designed and modelled in a 3D CAD file on the computer. The 3D CAD model is then sliced into multiple 2D layers and the regions with the nanoscale grating features are identified. Next, the fabrication process is split into two parts – (1) interference lithography, and (2) 3D printing. The interference lithography system would only fabricate the parts where the nanoscale grating features are necessary while the rest of the biosensors would be fabricated by the 3D printer. The sample would then be repositioned to another region so that other parts of the biosensor can be fabricated. After all the features for the first layer are fabricated, the sample can then be lowered, and the subsequent layers can be fabricated by repeating the same process as

before. The entire 3D structure of the biosensor would then be formed in a layer-by-layer fashion. For demonstration, a sample biosensor with internal microfluidic channels is model using CAD software as shown in Figure 6.3a. Figure 6.3b and Figure 6.3c shows the side profile and top view of the biosensor which is fabricated using an SLA 3D printer that is similar to the 3D printer in the proposed system.

Figure 6.3: (a) 3D model of a microfluidic channel design to be 3D printed, (b) side profile, and (c) top view of a 3D printed biosensor (blue dye is added to the water to highlight the internal channels)

By integrating both fabrication technologies in a single system, the biosensor can be fabricated from a single substrate which would greatly reduce the manufacturing cost per biosensor chip. Additionally, the microfluidics channels which are integrated to the biosensor chip can also be easily customised to suit different applications. Furthermore, grating features which varying periodicities and geometries can also be easily fabricated in a single biosensor chip to allow multiplex sensing. Therefore, the proposed system would be a highly useful 3D printing tool for fabricating grating coupled biosensors at a low cost. It is envisioned that the proposed system would allow biosensors chips to be

more affordable which is critical for biomedical applications, especially for disease diagnostics.

6.3.2 Optimisation of the Beam Profile of the IL System

The optical fibre employed in the OFIL system is a single-mode optical fibre, which means that it only supports a single transverse propagation mode. The guided propagation mode within a single-mode optical fibre is described as an LP_{01} mode which is illustrated in Figure 6.4a. The intensity distribution of such a beam, when measured across the central axis of the beam, would be similar to a Gaussian beam as shown in Figure 6.4c.

Figure 6.4: Beam profile of (a) Gaussian beam, (b) flat-top beam, and (c)-(d), the respective intensity distribution along the central axis

This means that there is a variation in the intensity along the radial position of the beam. This is not very desirable in IL as the resulting interference pattern would not be uniform which would result in deviation in the exposure dose within the photosensitive medium. Due to this, the grating features formed by the IL system would have varying line thickness or sidewall profiles which may cause some discrepancy in the signal when used as an SPR biosensor. This could be minimised by adding a beam shaper to transform the Gaussian beams into beams with a flat-top beam profile as shown in Figure 6.4b. From the intensity distribution which is given in Figure 6.4d, the centre portion of the beam has a very uniform intensity distribution which suggests that the exposure dose would not vary too much and the grating features would be exactly similar to each other.

Additionally, using such flat-top beams in the DLW system would be highly beneficial as it would be possible to fabricate features with very steep and smooth sidewalls as compared to a Gaussian beam. This would greatly improve the quality of the fabricated biosensors and the resolution of the DLW system. This would be especially beneficial for the fabrication of microfluidic channels and allow the sample fluid to flow more smoothly.

Alternatively, besides using beam shapers to achieve a uniform, flat-top beam profile, this could be achieved using speciality optical fibres such as shaped-core fibres, long-period gratings or multimode optical fibres with multimode interference devices. This would be an interesting research direction to explore for extending the efficiency of the OFIL system and improve the quality of the fabricated features.

6.3.3 Improve Biosensing Sensitivity of Grating Coupled SPR Biosensor

In the thesis, nanoparticles were demonstrated as a potential method for amplifying the signal for a prism coupled SPR biosensor. The results show a promising improvement in the detection limit which enables smaller concentrations of analytes to be detected. In this context, this work may be extended to investigate the improvement in a grating coupled SPR biosensor as shown in Figure 6.5. The Au nano-urchins which were employed in the thesis can be immobilised directly on top of the grating features to amplify the SPR signal.

Figure 6.5: Schematic of the Au nano-urchins enhanced grating coupled SPR biosensor

However, this work would still be challenging as further theoretical simulations are still necessary to optimise the grating parameters to ensure maximum coupling between the surface plasmons wave generated by grating and the localised surface plasmons at nanoparticles. There is also a need to ensure the nanoparticles are uniformly distributed over the gratings with no aggregation as this could reduce the coupling efficiency of the grating. The nanoparticles should also be immobilised on both the top layer and bottom layer of the gratings to maximise the surface area.

Nonetheless, this is an interesting research direction as grating coupled SPR biosensors are typically more compact as compared to prism coupled SPR biosensors. The grating features can also be fabricated economically using the developed OFIL system which may

further reduce the costs of such biosensors. Hence, this could potentially be used for the development of highly sensitive and low-cost biosensors which may better fulfil the requirements for the early detection of diseases.

6.3.4 Electrically Controlled Grating Coupled SPR Biosensor

The addition of a graphene layer allows for the development of a novel electrical controlled SPR sensor. This unlocks new avenue for the SPR biosensors as the cost benefits of an electrical biosensor and the high sensitivity of optical biosensors can be achieved in one biosensor. In the thesis, the electrically controlled SPR sensor was demonstrated in a prism coupled configuration. This method should also be possible to realise in a grating coupled SPR sensor. Hence, in this context, another possible future research work is to explore the potential of developing an electrically controlled grating coupled SPR biosensor. The graphene layered heterostructure which is necessary to achieve this is illustrated in Figure 6.6 where the graphene layer is coated uniformly over the entire surface of the grating features.

Figure 6.6: Schematic of graphene/metal/dielectric heterostructure with the grating features

One possible barrier to realise this structure is the difficulty in the transfer of the graphene layer over the entire surface of the grating. As the graphene is synthesised on a separate growth substrate, a subsequent process step is necessary to transfer the graphene over the grating features. However, since graphene is a 2D material, it may not

conform nicely to the geometrical shape of the grating and may instead only adhere to the top and bottom surface of the grating features. This would result in broken graphene and may reduce the effectiveness of the externally applied electric field on the tuning of the SPR condition.

Nonetheless, the development of an electrically controlled grating coupled SPR biosensor is highly desirable as the cost can be significantly reduced as compared to a conventional grating coupled SPR biosensor. Additionally, the electrical components are typically much more compact than optical components and much more cost-effective, hence, these biosensors would also be more advantageous to meet the requirements for the early detection of diseases.

6.3.5 Multiplex Grating Coupled Biosensor

The advantage of an OFIL system is the relative ease in fabricating grating features with varying periodicities (Λ). Therefore, it would be easy to fabricate an array of grating features with different periodicities (for example, Λ_1 , Λ_2 , Λ_3) on a single sensor chip as shown in Figure 6.7.

Figure 6.7: Schematic illustration of a multiplex grating coupled biosensor

Due to their varying periodicity, each grating features would have a different resonance wavelength across a wide spectrum. Therefore, by monitoring the entire spectrum of light, it would be possible to detect and identify any changes in the localised region near each grating features. Different bioreceptors can then be functionalized on each sensing sites for the detection of multiple analytes using a single biosensor chip.

This could be especially useful for the early detection of diseases as multiple disease biomarkers can be detected using a single biosensor chip. This would greatly reduce the time needed for screening as compared to taking multiple individual tests for individual diseases. Furthermore, the multiplexed biosensor can screen for other biomarkers associated with the diseases and assist clinicians in making a more accurate diagnosis.

However, the grating features need to first be optimised to ensure that their resonance wavelengths are spaced out sufficiently to ensure that their operating range does not overlap with each other. This would ensure that the signal from the individual sensing sites can be distinguished from the overall signal. Hence, further work is necessary to develop a good design multiplex grating coupled biosensor.

6.3.6 Guided Mode Resonance

Besides being developed for use in SPR sensors, the periodic nanoscale grating features can also be developed for use in guided-mode resonance (GMR) devices. GMR devices by virtue of their narrow linewidth, high efficiency, and high phase sensitivity, have been used for many applications such as bio-sensors, tunable optical filters and electro-optic switches [222–224]. The electric field corresponding to the resonant mode of the GMR structure is strongly confined within the waveguide that can be used to trap light and hence can enhance the absorption in 2D functional materials such as graphene monolayer

Graphene is an ideal material for optoelectronic devices such as photodetectors, absorbers, and modulators. However, monolayer graphene lacks the property of spectral selectivity due to its ultra-broadband uniform spectral response from ultraviolet to terahertz range. In addition, its absolute absorption is too low (2.3%) in this wavelength range that results in low photoresponsivity [225]. To overcome these issues, several mechanisms have been proposed to enhance absorption in a graphene layer by embedding it in photonic crystals and subwavelength gratings. Most of the reported works have used critical coupling by guided resonance and achieved more than 70% absorption both theoretically and experimentally [226]. However, all these reported works have used some sort of back reflectors such as metal films and Bragg mirrors to achieve perfect absorption [226,227]. The use of a metallic reflector, however, results in significant suppression of absorption in graphene, due to metallic losses. On the other hand, dielectric Bragg mirrors don't have this kind of loss issue. However, they increase the physical footprint of the device and makes the fabrication process more complicated since it requires material deposition of several layers. In addition, incorporation of these back reflectors makes the device complicated for certain applications. On the other hand, the optical absorption is very low (30-50%) without any back reflectors [228]. Therefore, absorption enhancement in a monolayer-graphene without using any back reflectors is still rare and in urgent need.

In this context, the possibility of using IL as a cost-effective method to fabricate the nanoscale grating features necessary for the GMR structures was explored. The GMR structure will be used to enhance the absorption of a monolayer graphene embedded within the structure. The proposed GMR structure consists of a 1D grating of depth 'h' and periodicity ' Λ ' coupled with a thin film waveguide (silicon nitride) of thickness 'd' on

a quartz plate as shown in Figure 6.8. The refractive index of the material forming the grating is n_g . The grating has a duty cycle (f) defined as w/Λ , where w is the line width of the grating.

Figure 6.8: Schematic of the proposed GMR structure with an embedded graphene monolayer

First, the necessary theoretical formulae based on coupled-mode theory was derived to predict and maximize the absorption. Based on the developed formulae, the peak absorptance at the resonance wavelength can be calculated and compared against the results from the FDTD simulation. The optimized GMR structure was then fabricated using IL and graphene was embedded within the GMR structure as described in the main text of the thesis. Refer to Appendix I for the full steps taken to develop the formulae and fabrication of the proposed GMR structure.

The absorption spectrum of the fabricated GMR sample was measured using a spectrometer and a broadband (halogen) light source as shown in Figure 6.9a. Multimode optical fibre and lenses are used for light coupling collimation purposes. S-polarized light was obtained using a polarizer and is incident normally to the sample. The absorption spectrum for the graphene monolayer on the SiN coated quartz substrate was first measured before any patterning. The obtained transmittance (blue), reflectance (green) and absorptance (red) spectrum are shown in Figure 6.9b. As can be seen from the figure,

the graphene monolayer has a uniform absorption of approximately 2.3% throughout the entire spectrum. The absorption spectrum with the grating pattern is shown in Figure 6.9c and resonance is observed at approximately 762.16 nm with peak absorbance value of 0.5952. As can be seen from the figure, the reflectance of the structure at or near the resonance wavelength is greatly reduced because of the critical coupling condition. To further validate the theory, the experimentally obtained absorption spectrum was compared with that of the simulated GMR structure modelled using FDTD. The result is shown in Figure 6.9d, where the experimentally obtained absorption spectrum is fitted with a Lorentzian function of frequency.

Figure 6.9: Schematic of the experimental set up for measuring the absorbance of the GMR structure, (b) R, T and A of a graphene monolayer on the quartz substrate without any GMR structure (c) R, T and A of a graphene monolayer when embedded in the GMR structure. (d) Comparison of the experimentally obtained absorption spectrum and its Lorentz fit with the simulated spectrum

Both the results show similar absorption spectrum except that the simulated resonance wavelength is red-shifted from the experimental spectrum. The reason for this discrepancy can be due to the deviation in experimental SiN film thickness and/or due to a change in duty cycle or nonuniform resist thickness throughout the patterned area of

the sample. Nevertheless, the simulated absorptance value (0.595179) is well-matched with the experiment (0.5952) and theoretical value (0.5962).

Therefore, it can be concluded that the absorption of graphene embedded in our developed GMR structure is well-matched with the theoretically calculated derived from the FDTD model and the formula developed based on the coupled-mode theory. The proposed model does not require any back reflector such as a metal film or Bragg mirror. Using this formula, the GMR parameters were optimised to enhance the absorption up to 70% which is higher than the reported values without back reflectors and comparable with results with back reflectors. The developed model can be applied for binary as well as other grating profiles such as pyramidal grating profile obtained using interference lithography.

Hence, the result of this work can be another possible research direction for future work where the flexible IL system can enable the fabrication of other GMR structures with tuneable operating wavelengths.

APPENDICES

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APPENDIX A

Global disease burden and Singapore's disease burden

With the findings of the report from the GBD, Singapore's Ministry of Health published a report on The Burden of Disease in Singapore (1990-2017) [229]. The metrics and concepts used are similar to the GBD, but the data is presented in more detail to highlight the current and potential health challenges of Singapore. Figure A1 is a graphical representation of the distribution of Singapore's disease burden for the year 2017 by the total DAYLs. Figure A2 shows the distribution of Singapore's disease burden in 2017 as compared to 1990 and also the change in the DALYs.

Figure A1: Graphical distribution Singapore's disease burden in 2017 by cause, all genders (graph production from [229])

Figure A2: Distribution and change in DALYs for different diseases in Singapore from 1990-2017, all genders (graph production from [229])

APPENDIX B

Published data from WHO on the global cancer burden in 2018

Data from the WHO reveals that breast cancer is the most prevalent and the deadliest form of cancer in 2018. Table B1 shows the ten most prevalent form of cancers in the last 5-years (from 2018) and Figure B1 shows the distribution in a pie chart.

Table B1: Estimated number of prevalent cases (5-year) in 2018 (worldwide, all ages, all genders)

Cancer sites	Number of prevalent cases (5-year) in 2018
Breast	6 875 099
Non-melanoma skin cancer	5 207 882
Prostate	3 724 658
Colon	2 785 583
Lung	2 129 964
Thyroid	1 997 846
Rectum	1 876 453
Bladder	1 648 482
Stomach	1 589 752
Cervix uteri	1 474 265
Other cancers	14 531 318
Total	43 841 302

Figure B1: Distribution of prevalent cases (5-year) in 2018 (worldwide, all ages, all genders)

Table B2 shows the ten deadliest form of cancers in 2018 among all females and the distribution is given in a pie chart as shown in Figure B2.

Table B2: Estimated number of cancer-related deaths in 2018 (worldwide, all ages, females only)

Cancer sites	Number of deaths in 2018
Breast	626 679
Lung	576 060
Cervix uteri	311 365
Stomach	269 130
Colon	260 760
Liver	233 256
Pancreas	205 332
Ovary	184 799
Oesophagus	151 395
Leukaemia	129 488
Other cancers	1 221 123
Total	4 169 387

Figure B2: Distribution of cancer-related deaths in 2018 (worldwide, all ages, females only)

Data source: GLOBOCAN 2018 database, International Association of Cancer Registries
 Graph production: Global Cancer Observatory, International Agency for Research on Cancer, World Health Organization [29]

APPENDIX C

Five most frequently diagnosed cancers among Singaporean females by 5-year period (1968 – 2017)

Table C: Five most frequently diagnosed cancers among Singaporean females by 5-year period from 1968-2017 (data source [50])

Cancer Site	No.	ASIR	Cancer Site	No.	ASIR
1968-1972			1973-1977		
Female breast	672	20.1	Female breast	861	22.1
Cervix uteri	603	18.0	Colon & rectum	715	19.6
Stomach	542	17.4	Cervix uteri	675	17.6
Lung	489	16.2	Lung	663	18.5
Colon & rectum	478	15.4	Stomach	610	16.6
1978-1982			1983-1987		
Female breast	1237	26.9	Female breast	1737	31.1
Colon & rectum	1084	24.6	Colon & rectum	1393	26.1
Lung	893	20.8	Lung	1072	20.4
Cervix uteri	751	16.6	Cervix uteri	897	16.2
Stomach	643	14.6	Stomach	772	14.3
1988-1992			1993-1997		
Female breast	2631	38.6	Female breast	3598	43.5
Colon & rectum	1848	28.3	Colon & rectum	2300	29.5
Lung	1174	18.0	Lung	1444	18.3
Cervix uteri	1002	15.3	Cervix uteri	1128	13.9
Stomach	826	12.5	Stomach	917	11.4
1998-2002			2003-2007		
Female breast	5577	55.6	Female breast	6856	58.9
Colon & rectum	2795	29.1	Colon & rectum	3350	28.8
Lung	1603	16.4	Lung	1905	16.3
Ovary & fallopian tube	1061	11.1	Corpus uteri	1356	11.9
Cervix uteri	1038	10.7	Ovary & fallopian tube	1347	12.2
2008-2012			2013-2017		
Female breast	8560	63.0	Female breast	10824	69.8
Colon & rectum	3920	27.0	Colon & rectum	4835	27.2
Lung	2262	15.4	Lung	2788	15.4
Corpus uteri	1787	13.1	Corpus uteri	2610	16.9
Ovary & fallopian tube	1625	12.7	Ovary & fallopian tube	1874	13.1

APPENDIX D

Breakdown of Singapore's cardiovascular disease burden (1990 – 2017)

The breakdown of Singapore's cardiovascular disease burden from 1990 to 2017 is given in Figure D. The age-standardised disability-adjusted life years (DALYs) is used here to account for Singapore's ageing population [229].

Figure D: Breakdown of age-standardised DALYs due to different forms of cardiovascular diseases in Singapore, all genders, 1990-2017 (graph production from [229])

APPENDIX E

Calculation of the grating periodicity by finding the average distance between each peak

In order to calculate the periodicity from the simulated interference pattern, the line profile plot along the x-direction is first extracted from the image. The peak centres are then identified by a fitting program. With this, the periodicity can be calculated by finding the average distance between each peak as shown in Table E1.

Table E: Calculation of the periodicity by finding the average distance between each peak

Peak position (μm)	Distance to next peak (μm)
-1.98565	0.23924
-1.74641	0.25518
-1.49123	0.25519
-1.23604	0.25518
-0.98086	0.23923
-0.74163	0.23924
-0.50239	0.23923
-0.26316	0.25519
-0.00797	0.27113
0.26316	0.23923
0.50239	0.23924
0.74163	0.23923
0.98086	0.25518
1.23604	0.25519
1.49123	0.25518
1.74641	0.23924
1.98565	-
Average periodicity (μm)	0.2482

APPENDIX F

Substrate preparation for recording of the interference pattern in the photoresist

The substrates used are 100 mm silicon (Si) wafers and are prepared as follows:

- 1) Cleaning of wafer in an acetone bath with sonication followed by rinsing in (DI) water
- 2) Cleaning in isopropanol alcohol (IPA) bath with sonication and rinsing in DI water
- 3) Drying of wafer on a hotplate at 100°C
- 4) Coating of hexamethyldisilane (HMDS) to promote photoresist adhesion
- 5) Spin coating of photoresist (Merck KGaA, AZ® MiR™ 701 - 14cPs) at 3,000 rpm (refer to Figure F for the photoresist spin curves)
- 6) Soft bake at 90°C for 90s followed by cooling of sample at room temperature for 60s
- 7) Loading of wafer on sample stage followed by exposure of laser beams. The required exposure duration is achieved by a mechanical shutter with a temporal resolution of 10ms, which is installed directly at the laser output. The required duration is calculated by the following steps:
 - i. A power meter was first used to measure the power in each beam
 - ii. A variable neutral-density (ND) filter wheel was used to ensure both beams have equal intensities to achieve maximum contrast
 - iii. Both beams were observed to have power around 1.5mW
 - iv. The beam spot diameter is measured to determine the irradiance of the laser to be around 45mW/cm²
 - v. The exposure duration can then be calculated by referring to the photoresist's required energy dose which is approximately 75 mJ/cm² for a 1 µm film thickness
 - vi. The required exposure time is approximately 1.6 s
- 8) After exposure, the wafer undergoes post-exposure bake at 110°C for 90s
- 9) Developing of photoresist in developer bath (MicroChemicals GmbH, AZ® 326 MIF) for 60s
- 10) Sample is rinsed in DI water to stop the developing process

Figure F: Spin curves for AZ® MiR™ 701 series positive-tone photoresist (source, Merck KGaA)

APPENDIX G

LabVIEW program for simultaneous control of the rotation stages and data acquisition

The Front Panel view of the LabVIEW program is given in Figure G1. The user would primarily interact with this window. Figure G2 shows the block diagram of the LabVIEW program. This is the actual code used to control the rotation stages and acquire the data.

Figure G1: Front Panel view of the LabVIEW program

Figure G2: Block diagram view of the LabVIEW program

APPENDIX H

Unscaled simulated electric field intensity

The unscaled electric field intensities of the various nanoparticles from the FDTD simulations are given in the follow few figures. Figure H1 is for the Au nano-sphere with 100 nm diameter. Figure H2 to Figure H5 shows the result from the Au nano-urchin with increasing particle size from 60 nm to 120 nm (in steps of 20 nm), respectively.

Figure H1: Electric field intensity of Au nano-sphere, diameter = 100 nm, $I_{\max} = 135$, $\lambda = 608$ nm

Figure H2: Electric field intensity of Au nano-urchin, particle size = 60 nm, $I_{\max} = 243$, $\lambda = 618$ nm

Figure H3: Electric field intensity of Au nano-urchin, particle size = 80 nm, $I_{\max} = 454$, $\lambda = 628$ nm

Figure H4: Electric field intensity of Au nano-urchin, particle size = 100 nm, $I_{\max} = 1054$, $\lambda = 637$ nm

Figure H5: Electric field intensity of Au nano-urchin, particle size = 120 nm, $I_{\max} = 804$, $\lambda = 659$ nm

APPENDIX I

Enhanced absorption in a graphene embedded 1D guided-mode-resonance structure without back-reflector and interferometrically written gratings

Referring to Figure 6.8, since the grating is comprised of high n_g and low index materials (air), the effective refractive index (n_{eff}) of the grating film for s-polarized light becomes

$$n_{\text{eff}} = \sqrt{f n_g^2 + (1 - f) n_{\text{air}}^2} \quad (7.1)$$

As the graphene monolayer is incorporated at the interface of the waveguide and grating layer, the amplitude reflection (Γ) and transmission (τ) coefficients can be obtained from the coupled mode theory which are given below.

$$\Gamma = \frac{r(\omega - \omega_0) + i(r\delta - \gamma)}{(\omega - \omega_0) + i(\gamma + \delta)} \quad (7.2)$$

$$\tau = t \left\{ \frac{(\omega - \omega_0) + i\delta}{(\omega - \omega_0) + i(\gamma + \delta)} \right\} \quad (7.3)$$

where r and t are the respectively the off-resonance (Fresnel) amplitude reflection and transmission coefficients when there is no resonance taking place in the structure, γ is the external leakage rate which is defined as the time rate of amplitude change in the resonator in the absence of input wave, δ is the intrinsic loss rate due to the embedded graphene layer. The corresponding (intensity) reflectance (R) and transmittance (T) can be written as

$$R = \Gamma \cdot \Gamma^* = \frac{r^2(\omega - \omega_0)^2 + (r\delta - \gamma)^2}{(\omega - \omega_0)^2 + (\gamma + \delta)^2} \quad (7.4)$$

$$T = \tau \cdot \tau^* = t^2 \left\{ \frac{(\omega - \omega_0)^2 + \delta^2}{(\omega - \omega_0)^2 + (\gamma + \delta)^2} \right\} \quad (7.5)$$

Thus, the absorption, $A = 1 - (R + T)$ as a function of incident frequency (ω) can be calculated as

$$A = \frac{(1 - r^2 - t^2)\{(\omega - \omega_0)^2 + \delta^2\} + 2\gamma\delta(1 + r)}{(\omega - \omega_0)^2 + (\gamma + \delta)^2} \quad (7.6)$$

This absorption spectrum takes the form of Lorentzian function of frequency whose peak value at the resonant frequency ($\omega = \omega_0$) is given by

$$A = \frac{(1 - r^2 - t^2)\delta^2 + 2\gamma\delta(1 + r)}{(\gamma + \delta)^2} \quad (7.7)$$

It can be inferred from Eqn (7.7) that the peak absorptance depends on the off-resonance reflection (r) and transmission (t) coefficients, the external leakage rate (γ) and the loss rate (δ). These parameters greatly depend on the grating parameters, h, f, and n_g . To validate this theoretical formula, the effect of these parameters was analysed for a fixed waveguide thickness ($d = 100$ nm) and grating period ($\Lambda = 500$ nm). The photoresist used to record the grating pattern on the silicon nitride coated quartz substrate is NR7-500P (Futurrex) and its refractive index (n_g) of is estimated to be 1.604 from 700 nm - 800 nm wavelength range.

First, f is fixed at 0.8 and h is from 100 nm to 600 nm. The obtained FDTD result is shown in Figure I1a. The absorptance value varies within the range of 0.4 to 0.7 and attains a maximum value ($A \approx 0.7$) when h is 225 nm and 500 nm. Keeping h value fixed at 225 nm and 500 nm, the effect of f was analysed, and the obtained results are shown in Figure I1b. As can be seen from this result, the absorptance value is maximum (≈ 0.7) when f is around 0.8 for both 225 nm and 500 nm depth. For further analysis, the grating depth is chosen to be around 225 nm due to the following three reasons. First, because NR7-500P is a thin photoresist and could achieve this film thickness easily. Second, photoresist material absorption (if any) will have reduced effect on the sole analysis of the graphene absorption. Thirdly, the results of the coupled mode theory are more accurate when the grating depth is thin. To understand the reason for maximum absorption at $f = 0.8$, the external leakage rate (γ) and the loss rate (δ) for $f = 0.2$ to 0.9 was calculated. γ is calculated by Lorentzian fit of the stored energy at the guided resonance. When there is no graphene layer in the GMR, the centre frequency of the Lorentzian (fitted as a function of frequency) gives the resonance frequency (ω_0) and the half-width at half maxima provides γ . The same procedure was repeated with a graphene embedded structure to find the half-width at half maxima, from which $\gamma + \delta$ and δ can be calculated. The obtained γ and δ values (Table I) are plotted in Figure I1c.

As can be seen from Table I, γ has different values for different duty cycles, whereas δ is almost independent of it. Both γ and δ have equal values at 0.8. This is the case of critical coupling where the condition for maximum absorption is achieved. The condition for maximum absorption can be explained as follows: when $\gamma > \delta$, photons decay away from the cavity before being absorbed by the graphene layer. On the other hand, when $\gamma < \delta$, photons cannot be coupled into the cavity. Thus, maximum absorption is attained when the critical coupling is achieved (when $\gamma = \delta$ at $f = 0.8$). After calculating the γ and δ values, the off-resonance reflectance (r) and transmittance (t) values can be obtained. The values of r and t are obtained by replacing the grating film with a uniform slab of effective refractive index, n_{eff} and thickness that is same as the grating depth (225 nm). n_{eff} depends on both f and n_g as per Eqn (6.1). Thus, using Eqn (6.1), n_{eff} can be calculated and used to derive r^2 and t^2 (Table I).

Table I: List of calculated parameters; γ , δ , r^2 and t^2

Duty cycle (f)	External leakage rate γ (10^{-6} nm^{-1})	Loss rate δ (10^{-6} nm^{-1})	Off-resonance reflectance (r^2)	Off-resonance reflectance (t^2)
0.3	6.0320	2.4239	0.1359	0.850693
0.4	7.2716	2.4224	0.1392	0.847516
0.5	7.0081	2.3621	0.1504	0.836448
0.6	5.7384	2.2929	0.1621	0.824899
0.7	3.9606	2.2886	0.1732	0.814005
0.8	2.1855	2.1019	0.1815	0.805782

After obtaining all these required parameters, the peak absorbance at the resonance wavelength (λ_0) can be calculated from Eqn (7.7). To validate the theoretically calculated absorption values, the GMR-graphene structure was also simulated in FDTD and compared against the theoretically calculated adsorption values. The obtained results are plotted in Figure I1d and it can be inferred that both the theoretical values and simulated values agrees well with each other.

Figure I1: Variation of absorbance with grating depth for $f = 0.8$, $n_g = 1.604$, $\Lambda = 500 \text{ nm}$ and $d = 100\text{nm}$.

(b) Variation of absorbance with duty cycle for two fixed grating depth, 225 nm and 500 nm.

(c) Leakage rate (γ) and loss rate (δ) plotted as a function of duty cycle (f).

(d) Comparison of theoretically calculated absorbance with simulated values as function of duty cycle

To validate the developed theory, the proposed GMR structure was fabricated on a quartz substrate. A 100 nm silicon nitride (SiN) film was first deposited on a quartz substrate by

plasma enhanced chemical vapor deposition (PECVD) method. Next, monolayer graphene was grown using the similar chemical vapor deposition method (CVD) system as described in the thesis. The grown graphene is then transferred onto the SiN coated substrate using the wet chemical etching method as explained previously.

Raman spectroscopy was used to confirm the successful transfer of the graphene as shown in Figure I2a. The characteristic '2D' peak ($\sim 2650 \text{ cm}^{-1}$) is clearly observed which indicates the presence of graphene. The 'G' peak ($\sim 1580 \text{ cm}^{-1}$) corresponds to the ordered hexagonal structure of the graphene. Additionally, the number of graphene layers can also be inferred from the relative intensity of the '2D' peak to the 'G' peak (I_{2D}/I_G). A high I_{2D}/I_G ratio of more than 2 indicates single-layer graphene and a lower ratio corresponds to a greater number of graphene layers. The transferred graphene has a I_{2D}/I_G ratio of approximately 4.53 which confirms that the graphene transferred on top of the SiN coated substrate is indeed monolayer.

Next, the nanoscale grating features are fabricated on top of the graphene/SiN coated substrate using the IL system which was developed as part of the research work. First, the required angle of incidence was derived based on the targeted periodicity of 500 nm. After modifying the IL system, a thin layer of NR7-500P negative-tone photoresist was spin coated on top of the graphene/SiN coated substrate and the interference pattern was recorded within the photoresist by exposing for 700 ms. After developing and drying, the substrate is investigated using a laser confocal microscope (Keyence, VK-X Series) as shown in Figure I2b. From the figure, the graphene area can be differentiated from the exposed quartz surface based on the slight difference in colour. The patterned area is well within the graphene area which forms the desired layered GMR structure.

Figure I2: (a) Measured Raman peak of the transferred graphene, (b) confocal image of the fabricated sample, (c) AFM image of the grating profile, and (d) AFM depth profile of the fabricated grating structure

Atomic force microscopy (AFM) was then used to measure the grating profile. The top view of the grating is shown in Figure I2c and the zoomed image is given in the inset. Based on the AFM measurement, the depth profile of the grating is plotted in Figure I2d. From the graph, the experimentally obtained grating features have a slanted side wall which differs from the FDTD simulation and the theoretical calculations. This is attributed to the sinusoidal interference pattern. The measured grating parameters have a period $\Lambda = 500$ nm, average depth $d = 240$ nm, base width = 470 nm and top width = 100 nm.

The off-resonance reflectance (r) and transmittance (t) values are needed to calculate the theoretical formula from Eqn (7.7) to validate the experimentally obtained value. Since the fabricated grating profile is not binary, Eqn (7.1) cannot be applied to find the effective index (n_{eff}) of the grating layer. For this purpose, the experimentally obtained grating profile is simulated in FDTD as shown schematically in Figure I3a. The effective refractive index (n_{eff}) (in a unit cell) for this grating (pyramidal) profile varies from the grating-waveguide interface to the grating-air interface. To calculate r and t , this grating is replaced with a uniform slab of thickness equal to the grating depth (240 nm as in experiment) with a gradient refractive index profile as shown in Figure I3b that is described with the equation, $n_{\text{eff}}(z) = 1.5824 - 0.0018z$.

Figure I3: (a) Schematic of the FDTD grating having a slanted side wall to replicate the experimentally obtained grating profile. (b) The corresponding n_{eff} profile calculated in one period of the grating

Therefore, the reflectance and transmittance values can be obtained as $r^2 = 0.115835$ and $t^2 = 0.870444$, respectively. The calculated leakage rate and loss rate for this geometry are $\gamma = 4.5014 \times 10^{-6}$ and $\delta = 2.2396 \times 10^{-6}$, respectively. Using these values, the absorptance at the resonance wavelength (762.16 nm) is calculated to be 0.5962 which is very similar to the experimentally obtained value (0.5952), thus validating our theoretical prediction. Therefore, it can be concluded that the absorption of graphene embedded in our developed GMR structure is well matched with the theoretically calculated derived from the FDTD model and the formula developed based on the coupled mode theory. Hence, the result of this work can be another possible research direction for future work where the flexible IL system can enable the fabrication of other GMR structures with tuneable operating wavelengths.

LIST OF PUBLICATIONS

Journals Articles

- [1] **Jian Yi Pae**, Rohit Medwal, Joseph Vimal Vas, Murukeshan Vadakke Matham and Rajdeep Singh Rawat, "Remote plasma-assisted low-temperature large-area graphene synthesis," Journal of Vacuum Science & Technology B 37, 041201 (2019) *[Editor's Pick]*
- [2] **Jian Yi Pae**, Pankaj Kumar Sahoo and Murukeshan Vadakke Matham, "Grating-Coupled Plasmonic Sensor for Sucrose Sensing Fabricated Using Optical Fiber-Based Interference Lithography (OFIL) System," IEEE Sensors Journal 19(22), 10477 (2019)
- [3] Pankaj Kumar Sahoo, **Jian Yi Pae** and Murukeshan Vadakke Matham, "Enhanced absorption in a graphene embedded 1D guided-mode-resonance structure without back-reflector and interferometrically written gratings," Optics Letters 44(15), 3661 (2019) *[Editor's Pick]*
- [4] Radhika Nair, **Jian Yi Pae**, Parasuraman Padmanabhan, Balázs Gulyás and Murukeshan Vadakke Matham, "Au nano-urchins enabled localized surface plasmon resonance sensing of beta amyloid fibrillation," Nanoscale Advances (2020)
- [5] **Jian Yi Pae**, Rohit Medwal, Radhika Nair, Avinash Chaurasiya, Marco Battiato, Rajdeep Singh Rawat, Murukeshan Vadakke Matham, "Electro-ionic control of surface plasmons in graphene-layered heterostructures," Nano Letters 20(11), 8305 (2020)

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- [6] **Jian Yi Pae**, Radhika Nair, Parasuraman Padmanabhan, R. Gayathri, Balázs Gulyás and Murukeshan Vadakke Matham, "Gold nano-urchins enhanced surface plasmon resonance (SPR) sensors for the detection of estrogen receptor alpha ($ER\alpha$)," IEEE Journal of Selected Topics in Quantum Electronics (2021) [*under review*]

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- [1] **Jian Yi Pae** and Murukeshan Vadakke Matham, "A review of interferometric techniques with possible improvement in pattern resolution using near-field patterning," Proc. SPIE 10449, Fifth International Conference on Optical and Photonics Engineering (icOPEN 2017), 104491B (2017)
- [2] **Jian Yi Pae**, Pankaj K. Sahoo, Rohit Medwal, Rajdeep Singh Rawat, Murukeshan Vadakke Matham, "Enhanced sensitivity in graphene-based nanoscale grating features for optical sensing applications," TPI-LUX Photonics@SG 2018 Conference (2018)
- [3] Murukeshan Vadakke Matham, **Jian Yi Pae**, Rohit Medwal, and Rajdeep Singh Rawat, "Synthesis and the development of graphene-layered substrates for flexible wearable biosensors," Proc. of the 3rd International Conference of Theoretical and Applied Nanoscience and Nanotechnology (TANN'19), 133 (2019) [*Best Poster Award*]
- [4] **Jian Yi Pae**, Rohit Medwal, Radhika Nair, Avinash Chaurasiya, Rajdeep Singh Rawat, Murukeshan Vadakke Matham, "Electro-optical interrogation of surface plasmons in an optical switch," OSA Advanced Photonics Congress (2020)

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